SEASONAL CHANGES IN MERCURY STOCKS AND METHYLATION RATIOS IN VERNAL POOLS IN THE NORTHEASTERN UNITED STATES

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ABSTRACT

Vernal pools play critical ecological roles by supporting diverse invertebrate communities and providing breeding habitat for globally pressured amphibian species. However, these systems may also be effective in transforming mercury (Hg) into a more toxic and bioavailable form, methylmercury (MeHg). Long-range atmospheric deposition of Hg, emitted primarily from industrial sources, has led to widespread ecosystem contamination. Upon entering aquatic systems, the anoxic conditions at the sediment-water interface in vernal pools favor anaerobic bacteria that facilitate the transformation of atmospherically deposited, inorganic Hg to MeHg. MeHg is a neurotoxin that has significant adverse effects on wildlife including reduced performance, maintenance, and reproductive capabilities. Vernal pools are also characterized by low pH conditions and high amounts of dissolved organic carbon and these environmental characteristics have been linked with increased methylation in other aquatic ecosystems. The variable hydrology of vernal pools may also favor increased methylation by shifting the redox cycle. Given the ecological role of vernal pools and their potential for efficient methylation, the uncertainty around methylation dynamics in these systems represents an important knowledge gap.

The purpose of this research was to quantify the concentrations of Hg and MeHg that biota are exposed to in vernal pools and to determine the methylation efficiency of these habitats. Eight sites in two national parks in the northeastern United States were utilized for this study. Water samples were collected and analyzed for parameters shown to influence methylation, including pH, temperature, conductivity, dissolved organic carbon (DOC), sulfate, as well as Hg and MeHg concentrations. Pressure transducers that continuously recorded water level were deployed at each site to quantify the hydrologic regime. These chemical and hydrologic parameters were also used to explore how the physical environment of these systems may affect methylation dynamics.

Mean total mercury concentrations at our study sites ranged from 0.53 ng/L to 8.27 ng/L. Mean methylmercury concentration ranged from 0.24 ng/L to 4.52 ng/L. The mean methylation efficiency averaged 43% and peaked at 58%. Methylation efficiencies exceeding 10% have been linked to elevated levels of MeHg in biota and each of our study sites regularly exceeded this threshold. Vernal pools play an integral role in energy cycling in forested ecosystems, exporting as much as 140 kg of biomass per hectare annually. Thus contamination of vernal pool biota not only has implications for these organisms, but also represents a vector for MeHg to move out of vernal pools and into other trophic webs. This potential contamination could negatively affect population dynamics for a wide range of species.

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1.1. LITERATURE REVIEW

MERCURY IN THE ENVIRONMENT

Mercury (Hg) contamination is a pressing ecological problem because of the widespread distribution of the contaminant, its toxic effects on biota, and its ability to bioaccumulate in food webs. The ubiquitous nature of Hg in the environment, as well as the presence of significant amounts of Hg in pristine natural areas, far away from potential sources, implicates long-range atmospheric deposition as the dispersal mechanism.[1, 2] Sediment and peat cores collected over a broad geographical range provide temporally and spatially consistent geochemical evidence that long distance transport of anthropogenic Hg and subsequent deposition is indeed an important source of Hg to the environment.[3]

Mercury is emitted to the atmosphere by natural geologic processes like volcanic eruptions, but also by a range of industrial activities including coal burning, mining, and the production of cement and chlorine.[4] More than 1000 tons of Hg were emitted to the atmosphere from anthropogenic sources in 2005 alone.[4] Considering this estimate, it isn't surprising that the mercury cycle has been substantially altered by anthropogenic activities. In fact, anthropogenic Hg now accounts for an estimated 64% of Hg in the atmosphere (Fig. 1-1). Measurements from lake sediment cores provide further evidence of anthropogenic alteration, indicating that the amount of Hg in the

atmosphere is three to five times greater than pre-industrial levels, further underlining how anthropogenic Hg has altered the Hg cycle.[5, 6]

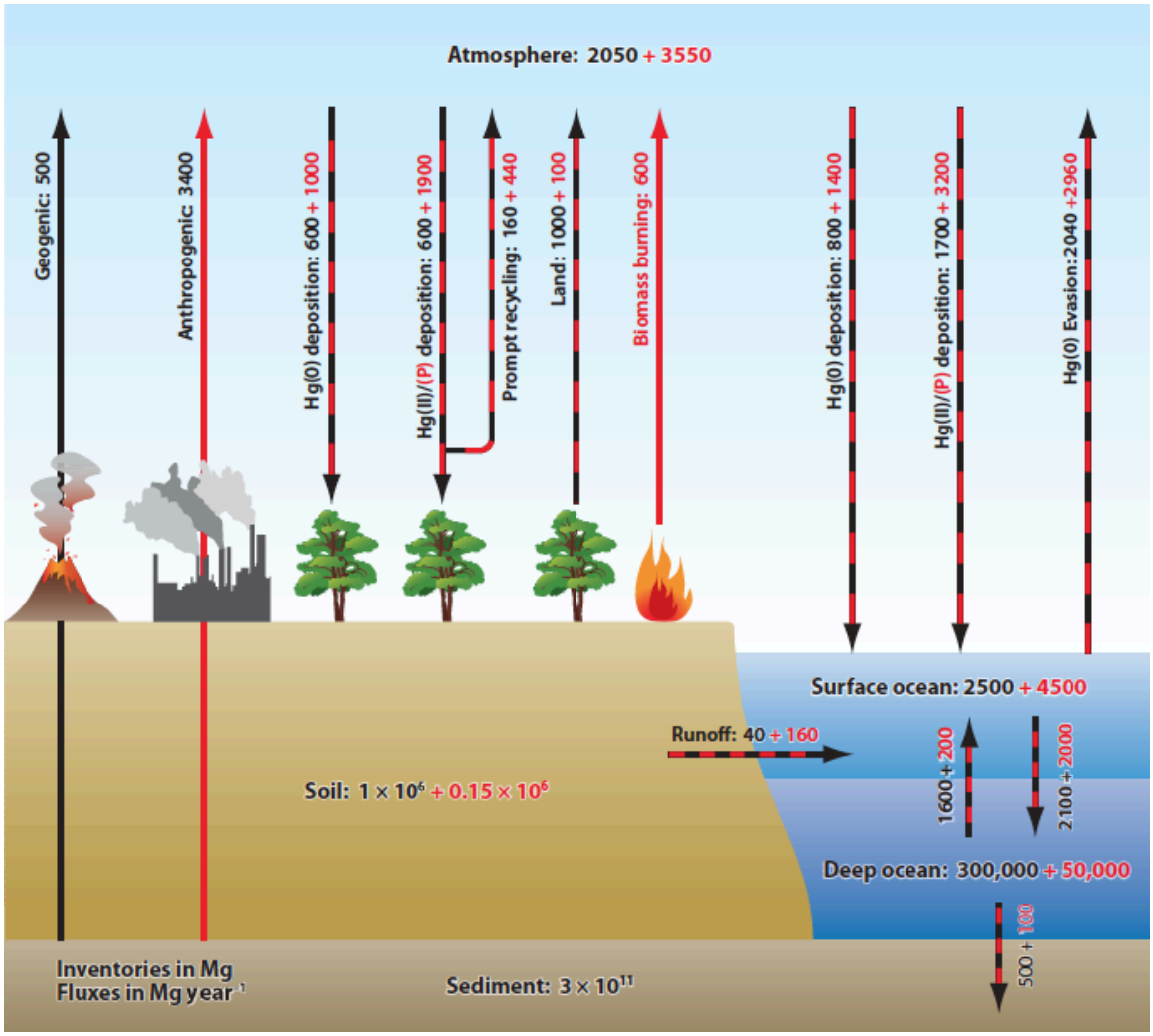

Figure 1–1. Hg fluxes to and from the atmosphere[6]

Upon entering aquatic ecosystems, atmospherically deposited, inorganic Hg is transformed into a more harmful and bioavailable form, methylmercury (MeHg), through the methylation process (Fig. 1-2).[7] The low oxygen conditions present at the soil-water interface favor anaerobic bacteria (principally sulfur reducing bacteria), which mediate this transformation.[8] Methylation is a complex process that is still not well understood,

but there is strong evidence that environmental factors such as temperature, pH, organic matter, sulfate, and redox conditions can influence the methylation process.[7]

