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ARTICLE

Preliminary Assessment of Mercury Accumulation in Massachusetts and Minnesota Seasonal Forest Pools

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Abstract Seasonal forest pools (SFPs) are common, widespread, and provide critical habitat for amphibians and invertebrates. The ephemeral hydrology of SFPs has been identified as an important factor in the production of biologically active methylmercury (MeHg). To investigate mercury (Hg) in SFPs, we collected water, fine benthic organic matter (FBOM), detrital materials, and faunal samples from 10 pools in Massachusetts and Minnesota, across a range of basin morphometry, water chemistry, canopy closure, and hydroperiod. Total Hg (THg) and MeHg concentrations were comparable to other small, lentic waters. MeHg was found in greater concentrations in fauna than in periphyton, detrital items, or FBOM. In spite of consistently lower pool acidity and higher THg deposition in Massachusetts, we found no significant differences in Hg concentrations between states. No significant relationship was found between MeHg concentration and canopy closure. A weak, positive relationship was identified between pool hydroperiod and the bioaccumulation of THg, suggesting that longer exposure may be a factor in increased Hg concentrations in basal resources, which is then propagated into consumer trophic levels. Further work on the mechanisms determining Hg trophic transfer and bioaccumulation is required to fully understand the influence of hydrogeochemical and landscape influences on Hg dynamics in SFPs.

Keywords Bioaccumulation · Methylmercury · Seasonal forest pools · Total mercury · Vernal pools

Introduction

Ephemeral lentic systems are common across most biomes around the globe (Williams 2006). In the northeastern and north central United States and adjacent Canada, seasonal forest pools (SFPs), commonly referred to as "vernal pools", are widespread and abundant (Burne 2001; Colburn 2004; Lathrop et al. 2005; Van Meter et al. 2008; Rheinhardt and Hollands 2009). They are the preferred breeding habitat of mole salamanders (*Ambystoma* spp. Tschudi) and wood frogs (*Rana sylvatica* Le Conte) and support a rich and abundant macroinvertebrate community (Colburn 2004; Colburn et al. 2009; Semlitsch and Skelly 2009). Due to their small size and spatial and hydrologic isolation, vernal pools are potentially affected by large-scale environmental perturbations. They have been shown to be sensitive to acid precipitation (Pough 1976; Cook 1983) and are predicted to be affected by climate change (Brooks 2009).

Mercury (Hg) deposition, originating principally from emissions from coal-fired combustion and large-scale incineration, is a major pollution issue in many locations across the United States (Miller et al. 2005; VanArsdale et al. 2005; Gbor et al. 2007, NADP Mercury Deposition Network [http://nadp.sws.uiuc.edu/mdn/]). Beyond deposition, several land use/cover, water chemistry, and climatic factors have

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been associated with Hg concentrations and bioaccumulation in aquatic systems (Chen et al. 2005; Kamman et al. 2005; Shanley et al. 2005). Mercury transformations in aquatic systems are complex with uncertain consequences for methylation and biological uptake (Gu et al. 2011).

Ephemeral waters are important sites for the conversion of ionic mercury to methylmercury (MeHg) (Grigal 2003; Galloway and Branfireun 2004). In aquatic environments, methylation is related to the chemical characteristics of the system, including redox, pH, dissolved organic carbon, sulfate concentrations, and the productivity of sulfate-reducing bacteria (Wiener et al. 2003; Driscoll et al. 2007). Water level fluctuations characteristic of ephemeral waters result in alternating cycles of reduction (anaerobic, flooded) and oxidation (aerobic, dry) conditions that can increase methylation (Evers et al. 2007). In SFPs, both chemical and physical characteristics would likely be important in Hg processes (Snodgrass et al. 2000; Chen et al. 2005; Unrine et al. 2005; Evers et al. 2007).

Increased MeHg production can result in MeHg bioaccumulation in aquatic food webs, particularly in predaceous fish, birds, and mammals (Snodgrass et al. 2000; Sorensen et al. 2005; Evers et al. 2007), posing potential health risks for wildlife and humans (Wiener et al. 2003; Driscoll et al. 2007; Wiener et al. 2007). MeHg trophic transfer can be diminished by increased algal productivity due to biomass dilution (Pickhardt et al. 2002). MeHg is also likely to move from aquatic systems via emigrating amphibian and invertebrate metamorphs entering terrestrial food webs through bird and mammal predation (Wolfe et al. 2007).

The goal of our study was to examine the effects of hydroperiod and canopy closure on Hg bioaccumulation in food webs of SFPs at two sites in the northern U.S, and to assess whether regional differences in water chemistry and atmospheric deposition affects Hg dynamics in these systems. Pool hydroperiod is largely determined by basin morphology and weather patterns and will be affected by climate change (Brooks and Hayashi 2002; Brooks 2004, 2009). To test the effects of pool hydrology on MeHg bioaccumulations, we measured Hg concentrations across trophic levels in pools with differing hydroperiods. Limited research from southern ephemeral wetlands suggests that MeHg bioaccumulation in pools that experience increased rates of wetting and drying (i.e., short hydroperiods) will be greater than in those that remain wetted longer (Snodgrass et al. 2000). This relationship could be negated in fauna by reduced MeHg bioaccumulation in faster-developing individuals inhabiting short hydroperiod pools due to more efficient growth (Rowe and Dunson 1995; Ward et al. 2010).

