

Mercury Bioaccumulation in Wood Frogs Developing in Seasonal Pools

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Abstract - Seasonal woodland pools contribute significant biomass to terrestrial ecosystems through production of pool-breeding amphibians. The movement of amphibian metamorphs potentially transports toxins bioaccumulated during larval development in the natal pool into the surrounding terrestrial environment. We documented total mercury (THg) in seasonal woodland pool water, sediment, litter, and *Lithobates sylvaticus* LeConte (Wood Frog) in Acadia National Park, ME. THg concentrations in pool water varied over the study season, increasing during April–June and remaining high in 2 of 4 pools upon October refill. Water in pools surrounded by softwoods had lower pH, greater dissolved organic carbon, and greater THg concentrations than pools surrounded by hardwoods, with seasonal patterns in sediment THg but not litter THg. THg increased rapidly from near or below detection in 1–2 week old embryos (<0.2 ng; 0–0.49 ppb wet weight) to 17.1–54.2 ppb in tadpoles within 6 weeks; 7.2–42.0% of THg was methyl Hg in tadpoles near metamorphosis. Metamorphs emigrating from seasonal pools may transfer mercury into terrestrial food webs.

Introduction

Amphibians are among the most threatened vertebrates globally (Wake and Vredenburg 2008), and 7 of 10 amphibians breeding in seasonal woodland pools (also known as vernal or ephemeral pools) in the northeastern United States are of conservation concern (Mitchell et al. 2008). Seasonal pool-breeding amphibian populations in human-dominated landscapes suffer losses from degradation or destruction of breeding pools as well as fragmentation or loss of mature forests serving as summer refugia and hibernacula (Windmiller and Calhoun 2008). Chemical pollution, even in intact habitats, also can threaten the viability of amphibians in seasonal woodland pools, although this issue is not particularly well studied in the northeastern US (Boone and Pauli 2008). Amphibian egg and larval stages may be sensitive to environmental conditions, and exposure to pollutants during these stages can lead to developmental abnormalities, low hatchability,

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delayed metamorphosis, and reduced metamorph and adult fitness in some species (Birge et al. 1979, Bridges et al. 2004, Britson and Threlkeld 1998, Terhivuo et al. 1984). Mercury (Hg) bioaccumulation has been documented in stream-dwelling *Eurycea bislineata* Green (Two-lined Salamander) (Bank et al. 2005; Bergeron et al. 2010a, b) and more terrestrial species such as *Anaxyrus americanus* Holbrook (American Toad) and *Plethodon cinereus* Green (Red-backed Salamander) (Bergeron et al. 2010a, b). Similarly, Unrine et al. (2004, 2005) demonstrated in *Lithobates sphenoccephalus* Cope (Southern Leopard Frog) that exposure in mesocosms to a diet with Hg concentrations ranging 54–3298 ng/g dry weight (reflecting in situ atmospheric contamination of their aquatic habitat) has the potential to affect amphibian development. Thresholds of these contaminants leading to impaired development and population level effects of reduced survival and reproductive success are unknown.

The amount of Hg deposition is large in the northeastern US when dry deposition in forested systems is accounted for (Johnson et al. 2007, Miller et al. 2005, Nelson et al. 2007, Rea et al. 1996). Wetlands in general are hotspots for conversion of Hg to the more biologically toxic methyl mercury (MeHg). Per unit area, wetlands are estimated to contribute up to 80 times more MeHg to receiving water bodies than do upland areas (St. Louis et al. 1994). The optimal chemical environment in wetlands for microbiota responsible for Hg methylation includes abundant dissolved organic carbon (DOC), low pH, low acid neutralizing capacity (ANC), and drying-wetting cycles (Benoit et al. 2002, Grigal 2003). For example, MeHg concentrations have been documented at levels potentially toxic to anuran larvae in seasonal wetlands (Carolina bays) in the southeastern US (Unrine et al. 2005). Similarly, seasonal woodland pools in the northeastern US may be hotspots for Hg methylation. In particular, many pools in Acadia National Park (ANP), ME, are characterized by low pH and relatively high DOC (Gahl and Calhoun 2010). In addition, ANP is an apparent hotspot for Hg deposition and accumulation in the environment, including bioaccumulation across trophic levels (Bank et al. 2005, 2007a, 2007b; Kahl et al. 2007, Longcore et al. 2007), in part owing to the interception of contaminated air masses within a landscape of $\approx 25\%$ wetland area with a high prevalence of DOC-rich waters.