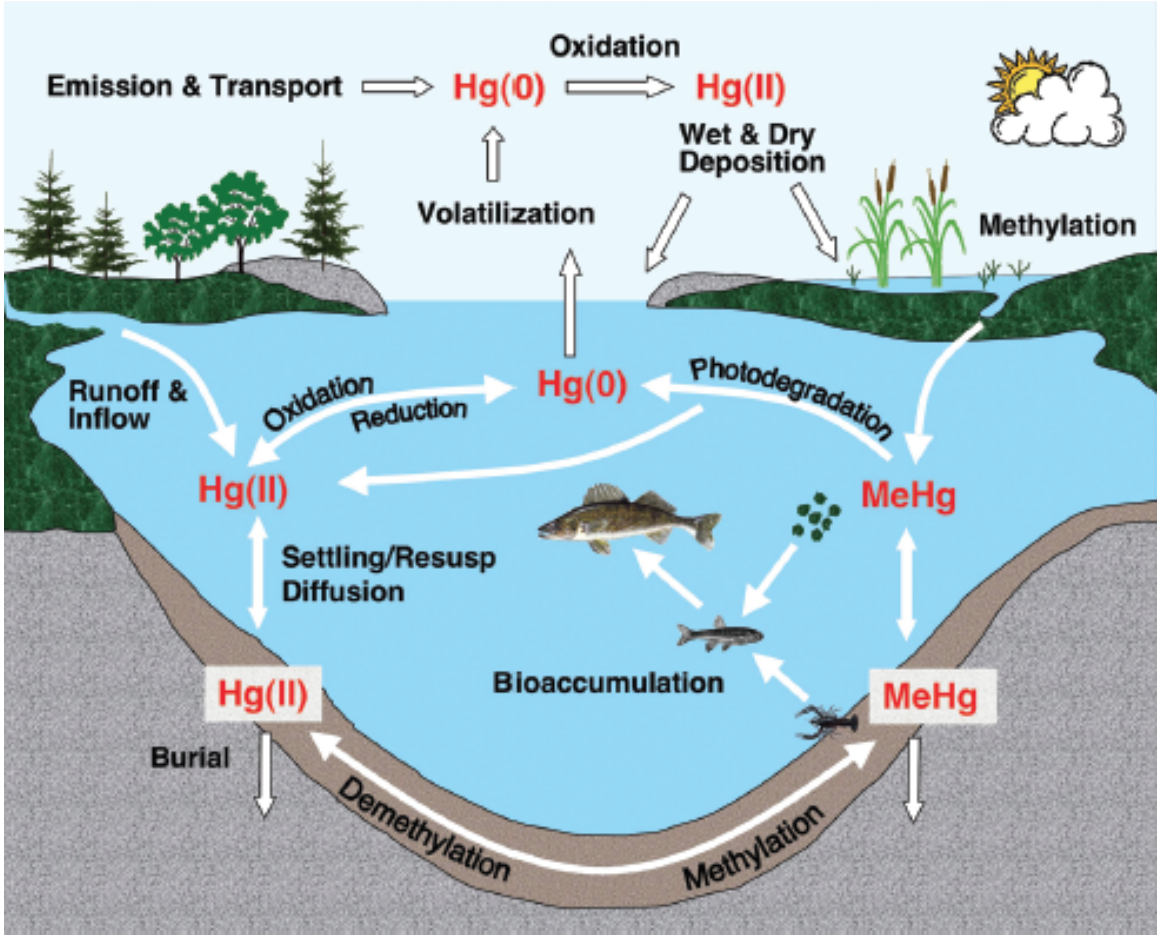

Figure 1–2. The aquatic Hg cycle[9]

Methylmercury first drew public attention in the 1950s after a mass poisoning event occurred in Minamata, Japan. Originally dubbed "Minamata Disease", symptoms of MeHg poisoning were marked by neurological effects including auditory and visual problems and difficulty walking.[10] In utero effects were even more severe and included mental retardation.[11]

Since MeHg garnered scientific attention, the effects of MeHg on wildlife have been extensively studied and have been found to affect performance, maintenance, and reproductive capabilities. This body of research has included species that consume amphibians. For instance, Bouton et al. fed Great Egret (*Ardea albus*) chicks a diet containing high amounts of MeHg and observed that the chicks displayed decreased appetite and strength and a reduced motivation to hunt.[12] A study of the Common Loon (*Gavia immer*) showed that elevated concentrations of MeHg in tissue was linked to reduced reproductive success.[13] Similarly, a study of American Black Ducks (*Anas rubripes*) showed impaired reproductive success through decreased egg production, hatching success, and embryo and duckling survival.[14]

While the negative effects of Hg contamination have been well documented in birds and mammals, effects on amphibians have received less attention even with reports of global population declines. Additionally, amphibians are more susceptible to environmental contaminants given their permeable skin and life histories, which include diverse diets, an aquatic larval stage, and use of a wide range of habitats.[15, 16] The body of literature documenting mercury contamination in amphibians is growing.[16-18] Recent work includes a laboratory study documenting the detrimental effects of MeHg accumulation on amphibians by comparing the speed, responsiveness, and prey capture ability of mercury contaminated salamanders to a reference group. This study ultimately determined that sublethal MeHg contamination negatively affects amphibians by reducing their ability to compete and survive.[19]

Methylmercury is an environmental contaminant of particular concern, not only due to its serious neurological effects, but also because of its persistence in biota, its tendency to bioaccumulate in organisms, and its tendency to biomagnify up trophic levels. When organisms ingest MeHg, it accumulates in tissues and organs and is stored rather than excreted. Higher trophic level organisms that feed on contaminated prey can therefore accumulate significantly higher concentrations of MeHg relative to concentrations present in the environment. For instance, Engstrom found concentrations of MeHg in predatory fish to be six orders of magnitude higher than concentrations of MeHg in the water.[9]

Studies have also shown that in geographic areas with similar Hg loading, MeHg concentrations in species at the same trophic level in different ecosystems can vary by an order of magnitude, indicating that the characteristics of individual ecosystems can influence bioaccumulation.[20, 21] In a study of lakes in the northeastern United States, it was determined that bioaccumulation was significantly affected by pH, acid neutralizing capacity, sulfate concentrations, land use, and zooplankton density.[22]

VERNAL POOL ECOLOGY

Vernal pools are distinctive aquatic ecosystems that play critical ecological roles in forested ecosystems. These ephemeral wetland systems and their common structural attributes create a unique habitat for biota. Vernal pools are commonly associated with forested ecosystems and this woodland context provides a stable input of organic matter into the pool's food web, helps to regulate temperature, and affects

hydrology by limiting evaporation and increasing transpiration.[23] These systems generally occur in depressions and lack continuous connections to surface water, so direct and indirect inputs from precipitation are an important water source, which often results in highly variable water levels.[24] In general, vernal pools are small and shallow resulting in water temperatures that increase rapidly in the spring, stimulating growth. Most importantly, the regular drying that is central to vernal pool hydrology creates a niche for biological communities specially adapted to the drying.[23]

Vernal pools have been described as "keystone ecosystems" for their important ecological role, which is larger than would be expected from their size.[23] They support a robust detritus-based food web that leads to high secondary production of biomass. These unique habitats are home to large numbers of obligate species specifically adapted to vernal pool conditions. Furthermore, the species composition and invertebrate community structure varies widely depending on the hydroperiod of each pool which results in high levels of diversity between sites, or beta diversity, and also contributes to greater overall regional diversity.[24, 25] The regular drying of these systems also excludes species (like fish) that would normally feed on vernal pool biota. This reduced predation pressure creates optimal breeding habitat for amphibian species, including frogs and ambystomid salamanders.

AMPHIBIANS

Amphibians play an integral role in forested ecosystems as critical vectors for energy and nutrient cycling. They function both as an important trophic base and as a

link between aquatic and terrestrial habitats. The biomass of salamanders in eastern forests has been estimated to be roughly twice that of birds during peak breeding season.[26] Amphibians represent a source of high quality energy for potential predators because of the efficiency in which they turn consumed energy into biomass.[27] Raccoons, snakes, owls, birds, and turtles are known to eat amphibians of various life stages.[28]

Declines of amphibian populations have been widely reported since the 1970s even in areas considered to be pristine.[29, 30] This trend is so distinct that herpetologists recognize the phenomena as 'global amphibian decline'.[31, 32] Globally, there are a plethora of stressors negatively affecting amphibian populations including climate change, UV radiation, acid rain, pesticide use, and disease.[15, 33] Presently, the International Union for Conservation of Nature estimates that one in three amphibian species worldwide is in danger of extirpation.[34]

In the northeastern United States, the primary agents of amphibian decline are believed to be the cascading effects stemming from habitat loss and degradation.[35] Given the importance of precipitation to vernal pool hydrology, these systems may be more heavily affected by the atmospheric deposition of contaminants than other aquatic ecosystems. Semlitsch and Bridges note that chemical contaminants directly entering aquatic habitats act as stressors on critical regulatory processes of amphibians and can reduce the probability of a population's persistence by impeding metamorphosis and reducing juvenile recruitment.[36] In particular, methylmercury contamination and its adverse effects on reproductive success have been shown to affect population

dynamics.[37] Hg contamination, especially combined with other anthropogenic stressors, may be a significant factor driving local amphibian decline.[38]

Pond breeding amphibians in particular have accounted for a large portion of the amphibian declines in North America. They rely heavily on recolonizing sites through dispersal, which is a strategy that is impeded by habitat loss and fragmentation.[38, 39] Vernal pool breeders may also be more prone to extirpation because historically these habitats have not been protected from development. Vernal pools are also sensitive to changes in hydrology and this is often impacted by development.[23] Several of the pools included in this study are used by Jefferson Salamanders (*Ambystoma jeffersonianum*). Jefferson Salamanders have been designated a species of greatest conservation need in Vermont because a high proportion of the global population occurs in the Northeast and species that have small or restricted ranges may be particularly vulnerable to environmental stressors.[36, 40]

METHYLATION AND VERNAL POOLS

The inherent characteristics of vernal pool ecosystems may lend themselves to efficient methylation of Hg. As in other aquatic ecosystems, the anoxic conditions present at the soil-water interface when inundated favor anaerobic organisms, like sulfur-reducing bacteria. Additionally, vernal pools are characterized by low pH conditions and high concentrations of dissolved organic carbon (DOC). Both parameters have been positively correlated with increased methylation in aquatic ecosystems.[7, 17] The unique hydrology of vernal pools may also increase the efficiency

of these systems to methylate Hg, as the drying and rewetting that result from water level changes may shift the redox cycle in a manner that favors sulfate-reducing bacteria.[41] As a result of these chemical and hydrologic factors, vernal pool ecosystems have the potential to be particularly efficient methylators of Hg.