MeHg accumulation may be greater in open-canopy pools with greater potential autotrophic production compared to closed-canopy pools. The greatest increase in MeHg concentrations occur in the trophic step between water and algae (Bell and Scudder 2005; Wiener et al. 2007; Driscoll et al. 2007). Thus open-canopy (i.e., high-light) pools could exhibit greater levels of primary productivity and greater MeHg bioaccumulation than in more shaded ponds. The ratio of MeHg to total mercury (THg) is low (1–6 %) in foliage (Heyes et al. 1998; Grigal 2003), the basal food source in detritus-based food webs such as SFPs (Colburn 2004). Very little has been published on MeHg bioaccumulation in detrital-based food webs and only one study examined Hg dynamics in glaciated forested pools in the northern United States (Loftin 2010). Finally, the lower Hg deposition and higher soil and water pH, characteristic of the north central region (e.g., Minnesota) of the U.S., should lead to lower Hg concentrations in SFP food webs compared to the greater Hg deposition and lower soil and water pH of the northeast (e.g., Massachusetts). Both deposition and acidity have been positively associated with high Hg accumulation in lentic and lotic ecosystems (Driscoll et al. 2007; Evers et al. 2007; Ward et al. 2010).

Study Sites

Ten SFPs, representing a range of hydroperiods, from short- to long-duration, were selected on both the Quabbin Reservation in central Massachusetts and on the Chippewa National Forest in northern Minnesota (Table 1). Pools were selected from a larger samples of pools in a MA study of pool basin morphology and hydroperiod (Brooks and Hayashi 2002) and a MN study of the effects of forest disturbance on SFPs (Palik et al. 2001). Detailed descriptions of the study areas are provided in these papers. Broadly, the pools are precipitation fed, and located in mixed conifer-deciduous forests on soils of glacial origin.

Methods

Physical and Chemical Characteristics of Pool Habitat

Pool basin morphology of the MA pools was available from the Brooks and Hayashi (2002) study. The morphology of MN pool basins was estimated from the maximum length of the short and long axes and maximum depth of each pool, using the formulae for ellipses for surface area and perimeter or ellipsoids for volume. Maximum pool depth was measured at the deepest location of each pool basin. Forest canopy closure over each pool basin was sampled using a spherical densiometer in July 2010 at systematically located points across each pool. The number of points was proportional for maximum pool surface area, with no less than five points at any pool. Canopy cover surveys were completed in mid-summer, rather than spring, to quantify full-canopy conditions and for safe access to the entire pool basin.

Table 1 Maximum pool basin morphology, overstory canopy closure, 2010 date last recorded surface water, and assigned hydroperiod class, by state (Massachusetts[a] [MA], Minnesota [MN]) and pool

State and pool	Maximum				Canopy closure (%)	2010 date last surface water	Hydroperiod class
	Depth (cm)	Area (m^2)	Volume (m^3)	Perimeter (m)			
MA401	31	146	19	55	77.9	20 Apr	Short
MA489	31	283	25	83	88.0	19 Apr	Short
MA505	29	140	20	45	79.9	20 Apr	Short
MA243	51	318	70	77	89.7	23 May	Intermediate
MA400	58	1,073	201	176	96.9	17 May	Intermediate
MA508	11	129	6	46	59.7	4 May	Intermediate
MA236	53	1,262	223	164	78.6	n/a[b]	Long
MA246	38	2,941	331	388	85.2	12 Jul	Long
MA391	68	843	193	124	92.5	24 Jul	Long
MA428	48	701	175	115	69.6	5 Jul	Long
MN21	49	452	147	80	91.4	n/a	Short
MN267a	34	277	63	59	95.1	n/a	Short
MN941	63	672	281	98	91.9	n/a	Short
MN974	75	672	334	94	88.9	n/a	Short
MN20	37	1,304	326	128	93.4	n/a	Intermediate
MN929a	78	897	466	107	86.6	n/a	Intermediate
MN974a	39	1,401	364	165	89.4	n/a	Intermediate
MN974b	79	835	439	110	91.4	n/a	Intermediate
MN267	45	2,599	786	181	91.4	n/a	Long
MN1073	49	2,851	940	200	88.8	n/a	Long

[a] Massachusetts pool morphology data from Brooks (2004)

[b] n/a—pools held standing water continuously until depth loggers were removed

Water depth and temperature were measured at 15-min intervals using Solinst Leveloggers® and 1-m staff gauges, installed at the deepest location of each pool. A Solinst Barologger® was installed adjacent to the pool of median elevation to record barometric pressure at 15-min intervals, which was used to correct water depth measurements. Leveloggers were recovered after a pool dried or at the end of the field season. Water levels were also recorded from the staff gauge at each pool visit. Dissolved oxygen, pH, conductivity, and temperature of pool water were measured once (MA) or twice (MN) at each pool using calibrated meters.

Monthly precipitation and annual mercury deposition were characterized respectively from NADP NTN (National Atmospheric Deposition Program, National Trends Network [http://nadp.sws.uiuc.edu/NTN]) and MDN (NAD Program, Mercury Deposition Network [http://nadp.sws.uiuc.edu/MDN]) sites geographically closest to the pool locations in each state: MN16 (Marcell Experimental Forest, Itasca County), approximately 100 km northeast of the Minnesota pool site and MA08 (Quabbin Reservation, Hampshire County) for precipitation and NY68 (Biscuit Brook, Ulster County) for mercury deposition, approximately 184 km southwest of the Massachusetts pool site.