Krabbenhoft found that ecosystems with methylation efficiencies exceeding 10% commonly contained biota with elevated levels of MeHg in their tissue.[42] As such, determination of Hg and MeHg concentrations in the water column of vernal pools will not only provide data about the exposure of amphibians to MeHg, it will also allow for inferences to be drawn about the potential for MeHg contamination in vernal pool biota. Vernal pool ecosystems export a significant amount of biomass, as much as 140 $kg/ha^{-1}yr^{-1}$, to surrounding upland ecosystems.[43] Given their importance as a quality food source, bioaccumulation of MeHg in amphibian species could be an important vector of Hg export.

While vernal pools are small ecosystems by area, they are extremely prevalent in the landscape of the glaciated Northeast. An inventory of the State of New Jersey using remote sensing techniques mapped 13,000 potential vernal pools in a total area of 19,500 km^2.[44] However, contemporary remote assessment techniques may grossly underestimate the number of vernal pools on the landscape, particularly in forested areas.[45] Given the large number of vernal pools on the landscape, efficient methylation and subsequent bioaccumulation could result in significant amounts of MeHg becoming bioavailable and mobile across the landscape.

JUSTIFICATION

At present, there are no published data reporting concentrations of MeHg in the water column of vernal pools, so the exposure of amphibians to MeHg in their breeding habitats has not been quantified. This is a significant knowledge gap, especially considering global amphibian decline. While there is limited data reporting MeHg concentrations in the tissue of amphibians, the methylation process is not well understood. This step between deposition and subsequent bioaccumulation represents a critical process that needs to be better understood, and the physical environment of these systems may provide important insights into this process.

Previous hydrologic studies have made important contributions to the existing knowledge base of vernal pool hydrology, including the effects of variable weather conditions on vernal pool hydrology, the effect of hydroperiod on macroinvertebrate composition, and the relationship between vernal pool size and area on hydroperiod.[25, 46-48] However, these studies have primarily employed periodic site visits, often at one-week intervals. Our research utilized continuous recording data loggers that allowed for finer scale hydrologic monitoring and a more precise determination of hydroperiod and characterization of the hydrologic regime. Hydrology influences many aspects of wetland ecology including biogeochemical cycling by shifting the metabolic processes that are favored by bacteria.[49] Precisely quantifying aspects of vernal pool hydrology may provide added insight into the biogeochemical processes of vernal pools.

The purpose of this study was to quantify the efficiency of Hg methylation in vernal pools and to explore selected factors that likely influence this process. The

determination of methylation efficiency included directly quantifying MeHg concentrations, which provided much needed data about exposure of vernal pool biota to MeHg. This study also characterized the chemical and hydrological environment of selected vernal pools to better understand how these factors influence methylation.

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1.2. SEASONAL CHANGES IN MERCURY STOCKS AND METHYLATION RATIOS IN VERNAL POOLS IN THE NORTHEASTERN UNITED STATES

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ABSTRACT

Vernal pools play critical ecological roles by supporting diverse invertebrate communities and by providing breeding habitat for globally pressured amphibian species. However, these systems may also be effective in transforming mercury (Hg) into a more toxic and bioavailable form, methylmercury (MeHg). The purpose of this study was to quantify the concentrations of Hg and MeHg that biota are exposed to in vernal pools and to determine the methylation efficiency (water MeHg to total Hg ratio) of these habitats. We utilized eight sites in two national parks for this study. Water samples were collected and analyzed for parameters known to affect methylation, including temperature, pH, conductivity, dissolved oxygen, dissolved organic carbon (DOC), sulfate, as well as Hg and MeHg concentrations. Pressure transducers that continuously recorded water level were deployed at each site to precisely quantify the hydrologic regime. Mean total mercury concentrations ranged from 0.53 ng/L to 8.27 ng/L, while mean methylmercury concentrations ranged from 0.24 ng/L to 4.52 ng/L. Mean methylation efficiency averaged 43% and peaked at 58%. Methylation efficiencies exceeding 10% have been linked to elevated levels of MeHg in biota and each of our study sites regularly exceeded this threshold. Vernal pools play an integral role in energy cycling in forested ecosystems, exporting as much as 140 kg of biomass per hectare annually. Thus contamination of vernal pool biota not only has implications for these organisms, but also represents a vector for MeHg to move out of vernal pools and into other trophic webs. This potential contamination could negatively affect population dynamics for a wide range of species.

Key Words

Vernal pools, methylmercury, mercury, hydrology, amphibians

INTRODUCTION

Mercury (Hg) contamination is a pressing ecological concern because of its widespread geographical distribution, toxic effects, and tendency to bioaccumulate in organisms and subsequently biomagnify at higher trophic levels. Mercury enters the atmosphere through natural geologic processes and a range of industrial activities including coal burning, mining, and through the production of cement and chlorine.[1] Anthropogenic emissions have significantly altered the amount of Hg in the atmosphere. Lake sediment cores indicate that the amount of Hg in the atmosphere is three to five times greater than pre-industrial levels.[2, 3] Sediment and peat cores collected over a broad geographical range provide strong geochemical evidence that the long-range atmospheric transport and deposition of anthropogenic Hg is an important source of Hg to the environment.[4] The presence of significant amounts of Hg in pristine natural areas, far away from potential sources, also implicates long-range atmospheric deposition as an important dispersal mechanism.[5, 6]

Upon entering aquatic ecosystems, atmospherically deposited, inorganic mercury can be transformed into more harmful methylmercury (MeHg) through a complex biogeochemical process.[7] Methylation is primarily mediated by sulfur reducing bacteria that are favored when anoxic conditions persist, often at the soil-water interface of aquatic ecosystems.[8] The neurological effects of MeHg have been well studied in wildlife and include adverse effects on performance, maintenance, and reproduction[9-12].

Methylmercury is also of particular concern because of its persistence in food webs. Methylmercury is bioavailable, so it is stored by organisms in fatty tissue and organs rather than excreted. This bioaccumulation in prey organisms leads to biomagnification at each higher trophic level as higher level consumers ingest organisms with higher amounts of stored MeHg.[13]

Vernal pools are unique aquatic systems and they play critical ecological roles in forested ecosystems. These ephemeral wetlands are fed either directly or indirectly by precipitation, leading to highly variable water levels that peak in the spring.[14] Given this dynamic, vernal pools are characterized by a regular regime of inundation and drying which makes them home to a specialized group of biota that are adapted to the regular drying cycle.[15] They support diverse communities of macroinvertebrates and because community structure is dependent on hydroperiod, pools that are close geographically can be home to widely varying communities of invertebrates.[16] This dynamic leads to high beta and gamma diversity.[14] Vernal pools are typically found in forested surroundings and this setting provides steady inputs of inorganic matter through litterfall. These inputs support detritus-based food webs that lead to high secondary production. Much of this biomass integrates into the surrounding forested ecosystem through secondary production, potentially as much as 140 kilograms per hectare per year.[15, 17]

The drying regime of vernal pools also provides optimal breeding habitat for amphibian species. These species, like frogs and ambystomid salamanders, play an integral role in energy and nutrient cycling in forested ecosystems. Amphibians represent a source of high quality energy for potential predators because of the efficiency in which

they turn consumed energy into biomass. Their 60% growth efficiency rate allows salamander populations to add more new tissue each year than birds or mammals and this new tissue is high in protein.[18] They also comprise a significant energy base at a low trophic level, with their biomass estimated to be roughly twice that of birds during the breeding season.[19] A wide range of animals, including raccoons, snakes, owls, birds and turtles are known to eat various life stages of amphibians. Given that amphibians provide linkages between trophic levels and terrestrial systems, it is possible that contamination of amphibians could represent an important vector of MeHg moving out of vernal pool systems.

Amphibian populations have been declining since the 1970's and the phenomena has become known as "global amphibian decline".[20, 21] Worldwide, it is estimated that one in three amphibian species are in danger of extirpation.[22] In North America, pool breeding amphibians have accounted for large portions of the decline.[23] This is in part due to fragmentation and habitat loss, but Hg contamination could also be an important factor driving local decline. The permeable skin of amphibians makes them particularly susceptible to soluble contaminants like MeHg, which has been shown to negatively affect amphibian population dynamics by impeding metamorphosis and reducing juvenile recruitment.[23, 24]

Vernal pools may be more acutely affected by atmospherically deposited contaminants like Hg given the important role precipitation plays in their hydrology.[23] They may also be particularly efficient methylators of Hg because vernal pools have high levels of dissolved organic carbon (DOC) and sulfate and low levels of pH and oxygen,

all factors that are known to enhance methylation efficiency, which is expressed by the ratio of water MeHg concentration to total Hg concentration.[7] In addition, the highly variable hydrology of vernal pools causes fluctuations in redox conditions that may enhance methylation efficiency.[25]

The specific objectives of this research were to 1) quantify the concentrations of Hg and MeHg to which vernal pool biota are exposed, 2) determine the methylation efficiency of these systems, and 3) investigate how the physical environment influences methylation dynamics.

METHODS

Study Area

Vernal pools in two national parks in the Northeastern United States were utilized for this study, Marsh-Billings-Rockefeller National Historical Park in Woodstock, Vermont and Saratoga National Historical Park in Stillwater, New York (Fig. 2-1). Marsh-Billings-Rockefeller is a 550-hectare park in Southern Vermont that encompasses a diverse landscape, ranging from 245 meters to 370 meters in elevation. It is dominated by Northern Hardwood forest but also includes non-native plantations and hay fields. The park is underlain by calcareous bedrock resulting in alkaline waters.[26] Saratoga National Historical Park is a 560-hectare park in northeastern New York. The park is approximately 150 meters in elevation and is a mixture of mixed and deciduous forest along with open fields. The area is underlain with unconsolidated glacial till on the surface and thick bedrock formations below.[27]

Sampling Design

This study utilized a tiered sampling design, with the base tier including five samples from each site. Base sampling included one sample in the late fall of 2010 before the pools froze, two samples during spring 2011, with one sample collected at the onset of spring fill and another collected before the pool dried in early summer, and two additional samples in fall 2011, with one collected at the onset of fall inundation and the final sample collected later in the season before the pools froze. The base sampling tier was designed to draw inferences about mercury concentrations and efficiencies between seasons and between the beginning and end of the season. Half of the study sites, two at each park, were sampled two additional times throughout the spring season to draw finer scale temporal inferences about methylation dynamics during the period in which the systems are being utilized by amphibians and macroinvertebrates.

Sample collection

Hg samples were collected in new, certified trace-clean polyethylene terephthalate copolyester, glycol-modified (PETG) bottles. Samples were double bagged in the field to prevent contamination and transported inside a cooler to prevent photo-degradation of Hg. Hg samples were collected primarily by grab sample, in which a new, unsealed bottle was uncapped and recapped below the water surface. Where hydrologic conditions would not allow for a grab sample to be collected because of shallow water levels, samples were collected by suspending Teflon tubing connected to a peristaltic pump into the pool to

avoid disturbing sediment. Teflon tubing was cleaned in a trace clean room according to the USGS protocol for low-level Hg sampling.[28] Hg Samples were preserved within 24 hours of collection with 5 ml ultra-trace pure 6 N hydrochloric acid per 250 ml of sample.[29] DOC samples were collected in acid washed 120 ml amber glass bottles by the same method utilized to collect the Hg sample. Samples were preserved within 48 hours by the lab performing the analysis. Sulfate and nitrate samples were collected in 60 ml plastic bottles consistent with the method used to collect the Hg sample and were subsequently frozen until analysis. Electrical conductivity, pH, temperature and dissolved oxygen were determined on site using a Yellow Springs Instruments 600 XL water quality sonde.

Lab analysis

Hg speciation analysis, which yields both inorganic and methylmercury concentrations, was performed at the Trace Element Analysis Laboratory at Dartmouth College according to an adaptation of the Environmental Protection Agency's 1630 method for methylmercury in water. The method was adapted to reduce analysis time and improve on the minimum detection limits (MDL) enabling ultra trace Hg speciation of small volumes of natural waters.[30] DOC analysis was performed by the Sawyer Environmental Chemistry Laboratory at the University of Maine-Orono. An OI analytical model 1010 wet oxidation carbon analyzer was used for analysis. Sulfate and nitrate analysis was performed by the Agricultural and Environmental Testing Laboratory at the

University of Vermont. Samples were analyzed with a Dionex 600DX Ion Chromatograph.

Hydrologic Monitoring Design

Each study site was instrumented with a HOBO pressure transducer (Onset Corporation) that continuously recorded water level and temperature at fifteen minute intervals. Before inundation in fall 2010 a PVC tube was driven a foot below the surface at the deepest point of each pool. The PVC tubes were capped at the bottom and allowed for water exchange by combining thin slits for the portion of the well extending below ground and larger drill holes for the portion above ground. The pressure transducers were suspended from the cap, so that they were returned to the same vertical position after each site visit. An additional pressure transducer was installed at an elevated position in each park to collect data for correcting the water level loggers for atmospheric pressure. Stage was measured manually at each site visit to ensure the loggers were accurately recording water level. The manual stage measurements were also used to correct recorded water level for their position below the soil surface to accurately reflect pool water level.

Statistical Analyses

The hydrologic data were exported from the proprietary data management software and concatenated in Microsoft Excel. Basic characterization of these data were also performed in Excel to extract variables describing the hydrologic regime of the study sites. For each point in time in which a methylmercury sample was collected, three

variables were derived from the hydrologic data: relative water level change, water level coefficient of variation, and length of inundation. Relative water level change was calculated by subtracting present water level from the mean value for the preceding 7 days to describe whether the pool was expanding or contracting at the time of sample. Water level coefficient of variation was calculated based on the fifteen minute water level data for the preceding 7 days to describe the variability of pool water level. Length of inundation was calculated by a count of days since water level was greater than zero to describe the length of time in which the system was inundated.

The correlation of the predictor variables characterizing the chemical environment, hydrologic environment, and Hg loading were analyzed in SPSS by calculating Spearman's Rank-Order Correlation (IBM Corporation). For the purpose of this study, tests will be deemed statistically significant at $p<0.10$. Further exploratory analysis of the predictor variables utilized principal component analysis and was performed in JMP Pro 10 (SAS Institute).

RESULTS

Water chemistry and hydrology

Mean specific conductance was generally higher at the Vermont sites, ranging from 92.8 µS/cm to 232.6 µS/cm compared to a range of 42.3 µS/cm to 69.6 µS/cm at the New York sites (Table 1). Mean pH values were also generally higher at the Vermont sites ranging from 6.64 to 7.17 compared to a range of 5.23 to 6.18 at the New York sites. Conversely, mean DOC values were generally higher at the New York sites ranging from

14.17 mg/L to 35.54 mg/L compared to a range of 1.55 mg/L to 5.52 mg/L at the Vermont sites.

The hydrographs indicate hydrologic differences exist between study pools (Figure 2-2), mostly along their morphological classification in Table 1. Pools classified as confined because of their defined bowl shape generally had higher maximum waters and more variable water levels. Pools classified as expansive due to their more gradual slope generally had lower maximum water levels and less variable water levels.

Total mercury concentrations, methylmercury concentrations, and methylation efficiency

The mean of total Hg concentrations and the mean of MeHg concentrations were higher at the New York sites (Table 2). The mean total Hg concentrations of the New York sites ranged from 4.86 ng/L to 8.19 ng/L while mean total Hg concentrations at the Vermont sites ranged from 0.53 ng/L to 2.98 ng/L. Similarly, mean MeHg values of the New York sites ranged from 1.50 ng/L to 4.52 ng/L while mean MeHg concentrations at the Vermont sites ranged from 0.24 ng/L to 1.41 ng/L. Conversely, methylation efficiencies were generally higher at the Vermont sites. Mean efficiencies ranged from 43% to 58% in Vermont while the range from the New York sites was 21% to 58% (Figure 2-3).

Methylation dynamics through spring

Over the course of the spring season, total Hg concentrations, MeHg concentrations, as well as methylation efficiency increased across all sites sampled

(Figure 2-4). On average across all sites, total Hg concentrations increased by 70%, while MeHg concentrations increased by 200%. The relatively larger increases in MeHg concentrations resulted in the methylation efficiencies of the systems increasing on average by 76%. Our tiered sampling design provided additional data points regarding temporal methylation dynamics throughout the course of spring (Figure 2-5). While the trend of increasing concentrations of total Hg, MeHg, and methylation efficiency continued, in 3 of the 4 more intensively sampled sites MeHg concentrations and methylation efficiency peaked before the last sampling date.

Chemical and hydrologic characteristics

The Spearman's Rank-Order Correlation matrix yielded twenty-six significant relationships among our eleven predictor variables (Table 3). Some relationships were expected because of the physical processes involved, like the negative correlation between temperature and percent dissolved oxygen and between temperature and relative water level change. Relationships detected in our analysis also support those reported in other Hg studies, specifically the negative correlation between pH and DOC concentrations and between pH and total Hg concentrations, as well as the positive correlation between Total Hg and DOC. We also detected a negative correlation between period inundated and sulfate concentrations.

Principal component analysis yielded four principal components with eigenvalues greater than one and together they accounted for 77% of the variation amongst the variables (Table 4). The first principal component accounted for 33% of the variability.

DOC and Total Hg loaded heavily negative on this component with pH and specific conductivity loading heavily positive. The second principal component accounted for 19% of the variability. Sulfate concentrations loaded heavily positive on this component while period inundated loaded heavily negative. The third principal component accounted for an additional 15% of the variation. Temperature loaded heavily positive on this component while relative water level change loaded heavily negative. The final principal component accounted for an additional 9.5% of the variability. Dissolved oxygen and water level coefficient of variation loaded heavily positive on this component.

DISCUSSION

Methylmercury through spring

From the beginning of the spring to the end of the spring, the time period in which biological activity peaks in vernal pool systems, Hg concentrations, MeHg concentrations, and methylation efficiency increased substantially in the eight study pools (Figure 2-4). While increasing mercury and methylmercury concentrations are surely linked to decreasing pool volume, the ratio of MeHg to total Hg as represented by methylation efficiency is not dependent on pool volume. The increasing methylation efficiencies indicate that the spring season is marked by increases in the portion of Hg that is present in a methylated form. Given that this time coincides with the period in which amphibians are going through metamorphosis, increasing methylation efficiencies in late spring likely represent an increased risk for bioaccumulation in biota.

Another interesting trend that was apparent from our more intensive sampling regime was that in 3 of the 4 sites, MeHg concentrations and methylation efficiencies peaked before the last sampling date (Figure 5). If increasing MeHg concentrations were driven primarily by shrinking pool volume, we would have expected this trend to continue. That the trend does not continue may indicate that another factor important to the methylation process shifts over that time period. Given that we did document decreasing sulfate concentrations as length of inundation increased, this could indicate a shifting redox cycle that no longer favored sulfur-reducing bacteria. Volatilization of MeHg may also increase with decreasing water levels as the physical barrier between the soil water-interface and the atmosphere is reduced. Pool drawdown likely changes the ratio of the soil-water interface relative to pool volume as well, limiting the area where methylmercury production can take place. While the driver of this trend was unclear, it's possible that this effect was not captured at the fourth site due to measurement error in light of the extremely low Hg and MeHg concentrations.