Mercury Sampling

Water and food web sampling occurred during a 2-week period between late April and early May when pools were fully accessible and taxa were at their peak abundance but prior to emergence from the pools.

Water Sampling

Water samples for Hg analysis were collected using "clean hand, dirty hands" technique upon first arrival at a pool to avoid contaminating samples. Water samples were taken using the dip-sampling method (USGS method NFM 4.1.1.B) (Lewis and Brigham 2004). Four unfiltered samples were collected in 125 mL PETG bottles from near the center of each pool. In MN, an additional four samples were collected and immediately filtered. Samples were double-bagged in new zip lock bags and transported on ice and under dark conditions.

Water samples were preserved with 0.4 % trace-metal grade HCl within 24 h of sampling.

Food Web Sampling

We collected no less than 2 g wet weight of periphyton, leaves, wood without periphyton, and fine benthic organic matter (FBOM) for Hg analysis. Samples were collected widely across each pool and composited in 4 oz (~115 cm^3) specimen cups. Periphyton samples were collected from stable substrates (e.g. wood, rocks) using a clean stainless steel scalpel. FBOM samples were scooped from the upper surface of the pond bottom and strained through a 250 μm stainless steel sieve. Excess pond water was decanted from the specimen cups. Samples were rinsed with deionized water, decanted, and transferred to clean, labeled 20 mL polyethylene scintillation vials. Submerged wood and soft leaves (e.g., those already undergoing decomposition) of mixed species were collected, double bagged, and frozen upon arrival at the lab.

Beetle (Coleoptera: Dytiscidae), caddisfly (Trichoptera: Limnephilidae), and mosquito (Diptera: Culicidae) larvae were collected using clean nets from a broad range of locations across each pool. Organisms were sorted from detritus in the field and placed in specimen cups filled with pool water. We attempted to collect individuals of similar sizes within each taxon to reduce within taxa variability. Specimen cups were labeled, placed in zip lock bags to prevent contamination, and transported on ice packs in a dark cooler. In the laboratory, organisms were allowed to clear their guts for 4–6 h and samples were transferred to clean 20 mL polyethylene scintillation vials and frozen. Cases were removed from caddisfly larvae prior to freezing. Voucher specimens of each taxon were preserved in alcohol for species identification.

Wood frog larvae were collected using D-frame or vinyl coated aquarium nets and/or vinyl coated minnow traps. Individuals were collected from as broad a range of locations within each pool as possible. Samples were not staged (Gosner 1960) but were free-swimming and most likely in the late embryonic (stages 21–25) to early larval (stages 26–30) stages. Wood frogs had clearly bred much earlier than our first sample collection since development to free-swimming stage requires about a third of the total embryonic and larval development to metamorphosis (Herreid and Kinney 1967), which can last 52–135 days (DeGraaf and Rudis 1983). Samples were placed in specimen cups filled with pond water. In the laboratory, samples were transferred to clean 20 mL polyethylene scintillation vials, after allowing them to clear their guts for 48 h (Unrine et al. 2005), and frozen.

Chemical and Statistical Analyses

All inorganic mercury (IHg) and MeHg measurements were conducted at the Dartmouth Trace Element Analysis Core Facility using a magnetic sector inductively coupled plasma-mass spectrometer (ICP-MS ELEMENT2, Thermo-Finnigan, Waltham, MA). For water samples, two sets of samples were collected in Minnesota- one was filtered through acid-cleaned 0.45 um filters in the field and acidified with 0.4 % HCl, the other set was not filtered before acidification. Only unfiltered water samples were collected in Massachusetts and were also acidified 0.4 % HCl. All unfiltered water samples were measured for whole-water THg concentration. Filtered water samples from the MN pools were also measured for dissolved MeHg concentrations.

Biological samples were sorted, rinsed of detritus with deionized water, freeze-dried, and homogenized before being analyzed for IHg and MeHg concentrations. Samples were analyzed using isotope dilution gas chromatography-ICP-MS. Samples were spiked with an appropriate amount of enriched IHg and enriched methyl [^{201}Hg] mercury and then extracted in 2–3 mLs of KOH/methanol (25 % w/v). The methodology involved purging with inert gas and trapping on a Tenax trap which was thermally desorbed and Hg species were quantified by isotope dilution. Approximate detection limits for average sample mass were 5.6 ng·g^{-1} IHg and 0.4 ng·g^{-1} MeHg. Quality control was conducted through the analysis of standard reference material (NIST 2976, mussel tissue). Average measured values for reference samples were within 5 % of the certified value (MeHg mean±SE: 26.5±0.5 ng/g, n=17, certified value=27.8 ng/g; IHg mean±SE: 33.5±0.7 ng/g, n=17, certified value=33.2ng/g). Repeatability of analysis was assessed by duplicate measurement of 20 samples. Average relative percent difference (RPD) of duplicates was ca. 10 % for both MeHg and IHg (MeHg mean RPD±SE=10.7±3.4 %; IHg mean RPD±SE=9.4±2.9 %).

Hydroperiod of Massachusetts pools was classified as short, intermediate, or long, depending on both the long-term and 2010 hydrology (Table 1). Since no long-term hydrology records existed for the Minnesota pools, and all pools remained flooded throughout the 2010 season, hydroperiod was classified based on basin morphology and on the dynamics (i.e., variability) of 2010 daily mean water levels. Pool basin dimensions have been shown to be positively related to hydroperiod (Brooks and Hayashi 2002). Pools with the smallest basin dimensions and greatest variability in daily mean water levels were classified as short hydroperiod, and vice versa. The use of stage variability as a surrogate for hydroperiod is analogous to the correlation of stream flashiness to flow regime (Poff and Ward 1989; Clausen and Biggs 2000; Baker et al. 2004). Bioaccumulation factors were calculated for each vernal pool as the ratio of the concentration of total mercury (THg=IHg+MeHg) in a trophic level to the concentration of THg in pool water. The relationships between pool physiochemical attributes and MeHg concentrations were examined using Spearman, rank-order correlations. The effects of pool hydroperiod on

MeHg concentrations and THg BAFs were analyzed by analysis of variance while controlling for the effect of location (i.e., state). Hg concentrations were log transformed prior to analysis. Significant differences were assigned for $p \leq 0.05$.

Results

The MA sample included three vernal pools smaller than the smallest pool in the MN sample (Table 1), contributing to significant differences in maximum pool surface area and volume between the two states (Table 2). Pool hydroperiods in 2010 differed between states (Table 1). In Massachusetts, the smallest pools dried within two weeks of the installation of the Leveloggers in early April; all Minnesota pools remained wetted throughout the study. In MA, NADP monthly precipitation was below the 10-year average for every month of the 2010 field season, while NADP monthly precipitation in MN started below average in the months prior to the field season, but was at or above average between May and September. Pool water chemistry differed significantly between the states (Table 2): Minnesota pools had higher pH (mean MN=7.0, MA=5.2), greater conductivity (mean MN=136.2 μS/cm, MA=21.7 μS/cm) and greater dissolved oxygen (mean MN=6.9 mg/L, MA=2.2 mg/L) (Table 3). From 2005 to 2009 annual THg deposition at a NADP MDN Site in New York State was greater than at the Minnesota NADP MDN Site (mean NY=9.58 μg/m^2, MN=6.32 μg/m^2).

Table 2 Analysis of variance statistics by state and hydroperiod class, Massachusetts and Minnesota seasonal forest pools, 2010

Variable	State		Hydroperiod class	
	F	*p*	F	*p*
Maximum pool				
Depth	2.312	0.151	0.756	0.57
Area	8.807	0.01	8.172	0.001
Volume	88.082	<0.001	21.96	<0.001
Perimeter	0.207	0.656	2.835	0.065
Pool water chemistry				
pH	64.061	<0.001	0.52	0.677
Conductivity	45.954	<0.001	0.85	0.495
Dissolved oxygen	65.721	<0.001	1.872	0.193
Total mercury concentration				
Pool water	0.038	0.849	2.152	0.152
Methylmercury concentration				
Pool water	nc[a]		1.153	0.369[b]
Fine benthic organic matter	0.547	0.474	2.401	0.119
Periphyton	0.89	0.366	4.186	0.003
Leaves	0.0	0.994	0.638	0.644
Wood	0.25	0.625	0.608	0.663
Mosquito larvae	0.348	0.57	14.805	0.001
Wood frog larvae	7.844	0.014	3.736	0.029
Caddisfly larvae	4.14	0.065	3.785	0.032
Beetle larvae	0.745	0.405	4.414	0.02
Total mercury bioaccumulation factor				
Fine benthic organic matter	0.634	0.443	2.09	0.16
Periphyton	4.033	0.07	1.504	0.268
Leaves	0.796	0.391	1.988	0.174
Wood	1.263	0.285	2.194	0.146
Mosquito larvae	nc		1.583	0.271[b]
Wood frog larvae	3.421	0.091	1.193	0.357
Caddisfly larvae	1.474	0.257	0.931	0.461
Beetle larvae	1.814	0.211	2.489	0.127

[a] nc—no comparison for this effect

[b] for MN pools only

Methylmercury concentrations differed among components of vernal pool food webs (Table 4). No significant differences in MeHg concentrations were found between states (Table 2). In both states, mean MeHg concentrations were low in the basal trophic components (i.e., FBOM, periphyton, leaves, wood) and much greater in the higher, faunal trophic levels. MeHg concentrations in all food web components were poorly correlated with the pool morphometry or pool water chemistry. An exception were the strong (> 0.75) positive correlations of 2010 hydroperiod in Massachusetts pools with MeHg concentrations in periphyton, and mosquito, wood frog, and caddisfly larvae (Table 5). MeHg concentrations in food web components were generally greater in longer hydroperiod pools, but with considerable variation among pools in each hydroperiod class (Fig. 1). There were significant differences in MeHg concentrations among hydroperiod classes for periphyton, and mosquito, wood frog, caddisfly, and beetle larvae (Table 2).

Total mercury concentrations in Massachusetts pool water were low with the exception of pool MA400 (Table 3). THg concentrations in Minnesota pool water were slightly greater than for Massachusetts pools, but less than for MA400. MeHg concentrations in Minnesota pool water were low, typically < 1.0 ng·L^{-1} (Table 3). MeHg concentrations as a percent of THg varied considerably among Minnesota pools, from <1 % in pool MN21, to 19.4 % in pool MN974b. Differences in THg in pool water were not significant for the effects of hydroperiod class or state, or for the effects of hydroperiod class on pool water MeHg in Minnesota pools (Table 2).