Methylation Efficiencies

While our study did not directly sample bioaccumulation in biota, it is possible to draw some inferences in regards to potential contamination. Previous research in aquatic systems has found that when methylation efficiencies exceed 10%, biota are likely to have elevated concentrations of MeHg in their tissue.[31] This threshold was routinely exceeded at our study sites, often substantially. Mean methylation efficiencies across all sites averaged 43% and the maximum measured methylation efficiency reached 89%.

Methylation Dynamics

Due the highly correlated nature of our predictor variables, we used principal component analysis as an exploratory tool to investigate potential relationships among them.[32] Total Hg, DOC, pH, and specific conductivity, all chemical parameters, loaded heavily on principal component one. Specific conductivity and pH both loaded heavily positive indicating that they are positively correlated with each other but negatively correlated with the other two variables (DOC and Total Hg), which loaded heavily negative. This component also indicates DOC and Total Hg are positively correlated with each other. While this component largely breaks down according to the differences between water chemistry and Hg loading between parks (Figure 2-6), it seems to describe the methylation potential of a given pool. Additionally, the positive correlation between DOC and total Hg has been substantiated in other Hg studies.[33] Sulfate concentrations and period inundated were negatively correlated in principal component two. This component seems to be an indicator of the intensity of inundation; as anoxic conditions persist, sulfate concentrations decrease as anaerobic bacteria utilize it for metabolic processes. Temperature and relative water level change were negatively correlated in principal component three. This component describes the physical and hydrologic changes driven by the season. Specifically, as evapotranspiration increases, pool temperature increases and water level decreases. The fourth component consisted of a positive correlation between the water level coefficient of variation and percent dissolved oxygen. This component seems to describe the hydrologic variability. The more

frequently water levels change, the more oxygenated the water is. The exploratory analysis points to four processes that the correlations among our predictor variables describe. These processes could have important effects on methylation in these systems.

It is important to note that there are several characteristics of our dataset that limit the applicability of principal component analysis. Our samples lack independence because a relatively small number of pools were sampled multiple times. The substantial differences between the two clusters of sites in both water chemistry and Hg loading are also grouped together in principal component one, which makes it difficult to separate these effects. Given these limitations, the principal component analysis should be considered a descriptive effort to explore the highly correlated nature of our predictor variables.

Considering the exploratory principal component analysis identified four potential processes among our predictor variables, we performed an exploratory correlation analysis among these four predictor components and a response variable, methylmercury concentration (Table 5). In addition to examining these relationships among our complete dataset, we also examined the relationships by park and by site to explore the effects of our nested design. A statistically significant negative correlation was detected between the value of principal component one and methylmercury concentration over the complete dataset and in the Vermont sites. And while this relationship did not hold up in New York, the relatively strong relationships across pools suggest that this component may influence methylation. Considering the loading factors of this component, low values of principal component one would occur under high Hg loading and/or high DOC

concentrations, as well as low pH conditions. Low values of principal component one being linked with increasing methylmercury concentrations is not surprising.

The correlation matrix indicated a significant positive correlation with the value of principal component two and methylmercury concentration in the overall dataset and in the New York sites. However, given its changing signs across pools, principal component two likely accounts for a substantial amount of variation, but the influence on methylation seems less clear. And while principal component four is not significant at either park or overall, the strong effects at the pool level indicate a potential influence.

Implications

The ecological importance of both vernal pool habitats as well as amphibians can't be understated. Vernal pools provide optimal breeding habitat for species that are under substantial pressure. They also support diverse invertebrate communities and contribute to landscape scale biodiversity. Vernal pool biota represent a critical trophic input into surrounding forested ecosystems. Given the low trophic status of amphibians and macroinvertebrates, and the potential for efficient methylation of atmospherically deposited Hg in vernal pools, these systems have the potential to act as an important conduit between aquatic and terrestrial ecosystems and could represent a substantial source of methylmercury into other trophic webs.

Our study provided unique data regarding the concentrations of Hg and MeHg that amphibians are exposed to in their breeding habitats. From the methylation efficiencies we encountered, the possibility for contamination in biota exists and should

be researched further. Methylation is an extremely complex process and while this research pointed to processes that may influence methylation, more work needs to be done. Future studies should aim to increase sample size, include more diversity between sites, and measure bioaccumulation directly. Future work should continue to explore how methylation is influenced by chemical parameters, as anthropogenic stressors like acid deposition have the potential to increase methylation efficiency and bioaccumulation in these habitats. It is also important that future work continue to focus on vernal pool hydrology as a changing climate could bring more precipitation and higher temperatures, both of which could increase the methylation potential of vernal pools.

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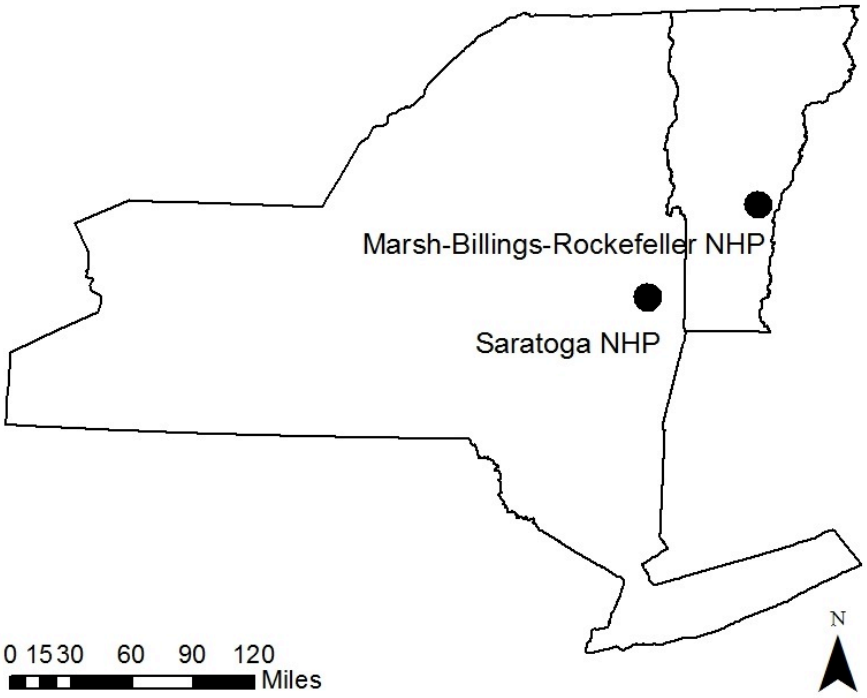