The bioaccumulation (BAF) of THg consistently increased with increasing pool hydroperiod however, the effect of hydroperiod class on BAF was not significant for any food web component (Fig. 2, Table 2). The bioaccumulation of THg was greatest from water to FBOM, intermediate for water to periphyton and mosquito and wood frog larvae, and least for water to basal resources. BAFs for caddisfly and

Table 3 Mean pool water chemistry and mercury (THg—total mercury, MeHg—methylmercury) concentrations by state (Massachusetts [MA], Minnesota [MN]), pool, and hydroperiod class[a] (Short, Intermediate, Long), 2010

State and pool	Hydroperiod class	pH	Conductivity (μS/cm)	DO (mg/L)	Temperature (°C)	THg ($ng \cdot L^{-1}$)	MeHg ($ng \cdot L^{-1}$)
MA243	I	5.29	20.0	0.84	13.0	4.5	[b]
MA400	I	4.90	31.5	1.68	12.0	48.7	
MA236	L	5.94	21.3	3.52	11.7	3.8	
MA246	L	4.74	20.4	3.92	11.5	5.0	
MA391	L	4.93	18.4	1.46	11.8	4.6	
MA428	L	5.23	18.4	1.85	12.3	5.3	
MN21	S	6.8	94.7	7.8	12.7	19.5	0.18
MN267a	S	6.5	121.1	8.0	14.1	22.6	0.44
MN941	S	7.3	165.3	6.8	10.7	8.6	1.28
MN974	S	7.3	182.8	7.9	12.8	8.4	0.53
MN20	I	6.6	111.4	6.6	13.7	5.2	0.91
MN929a	I	7.1	118.6	7.0	14.7	8.4	0.56
MN974a	I	7.4	158.6	5.7	13.5	8.1	1.18
MN974b	I	7.4	193.7	5.8	14.8	5.2	1.01
MN267	L	6.8	135.8	5.6	13.6	4.1	0.58
MN1073	L	6.6	79.8	7.9	10.7	15.8	0.93

[a] MA short hydroperiod pools 401, 489, and 505, and intermediate hydroperiod pool 508 were dry before water chemistry measurements were taken

[b] no methylmercury data available for Massachusetts pools

Table 4 Methylmercury concentrations ($ng \cdot g^{-1}$) and as a percent of total mercury concentrations (in parentheses) by state (Massachusetts [MA], Minnesota [MN]), pool, and hydroperiod class (HC: Short, Intermediate, Long), and seasonal forest pool food web component (FBOM—fine benthic organic matter, WOFR—wood frog, 2010)

State and pool	HC	FBOM	Periphyton	Leaves	Wood	Larval			
						Mosquito	Wood frog	Caddisfly	Beetle
MA401	S	nd[a]	nd	0.4 (1.2)	0.4 (2.3)	7.1 (15.9)	6.4 (9.0)	14.3 (18.7)	nd
MA489	S	nd	nd	0.6 (1.3)	0.3 (2.7)	17.1 (30.3)	19.3 (25.8)	nd	27.9 (78.7)
MA505	S	nd	nd	0.9 (3.1)	0.4 (2.1)	13.8 (34.4)	10.9 (15.7)	12.8 (31.3)	14.6 (45.3)
MA243	I	5.2 (5.6)	5.8 (8.1)	3.1 (7.5)	1.7 (8.2)	nd	18.9 (13.7)	32.1 (61.5)	40.4 (70.1)
MA400	I	3.2 (2.3)	1.7 (3.8)	2.4 (6.3)	2.7 (10.2)	nd	15.0 (10.5)	11.5 (37.1)	40.5 (76.9)
MA508	I	7.2 (7.5)	nd	3.5 (7.7)	7.0 (25.4)	165.8 (25.0)	27.1 (57.9)	38.1 (59.0)	82.5 (89.6)
MA236	L	8.8 (4.5)	15.8 (22.3)	5.8 (15.7)	8.4 (21.6)	nd	63.5 (32.4)	89.2 (73.4)	70.6 (91.1)
MA246	L	3.9 (2.3)	6.0 (7.3)	3.1 (7.3)	3.3 (10.2)	nd	25.0 (18.3)	nd	nd
MA391	L	7.0 (2.4)	11.4 (14)	8.8 (10.7)	1.8 (4.9)	nd	40.0 (21.9)	54.1 (68.5)	71.4 (87.3)
MA428	L	12.7 (11.1)	16.1 (24.3)	7.6 (14.3)	5.3 (23.4)	nd	60.9 (24.9)	41.8 (63.0)	192.3 (88.1)
MA	Mean	6.9 (4.7)	9.5 (12.3)	3.6 (6.7)	3.1 (9.6)	51.0 (26.1)	33.7 (22.0)	39.6 (51.9)	67.6 (79.7)
MN21	S	3.2 (0.9)	2.1 (1.4)	1.2 (1.6)	1.2 (3.6)	35.3 (28.0)	14.3 (7.7)	11.2 (14.5)	40.0 (44.4)
MN267a	S	5.0 (2.2)	3.4 (1.8)	1.9 (2.1)	2.0 (5.3)	41.6 (42.1)	40.8 (19.2)	24.8 (22.3)	102.0 (72.6)
MN941	S	3.1 (0.8)	5.3 (7.2)	2.7 (4.4)	4.1 (15.7)	66.3 (45.2)	38.7 (21.7)	75.3 (56.9)	71.4 (66.6)
MN974	S	2.7 (0.8)	2.7 (4.7)	2.8 (5.8)	2.5 (6.7)	33.0 (29.7)	58.9 (44.5)	28.5 (40.4)	55.2 (74.1)
MN20	I	7.4 (3.4)	8.5 (11.3)	7.9 (14.8)	4.8 (14.9)	71.9 (39.3)	32.9 (17.3)	84.4 (68.4)	45.3 (69.7)
MN929a	I	4.1 (1.2)	2.7 (5.8)	2.7 (5.8)	1.6 (6.9)	42.8 (40.7)	21.9 (24.7)	38.6 (51.7)	53.3 (61.8)
MN974a	I	7.0 (2.7)	5.5 (6.4)	3.8 (5.7)	3.9 (14.1)	68.8 (40.8)	71.2 (44.2)	68.5 (63.5)	71.8 (59.1)
MN974b	I	4.9 (1.7)	7.0 (10.0)	3.0 (5.7)	3.2 (12.3)	52.0 (40.3)	57.1 (30.1)	77.9 (66.0)	83.8 (66.9)
MN267	L	11.9 (5.3)	5.2 (9.7)	10.7 (15.9)	9.5 (21.0)	74.3 (42.4)	48.8 (35.7)	48.3 (55.2)	64.8 (64.3)
MN1073	L	4.4 (1.9)	6.6 (5.3)	3.4 (5.7)	4.8 (23.2)	39.4(34.7)	42.4 (46.0)	56.2 (56.0)	31.3 (77.3)
MN	Mean	5.4 (1.9)	4.9 (5.3)	4.0 (6.4)	3.8 (12.7)	52.5 (38.7)	42.7 (27.3)	51.4 (51.4)	61.9 (65.9)

[a] nd—no data, no specimens collected

Table 5 Spearman rank-order correlations among morphometric, chemical, 2010 hydroperiod, and methylmercury concentrations in seasonal forest pool food web components (FBOM—fine benthic organic matter, WOFR—woof frog), Massachusetts and/or Minnesota, 2010

Pool characteristic	Water		FBOM	Periphyton	Leaves	Wood	Larval			
	THg	MeHg[b]					Mosquito	WOFR	Caddisfly	Beetle
Maximum										
Depth	−0.013	0.152	−0.445*	−0.112	−0.027	−0.146	0.088	0.308	0.260	0.182
Area	−0.219	0.442	0.145	0.253	0.427*	0.358	0.380	0.471*	0.585**	−0.29
Volume	0.032	0.333	−0.128	−0.010	0.250	0.239	0.314	0.549**	0.564**	0.050
Perimeter	−0.196	0.576*	0.144	0.277	0.477*	0.455*	0.455	0.477*	0.538**	0.049
2010 hydroperiod[a]	−0.600		0.464	0.829*	0.450	0.274	0.800*	0.818*	0.786*	0.571
Canopy closure	0.496*	−0.092	−0.296	−0.393	−0.050	−0.127	0.311	0.033	0.003	0.053
pH	0.297	0.515	−0.205	−0.255	−0.174	−0.004	0.104	0.339	0.263	0.032
Conductivity	0.454*	0.394	−0.334	−0.398	−0.283	0.060	0.200	0.268	0.168	0.133
Dissolved oxygen	0.484*	−0.640*	−0.310	−0.445*	−0.315	−0.097	−0.191	−0.074	−0.083	−0.150
FBOM	−0.620**	0.152								
Periphyton	−0.725**	0.729*	0.675**							
Leaves	−0.718**	0.480	0.812***	0.727***						
Wood	−0.282	0.596*	0.598**	0.521*	0.781***					
Mosquito	−0.742**	0.552*	0.791**	0.565*	0.570*	0.581*				
WOFR	−0.288	0.394	0.431*	0.487*	0.517*	0.547**	0.495*			
Caddisfly	−0.520*	0.842**	0.405	0.722**	0.689**	0.649**	0.654**	0.657**		
Beetle	−0.258	0.133	0.379	0.428	0.150	0.237	0.636*	0.651	**0.327	

[a] MA pools only

[b] MN pools only

*$p<0.05$, **$p<0.01$, ***$p<0.001$

beetle larvae were low in short hydroperiod pools and intermediate in intermediate and long hydroperiod pools.

Discussion

Mercury concentrations varied greatly in the water, FBOM, detritus, periphyton, and fauna of seasonal forest pools in Massachusetts and Minnesota (Table 4). Comparable Hg concentration data for ephemeral, lentic systems are limited. Total Hg concentrations in seasonal forest pool water (3.8–48.7 ng·L^{-1}; Table 3) were frequently greater than from an Adirondack, New York beaver pond (2.3–2.5 ng·L^{-1}; Driscoll et al. 1998), an Ontario, Canada forest swamp (1.1–1.7 ng·L^{-1}; Galloway and Branfireun 2004), or Acadia National Park (ANP), Maine ponds (1.3–8.4 ng·L^{-1}; Bank et al.