Figure 2–1. Site Map

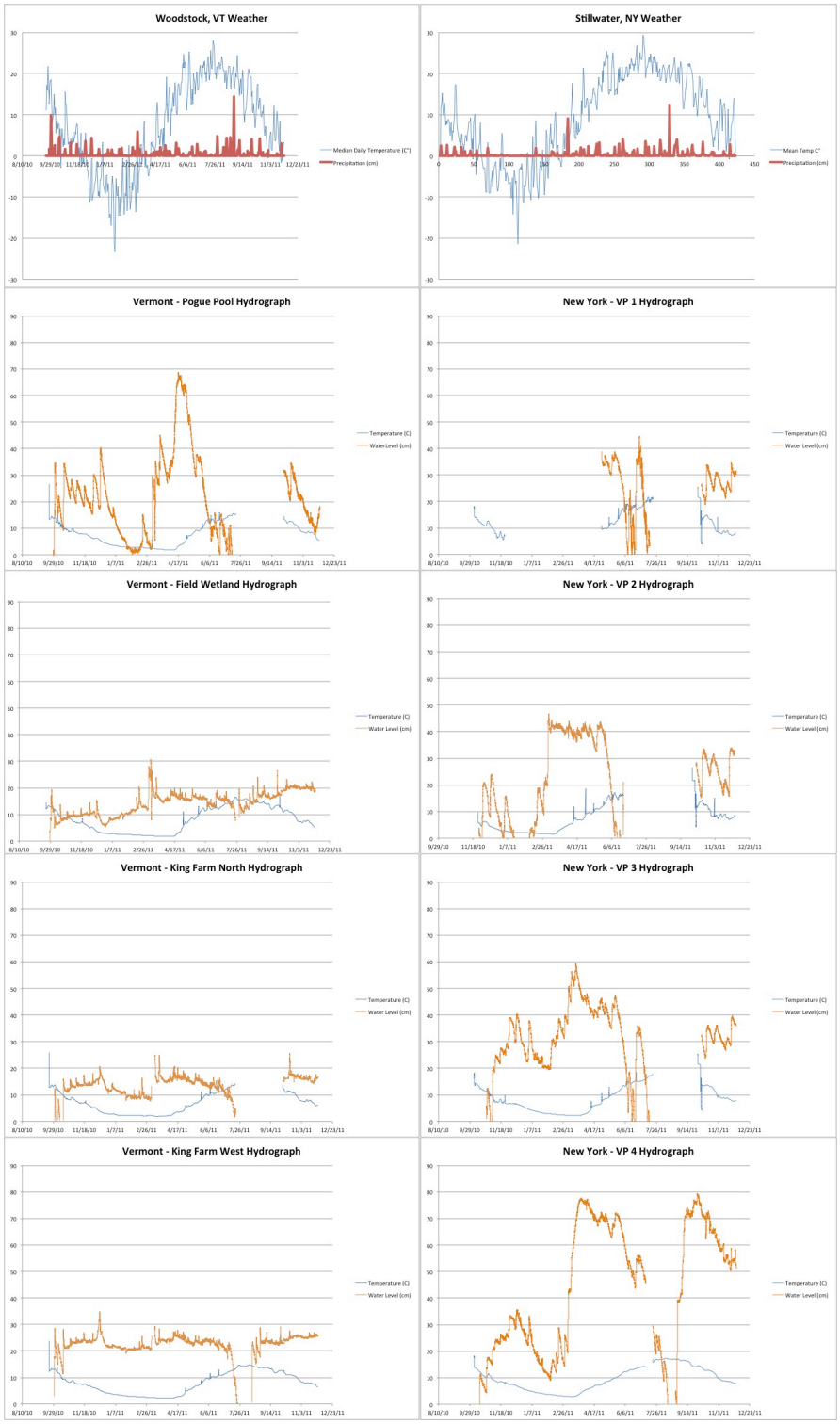

Figure 2–2. Hydrographs by Site

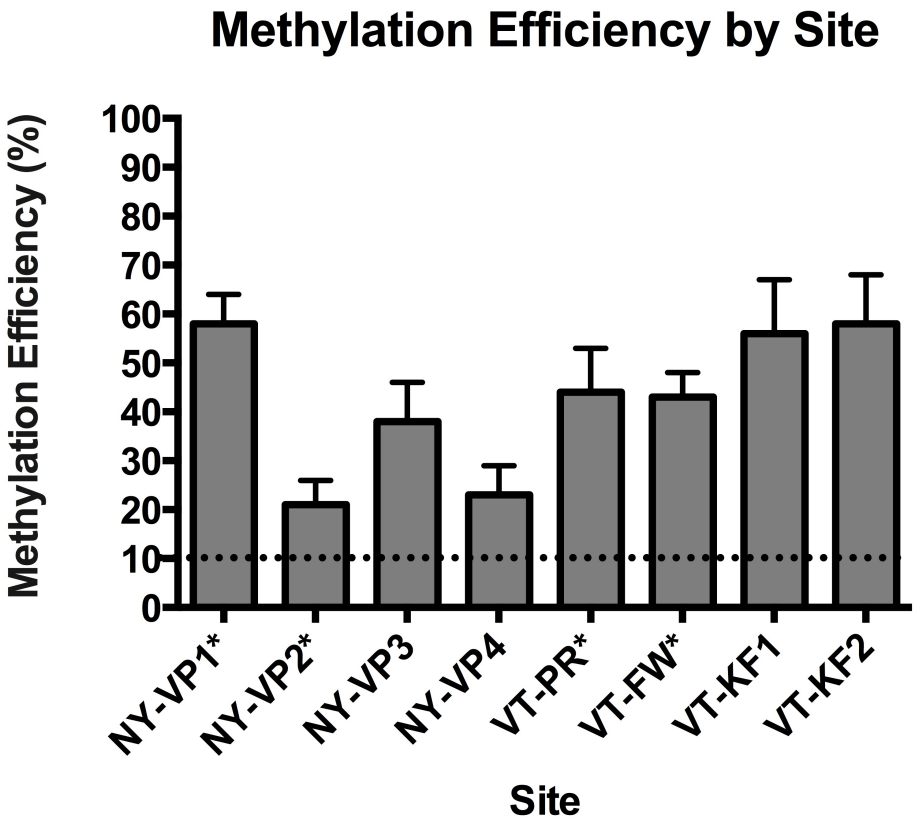

Figure 2–3. Mean methylation efficiency (± SE) across sites

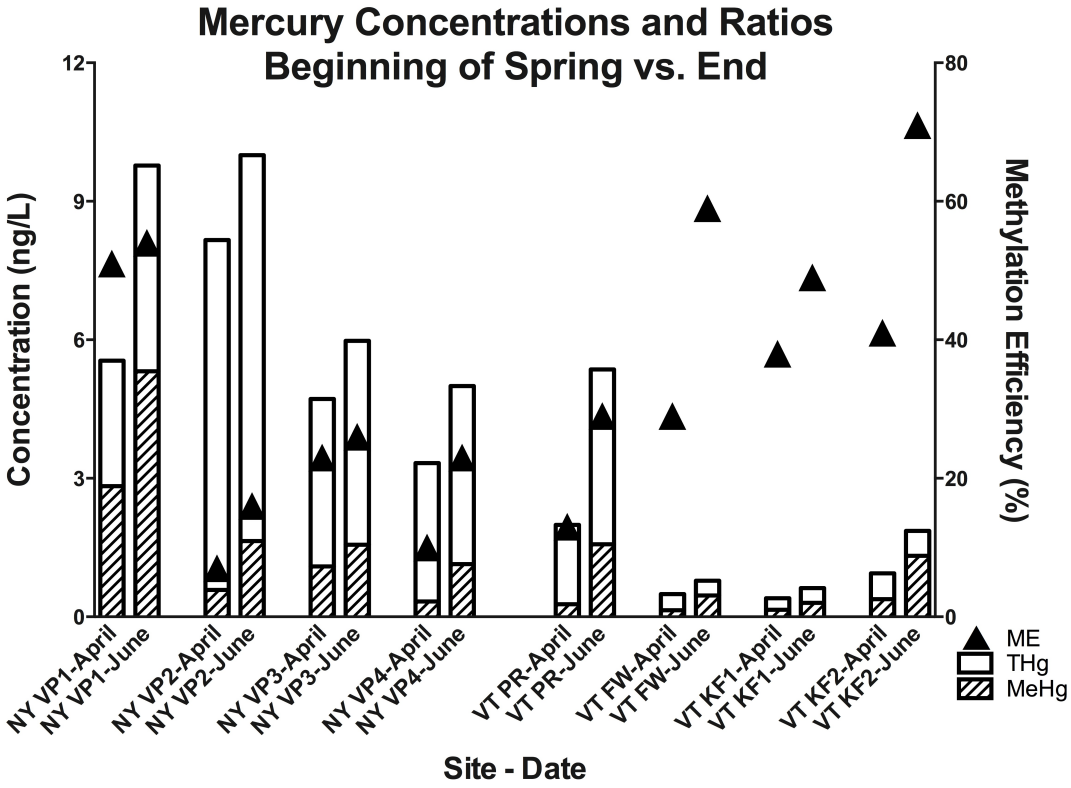

Figure 2–4. Total Hg, MeHg, and methylation efficiency; beginning and end of spring