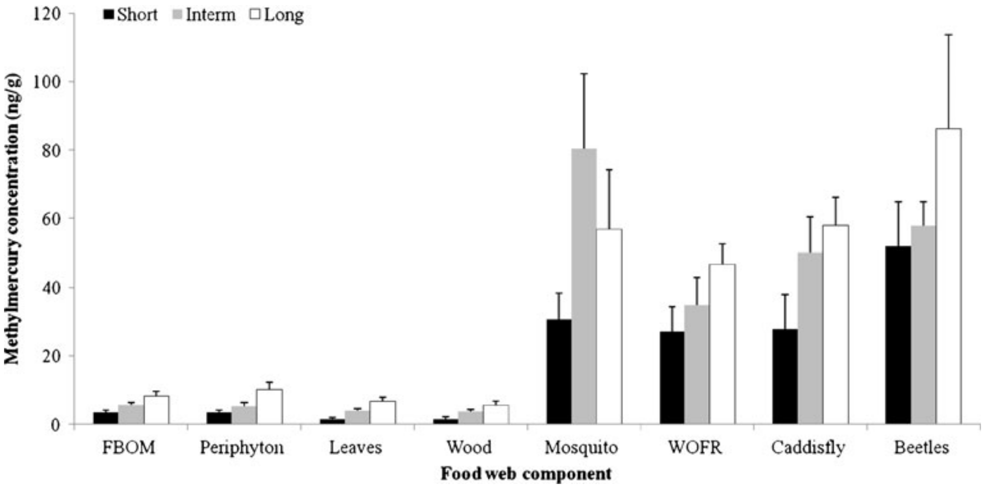

Fig. 1 Mean MeHg concentrations, with standard errors, by food web component (*FBOM* fine benthic organic matter, *WOFR* wood frog) and hydroperiod class (Short, Intermediate, Long), in Massachusetts and Minnesota seasonal forest pools, 2010

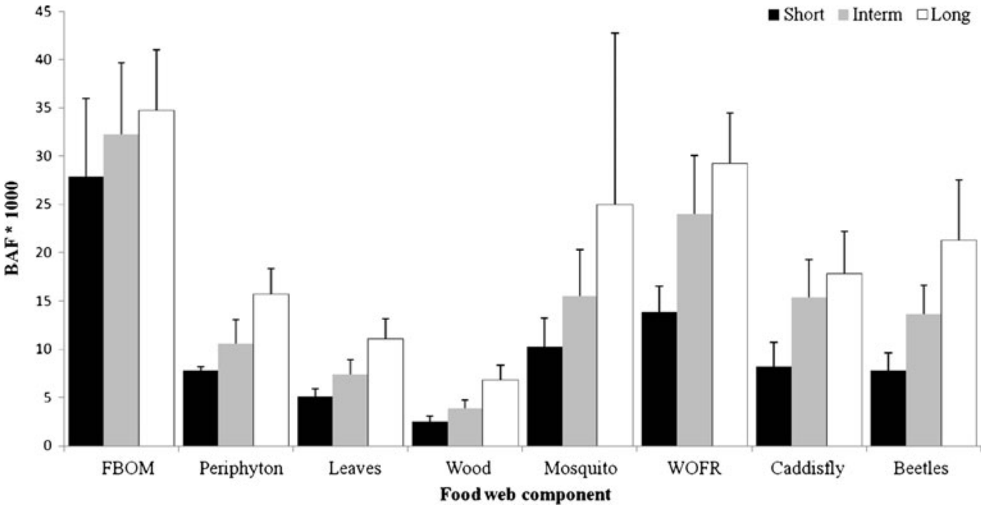

Fig. 2 Mean bioaccumulation factor (*1,000), with standard errors, based on the concentration of THg in pool water, by food web component (*FBOM* fine benthic organic matter, *WOFR* wood frog) and hydroperiod class (Short, Intermediate, Long), in Massachusetts and Minnesota seasonal forest pools, 2010

2007b), but within regional values for northeastern lakes and streams (Driscoll et al. 2007). Likewise, pool methylmercury concentrations in MN pool water (0.18–1.28 ng·L^{-1}) were often greater than for ANP ponds.

MeHg concentrations were low in SFP FBOM and detritus, and several times greater in faunal organisms. FBOM MeHg concentrations (Table 4) fell within the range of published values (Bank et al. 2007b). No data from comparable systems are available for leaves, wood, or periphyton. MeHg concentrations in predatory Odonates from ANP ponds (244 ng·g^{-1}; Bank et al. 2007a) were greater than those for predatory Coleoptera larvae from either MA or MN pools or shredder Trichoptera or collector Culcidae larvae from the pools (Table 4). THg concentrations in wood frog larvae were considerably greater (139–157 ng·g^{-1}) than for wood frog (15–44 ppb; Loftin 2010) or other ranid larvae (Bank et al. 2007b) from ANP ponds. Hg toxicity in amphibians is not well known (Wolfe et al. 1998; Scheuhammer et al. 2007). Boening (2000) reports that the environmental toxicity of IHg to two species of Ranidae ranges between 50 and 80 μg/L, much greater than the Hg concentrations found in our study (Table 3).