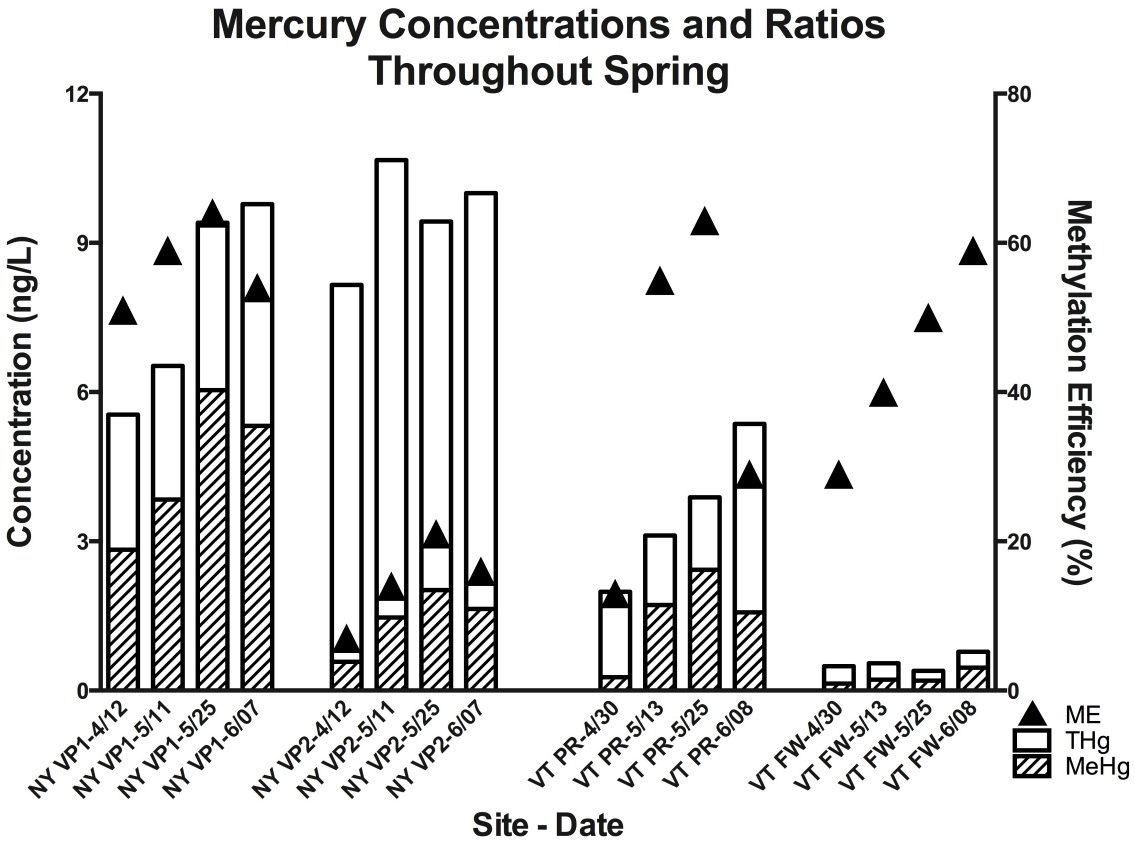

Figure 2–5. Total Hg, MeHg, and methylation efficiency throughout spring

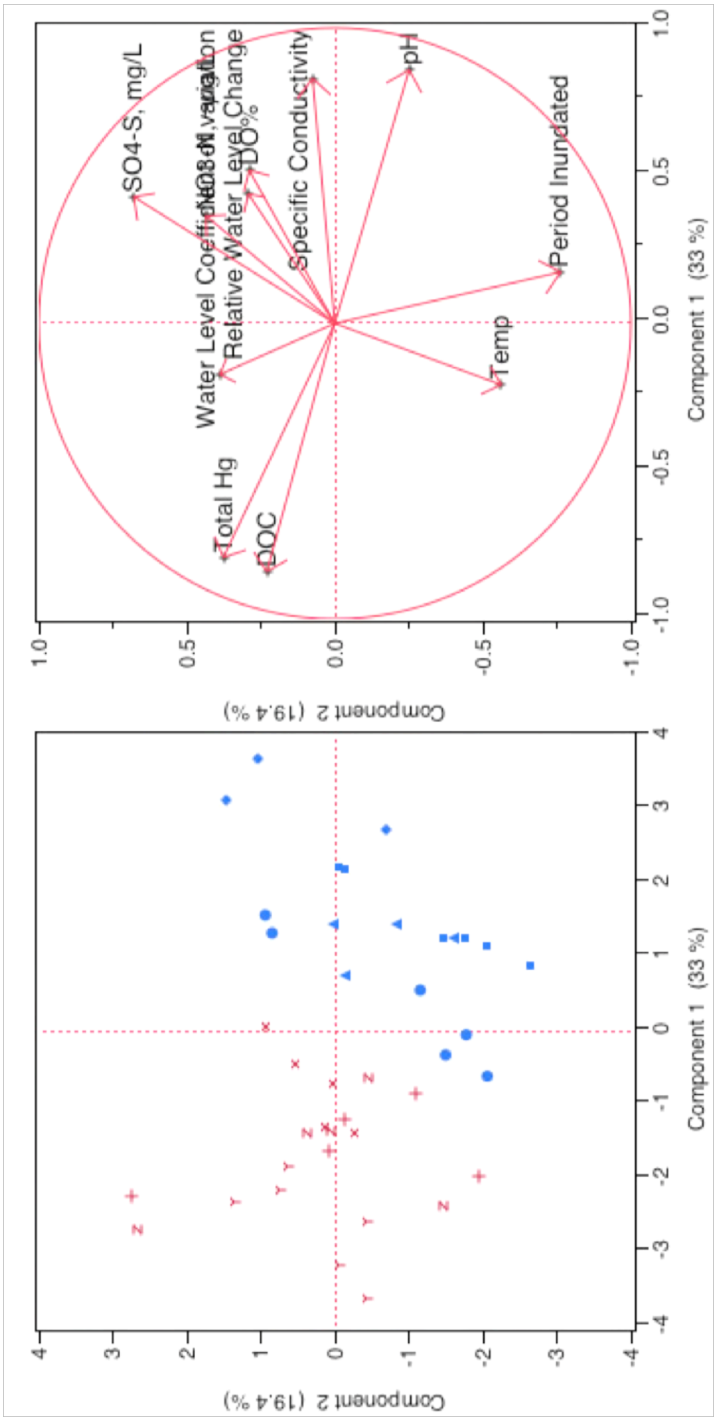

Figure 2–6. Biplot of principal component 1 vs. principal component 2

Table 1. Water chemistry data by site

Site	Pool Morphology	Specific Conductance (uS/cm)	Dissolved Oxygen (%)	pH	Dissolved Organic Carbon (mg/L)	Sulfate (mg/L)	Nitrate (ug/L)
VT -FW*	expansive	173.0 ± 10.9	31.6 ± 9.7	7.03 ± 0.06	3.81 ± 0.43	1.18 ± 0.20	3.2 ± 0.5
VT -KF1	expansive	232.6 ±15.9	43.1 ± 11.5	7.17 ± 0.05	1.55 ± 0.56	4.33 ± 0.67	73.1 ± 29.3
VT -KF2	expansive	161.1 ± 11.0	28.3 ± 5.3	6.81 ±0.05	5.31 ± 0.73	1.99 ± 0.26	3.1 ± 1.2
VT -PR*	confined	92.8 ± 13.4	28.3 ±8.5	6.64 ± 0.09	5.52 ± 1.01	1.27 ± 0.09	5.6 ± 2.8
NY-VP1*	confined	69.6 ± 6.9	43.1 ± 6.5	6.18 ± 0.09	14.17 ± 2.51	2.14 ± 0.62	13.5 ± 9.2
NY-VP2*	expansive	45.4 ± 3.8	19.2 ± 6.6	5.23 ± 0.17	35.15 ± 2.89	1.01 ± 0.20	13.5 ± 2.4
NY-VP3	confined	42.3 ± 6.4	28.7 ± 8.3	5.67 ± 0.09	34.10 ± 7.00	1.05 ± 0.60	10.0 ± 3.1
NY VP4	confined	54.6 ± 8.2	21.2 ± 4.6	5.78 ± 0.05	35.54 ± 8.11	1.01 ± 0.77	11.2 ± 2.9

values are presented as mean ± standard error

*7 samples (vs. 5)

Table 2. Hg and MeHg concentrations by site

Site	Mean Total Hg Concentration (ng/L)	Mean MeHg Concentration (ng/L)
VT -FW*	0.53 ± 0.05	0.24 ± 0.04
VT -KF1	0.88 ± 0.28	0.61 ± 0.28
VT -KF2	1.45 ± 0.35	0.98 ± 0.38
VT -PR*	2.98 ± 0.45	1.40 ± 0.32
NY -VP1*	8.19 ± 1.11	4.52 ± 0.58
NY -VP2*	8.27 ± 0.89	1.60 ± 0.25
NY -VP3	5.74 ± 1.71	1.92 ± 0.49
NY -VP4	4.86 ± 1.70	1.50 ± 0.95

values are presented as mean ± standard error

*7 samples (vs. 5)

Table 3. Spearman's Rank-Order Correlation matrix of predictor variables

	Temp	Specific Cond.	DO%	pH	DOC	SO_4	NO_3	Relative Water Level Change	Water Level Coefficient of variation	Period Inundated	THg
Temp	-										
Specific Cond.	-0.12	-									
DO%	-0.26*	0.23	-								
pH	0.11	0.83*	0.37*	-							
DOC	0.11	-0.71*	-0.48*	-0.88*	-						
SO_4	-0.20	0.53*	0.34*	0.36*	-0.48*	-					
NO_3	-0.08	0.12	-0.10	-0.16	-0.22	0.05	-				
Relative Water Level Change	-0.57*	0.44*	0.28*	0.29*	-0.44*	0.41*	0.01	-			
Water Level Coefficient of variation	0.09	-0.04	0.06	-0.02	0.01	-0.13	0.40*	-0.01	-		
Period Inundated	0.40*	0.01	-0.15	0.17	-0.13	-0.36*	-0.15	-0.37*	-0.14	-	
THg	0.18	-0.64*	-0.31*	-0.80*	0.81*	-0.15	0.15	-0.44*	0.18	-0.25*	-

*$p < 0.1$

Table 4. Principal component analysis summary

Principal Component	Eigenvalue	Variability Explained	Components (Directionality)	Process
1	3.63	33%	Specific Conductivity (+) pH (+) DOC (-) Total Hg (-)	Methylation Potential
2	2.13	19.38%	Sulfate (+) Period Inundated (-)	Intensity of Inundation
3	1.67	15.15%	Temperature (+) Relative Water Level Change (-)	Seasonal Drivers
4	1.05	9.52%	DO% (+) Water Level Coefficient of Variation (+)	Hydrologic Variability

Table 5. Spearman's Rank-Order Correlation matrix for principal component predictors and methylmercury concentration

	PCA1	PC 2	PC 3	PC 4
All Samples	-0.529**	0.346*	0.183	-0.084
By Park:				
Vermont	-.472*	-0.144	0.209	-0.089
New York	0.069	0.438*	0.333	0.099
By Site:				
VT – PR^	-0.486	-0.543	0.486	-0.943
VT – FW^	-0.029	-0.086	0.371	0.086
VT – KF1	-0.5	0.5	-0.5	-1
VT – KF2	-1	0	-0.2	-1
NY – VP1^	-0.086	-0.543	0.143	-0.829
NY – VP2^	-0.314	0.086	-0.257	-0.2
NY – VP3	-0.7	0.5	-0.1	-0.9
NY – VP4	-0.9	0.3	0.6	-0.5