Forest canopy closure over SFPs affects light exposure and various ecological conditions and processes such as temperature, dissolved oxygen, and food nutritional quality (Schiesari 2006; Felix et al. 2010), amphibian breeding pond selection (Werner and Glennemeier 1999; Felix et al. 2010), and larval amphibian performance (Skelly et al. 2002; Halverson et al. 2003; Schiesari 2006). Canopy closure was not correlated with MeHg concentrations (Table 5). While the range in canopy closure values was small across the pools in the study (60–97 %, Table 1), it likely reflects the natural range in undisturbed canopy closure for these small aquatic systems embedded in continuous forested landscapes (Skelly et al. 2002; Palik et al. 2007). Canopy closure was measured in mid-summer, after full foliage development. Sampling for Hg concentrations occurred in mid-spring, before deciduous foliage had developed. A concurrent measurement of closure and Hg would have resulted in a better assessment of the effect of canopy closure on Hg in SFPs. However, this design would frequently exclude short hydroperiod pools which often dry with foliage development and increased transpiration (Brooks 2009). Hg deposition is typically the principal source of Hg to aquatic systems (Driscoll et al. 2007). On an annual basis, average wet Hg deposition between 2005 and 2009 was 59 % greater at the New York NADP MDN site nearest the MA study location than at the NADP MDN site nearest the MN pools. Dry Hg deposition is likely equivalent in amount to wet deposition and is predicted to be highest in the northeastern U.S. (Driscoll et al. 2007). Dry Hg deposition would enter SFPs in throughfall from forest canopies which collect dry Hg deposition from the atmosphere (Driscoll et al. 2007). Greater Hg deposition at the MA pools did not result in greater THg concentration in pool water or greater MeHg concentrations in pool food web components. Likewise, despite the clear and substantial differences in water chemistry (Table 3), there were no significant differences in water THg or MeHg concentrations between the two sites due to the large variation in Hg concentrations among pools in each state (Table 2). Water pH, which has been shown to be inversely related to THg concentrations in fish (Driscoll et al. 2007), was lower for the MA SFPs than the MN pools. Nevertheless, other than for periphyton and caddisfly larvae, mean MeHg concentrations in vernal pool food-web components were similar between states (Tables 2 and 4). Influences other than Hg deposition and pool water chemistry appear to have greater influence on Hg concentrations in the study wetlands.

There is an implication in our results that hydroperiod may affect MeHg concentrations, especially in some food web components (e.g., periphyton and wood frog and insect larvae) (Fig. 1). Where a significant effect was found, MeHg concentrations generally increased with increasing hydroperiod and in the longer hydroperiod MN pools (Table 4). The increase in MeHg concentration in longer hydroperiod MN pools suggests continued methylation during flooded conditions. THg concentrations in pool water were greater in shorter hydroperiod pools than in both intermediate and long hydroperiod pools in MN, while no difference was seen in MA samples because of the high variability in the two intermediate hydroperiod pools (Table 3). The most likely cause of the decrease of THg concentration with hydroperiod is evasion of gaseous Hg to the atmosphere, which can result from both biotic (e.g., demethylation) and abiotic (e.g., photoreduction) processes. A study of Hg mass balance on a small lake near the MN SFP site found that 26 % of the Hg output from the lake was a result of evasion (Hines and Brezonik 2007), which could begin to explain lower concentrations in long hydroperiod pools. Other studies have shown decreases of THg concentrations following flooding (St. Louis et al. 2004; Hall et al. 2005), although we are aware of no other studies that have closed the mass balance and quantified the processes leading to those losses.

The bioaccumulation (BAF) of THg in SFP food webs appears to be positively related to hydroperiod (Fig. 2), but the variability in BAF among pools in hydroperiod classes was generally too great for the differences to be significant (Table 2). The BAFs of basal trophic levels (FBOM, leaves, wood, and periphyton) could be related to the duration of exposure of these resources to Hg in pool water since these food web components were present from when pools were initially inundated. While all pools were fully inundated when sampling was initiated in the spring 2010, some of the Massachusetts pools were partially inundated in late October 2009. The long hydroperiod MA pools held surface water at that time, intermediate pools had shallow groundwater just below the basin surface, and there was no surface or shallow groundwater in short hydroperiod pools. This pattern of inundation results in longer Hg exposure for basal trophic levels in longer hydroperiod pools. Faunal food web components had similar exposure to Hg regardless of pool hydroperiod, since they entered and were removed (i.e., sampled) from pools over the same time periods. BAF patterns in basal trophic levels related to hydroperiod and exposure duration could be passed along to higher, consumer trophic levels. Repeated sampling in longer hydroperiod pools could have revealed whether longer inundation and exposure would result in increased MeHg concentrations.

MeHg in vernal pool fauna could enter terrestrial or other aquatic food webs through the emigration and predation of adult insects and amphibians and the predation of larval, vernal pool fauna by species such as spotted turtles (*Clemmys gutta* Schneider), green frogs (*R. clamitans* Latreille) and bullfrogs (*R. catesbeiana* Shaw), shrews (Soricidae), and blackbirds (Icterinae) (Doyle 1997; Mitchell et al. 2009). While the export of MeHg from any single pool may be minor, the abundance of pools across the northern forest landscape (e.g., Brooks et al. 1998; Burne 2001; Lathrop et al. 2005; Van Meter et al. 2008) and the large numbers of potential invertebrate and vertebrate emigrants from any productive pools would cumulatively result in a significant export of MeHg to terrestrial and other aquatic environments.

This study provides evidence of mercury methylation and bioaccumulation in SFPs; however, this preliminary study is insufficient to fully understand the factors that control the mercury methylation in these ephemeral, lentic systems. Further work is needed to understand the mechanisms determining bioaccumulation rates and trophic transfer of MeHg in these physically and biologically complex ecosystems. Additional research could lead to our ability to predict how climate and land use changes will influence hydrochemical regimes and their influence on Hg dynamics in these aquatic systems.

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