**p< 0.01; *p<0.05
^denotes 7 samples (vs. 5)

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APPENDICES

APPENDIX A: RAW SAMPLING DATA

Park Site	Date	Sp Cond (us /cm)	DO (%)	pH	DOC (mg /L)	Cl A	SO4 (mg /L)	NO3 (µg /L)	Relative Water Level Change	Water Level Coefficient of variation	Period Inundated (days)	THg (ng /L)	MeHg (ng /L)	ME
NY-VP1	11/24/10	49	51.7	5.67	14.10	3.7	5.79	15.00	-4.89	0.57	7	13.13	3.64	0.28
NY-VP1	4/12/11	103	66.5	6.29	9.25		1.82	1.06	-	-	39	5.55	2.83	0.51
NY-VP1	4/29/11	78	22.4	5.99	-	-	-	-	-	-	56	-	-	-
NY-VP1	5/11/11	53	43.7	6.25	12.6	3.0	1.43	2.86	-2.65	0.03	68	6.53	3.84	0.59
NY-VP1	5/25/11	61	12.3	6.25	15.5	3.5	1.96	3.71	-1.90	0.03	82	9.41	6.04	0.64
NY-VP1	6/7/11	96	39.1	6.59	28.2	13.7	1.01	67.3	-6.29	0.20	95	9.78	5.32	0.54
NY-VP1	6/22/11	47	73.1	6.03	-	-	-	-	4.70	0.67	6	-	-	-
NY-VP1	11/1/11	59	44	6.21	9.05	-	1.72	2.66	1.47	0.08	68	4.51	3.18	0.71
NY-VP1	12/1/11	80	32.5	6.31	10.5	-	1.25	1.57	-0.32	0.02	98	8.40	6.81	0.81
NY-VP2	4/12/11	46	44.6	5.37	31.00	3.7	1.30	8.04	-0.68	0.03	65	8.16	0.58	0.07
NY-VP2	4/29/11	39	36.5	5.73	-	-	-	-	0.68	0.03	82	-	-	-
NY-VP2	5/11/11	45	6.6	5.27	38.9	2.0	0.58	9.05	-4.15	0.04	94	10.67	1.47	0.14
NY-VP2	5/25/11	34	3.7	5.49	28.8	1.8	0.72	11.7	-2.43	0.03	108	9.43	2.02	0.21
NY-VP2	6/7/11	54	1.6	5.71	45.9	1.8	0.40	24.4	-10.73	0.24	121	10.00	1.64	0.16
NY-VP2	6/23/11	32	41.2	5.28	-	-	-	-	-	-	-	-	-	-
NY-VP2	11/1/11	65	9.7	4.43	38.4	-	1.48	15.2	2.12	0.09	68	5.67	2.36	0.42
NY-VP2	12/1/11	48	7.7	4.56	27.9	-	1.56	12.8	-1.25	0.02	98	5.68	1.48	0.26
NY-VP3	11/24/10	84	13.8	5.24	57.40		3.40	17.10	-0.47	0.03	24	12.17	3.74	0.31
NY-VP3	4/12/11	34	73.7	5.82	17.90	3.1	0.97	1.87	-2.44	0.05	163	4.72	1.09	0.23
NY-VP3	4/29/11	35	34	5.94	-	-	-	-	1.74	0.05	180	-	-	-
NY-VP3	5/11/11	35	8.6	5.75	-	-	-	-	-4.15	0.05	192	-	-	-
NY-VP3	6/7/11	52	15.3	5.96	41.8	<1	0.28	17.4	-7.49	0.14	219	5.98	1.56	0.26
NY-VP3	6/23/11	35	51.3	5.47	-	-	-	-	-0.47	0.03	-	-	-	-
NY-VP3	11/1/11	34	11.4	5.57	28.5	-	0.18	8.19	1.44	0.07	68	2.67	1.15	0.43
NY-VP3	12/1/11	29	19.4	5.57	24.9	-	0.41	5.48	-1.28	0.03	98	3.16	2.06	0.65
NY-VP4	11/24/10	105	23.7	5.51	64.40		4.03	20.00	-0.33	0.02	42	11.33	5.24	0.46
NY-VP4	4/12/11	33	31	5.78	14.20	4.6	0.84	1.63	-2.06	0.02	181	3.33	0.33	0.10
NY-VP4	4/29/11	37	32.4	6.06	-	-	-	-	1.41	0.02	198	-	-	-
NY-VP4	5/11/11	38	39	5.81	-	-	-	-	-3.81	0.03	210	-	-	-
NY-VP4	6/7/11	56	5.9	5.75	31.5	31.3	0.14	12.1	-5.09	0.04	237	5.00	1.14	0.23
NY-VP4	6/23/11	61	8	5.8	-	-	-	-	6.34	0.05	253	-	-	-

Park Site	Date	Sp Cond (us /cm)	DO (%)	pH	DOC (mg /L)	Cl A	SO4 (mg /L)	NO3 (µg /L)	Relative Water Level Change	Water Level Coefficient of variation	Period Inundated (days)	THg (ng /L)	MeHg (ng /L)	ME
NY-VP4	11/1/11	60	6.6	5.68	35.4	-	0.01	10.4	-2.32	0.03	68	2.87	0.51	0.18
NY-VP4	12/1/11	47	22.4	5.83	32.2	-	0.02	12.1	-2.82	0.02	98	1.74	0.29	0.17
VT-PR	11/19/10	78	-	6.96	2.58		1.62	0.50	2.20	0.12	53	2.50	1.07	0.43
VT-PR	4/15/11	60	64.9	6.06	3.10	<1	0.99	13.3	14.82	0.20	200	1.29	0.07	0.05
VT-PR	4/30/11	46	63.2	6.55	5.98	2.6	1.38	0.5	-3.00	0.02	215	1.99	0.27	0.13
VT-PR	5/13/11	71	21.3	6.47	5.05	-	1.39	0.511	-7.84	0.12	228	3.12	1.72	0.55
VT-PR	5/25/11	78	12.5	6.61	4.57	1.5	1.51	1.7	-3.49	0.04	240	3.89	2.43	0.63
VT-PR	6/8/11	103	12.6	6.83	11.9	1.9	0.82	5.12	-6.08	0.19	254	5.36	1.57	0.29
VT-PR	6/22/11	148	18.4	6.84	-	-	-	-	-5.47	0.53	0	-	-	-
VT-PR	10/3/11	84	4.2	6.72	5.86	-	1.22	1.08	-	-	50	3.43	2.52	0.74
VT-PR	11/29/11	167	22.8	6.7	5.08	-	1.22	22	5.49	0.22	107	2.26	1.59	0.70
VT-FW	11/19/10	181	-	7.30	2.29		1.88	3.56	-0.15	0.06	53	0.42	0.11	0.26
VT-FW	4/30/11	150	18.6	6.98	4.54	1.9	1.42	1.8	-1.05	0.04	215	0.49	0.14	0.29
VT-FW	5/13/11	154	24.2	6.86	4.63	1.8	1.00	1.63	-0.48	0.02	228	0.55	0.22	0.40
VT-FW	5/25/11	144	21.8	6.85	3.49	2.1	1.49	2.02	-0.57	0.02	240	0.40	0.20	0.50
VT-FW	6/8/11	151	9.2	7.03	5.21	1.5	0.19	4.11	-1.14	0.02	254	0.78	0.46	0.59
VT-FW	6/22/11	195	8.9	6.93	-	-	-	-	-0.75	0.04	268	-	-	-
VT-FW	10/3/11	235	61.9	7.12	4.15		1.23	5.43	-0.40	0.06	67	0.52	0.29	0.57
VT-FW	11/29/11	174	71.6	7.18	2.38		1.03	3.77	-0.59	0.05	124	0.58	0.23	0.40
VT-KF1	11/19/10	260	-	7.4	0.99		6.56	166.00	-0.13	0.05	36	0.31	0.09	0.29
VT-KF1	4/30/11	195	92.5	7.26	2.30	5.4	2.72	113	-0.34	0.03	198	0.40	0.15	0.38
VT-KF1	5/25/11	223	34.3	7.09	-	-	-	-	-0.46	0.03	223	-	-	-
VT-KF1	6/8/11	256	22.2	7.28	1.95	<1	4.33	18.9	-1.28	0.04	237	0.62	0.30	0.49
VT-KF1	6/22/11	161	24.1	7.08	-	-	-	-	-1.82	0.07	251	-	-	-
VT-KF1	10/3/11	252	51.1	7.02	1.37	-	3.25	12.5	-	-	50	1.33	1.05	0.79
VT-KF1	11/29/11	281	25.4	7.08	1.12	-	4.81	55	0.91	0.04	107	1.72	1.48	0.86
VT-KF2	11/19/10	146	-	6.94	3.08		2.96	1.10	-0.25	0.02	51	0.70	0.24	0.35
VT-KF2	4/30/11	113	44.6	6.89	4.13	1.0	1.94	2.48	-0.42	0.02	213	0.94	0.38	0.41
VT-KF2	5/13/11	154	28.7	6.8	-	-	-	-	-0.77	0.02	226	-	-	-
VT-KF2	5/25/11	183	28.8	6.79	-	-	-	-	0.1420	0.0173	238	-	-	-
VT-KF2	6/8/11	183	18.9	6.79	6.37	<1	1.85	1.96	-0.699171454	0.0189	252	1.86	1.32	0.71
VT-KF2	6/22/11	213	14.7	6.54	-	-	-	-	-1.10	0.03	266	-	-	-
VT-KF2	10/5/11	158	45.1	7.01	6.01	-	1.39	8	-0.43	0.03	52	1.14	0.64	0.56
VT-KF2	11/29/11	139	11.8	6.73	6.98	-	1.84	1.95	-0.18	0.02	107	2.60	2.32	0.89

APPENDIX B: COMPLETE TABLE OF LOADING FACTORS FROM PRINCIPAL COMPONENT ANALYSIS

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11
Temperature	-0.21	-0.56	0.67	0.04	0.12	-0.12	0.3	0.21	-0.05	-0.11	0.07
Specific Conductivity	0.83	0.08	0.25	-0.3	-0.2	-0.03	0.05	0.08	0.29	-0.08	-0.11
DO%	0.52	0.29	-0.04	0.47	0.57	-0.23	-0.15	0.1	0.11	-0.04	0.01
pH	0.86	-0.25	0.26	0.07	-0.14	-0.2	0.06	-0.02	0.02	0.25	0.07
DOC	-0.84	0.23	0.04	-0.31	0.16	0.08	-0.01	0.02	0.3	0.06	0.12
SO4	0.43	0.68	0.36	-0.14	-0.22	0.09	-0.26	0.22	-0.13	-0.06	0.11
NO3	0.36	0.44	0.51	-0.29	0.42	0.29	0.16	-0.21	-0.1	0.04	-0.04
Relative Water Level Change	0.44	0.29	-0.67	0.04	0.03	0.25	0.35	0.27	-0.01	0.03	0.03
Water Level Coefficient of variation	-0.17	0.39	0.36	0.71	-0.28	0.23	0.13	-0.15	0.11	-0.02	0.02
Period Inundated	0.17	-0.76	0.12	0.13	0.11	0.5	-0.25	0.15	0.05	0.04	-0.02
Total Hg	-0.79	0.38	0.3	0.08	0	-0.08	-0.01	0.3	-0.04	0.14	-0.14