Seasonal woodland pools in ANP are the preferred breeding habitat for *Lithobates sylvaticus* LeConte (Wood Frog) (Cunningham et al. 2007, Kolozyvary 2003) and may be hotspots for MeHg production during key developmental stages of these animals. Wood Frogs undergo development from eggs to larvae during the spring and early summer, coincident with seasonal flushing of Hg from soils and litter with snowmelt into these small, relatively shallow wetlands (Nelson et al. 2008, Shanley et al. 2002). By mid- to late summer many pools dry, and only animals that have metamorphosed survive. This rapid morphogenesis occurs concurrently with dramatic changes in the drying pool environment, including rising water temperature, fluctuating pH, declining oxygen, and increasing solute concentration (Colburn 2004 and references therein). These physical and chemical conditions may make amphibians in sea-

sonal pools particularly susceptible to non-point source pollutants such as Hg (Unrine et al. 2004). These same pools refill with autumn rainfall (Calhoun and deMaynadier 2008, Colburn 2004) that could be enriched with Hg, or could result in Hg-enriched runoff from soils and litter.

Little is known about the presence and disposition of atmospherically deposited Hg in seasonal woodland pools in the Northeast or the relationship of Hg transformation with pool conditions and characteristics of the surrounding landscape. Conifers capture Hg more efficiently than deciduous species and also generally deliver more Hg in throughfall (Demers et al. 2007; Grigal et al. 2000; Johnson et al. 2007; Kolka et al. 1999; Rea et al. 1996, 2001), suggesting that pools in softwood dominated landscapes may receive more Hg in litterfall and total (wet + dry) deposition than pools embedded in hardwood forests. Mercury assimilation into the pool food web and its potential transport into surrounding terrestrial systems through Wood Frog emigration are undocumented. Such transport may be important given that amphibians can contribute significantly to terrestrial carbon reserves in southeastern (Gibbons et al. 2006, Unrine et al. 2007) and northern US seasonal wetlands (Berven 2009, Windmiller 1996). Negative implications for both metamorph survival (Unrine et al. 2004) and transport of Hg to the terrestrial environment may emerge as Wood Frog metamorphs with bioaccumulated Hg move from seasonal pools into the adjacent uplands.

Previous studies of Hg in ANP have documented occurrence of Hg in the environment (including relationships among forest type and deposition dynamics) and selected amphibian species in permanently flooded systems (e.g., streams, lakes) (Bank et al. 2005, 2007b). Our study targeted short-hydroperiod (e.g., inundated 3–9 weeks) amphibian breeding pools and examined relationships among pool chemical and physical characteristics (e.g., pool substrate type, size, hydroperiod, perimeter forest cover type, and burn history) and concentrations of total Hg (THg) in developing Wood Frogs in these pools. We hypothesized that:

- 1) Developing Wood Frogs in ANP's seasonal pools contain detectable concentrations of THg that they have bioaccumulated in the natal pool.
- 2) THg concentrations in Wood Frog embryos and larvae are greatest in pools surrounded by softwood forests (compared to hardwood forests) because of high DOC and low pH in pool water and high THg concentrations in sediment, litter, and pool water.
- 3) Elevated concentrations of THg in the water persist throughout Wood Frog larval development, increasing the probability that THg is delivered into the adjacent terrestrial habitat with emigrating juvenile Wood Frogs.

Methods

Study area

We selected four small (<0.10 ha), short-hydroperiod (i.e., pools likely to dry by mid-June; Table 1) seasonal woodland pools in ANP based on existing information about the Park's pool-breeding amphibian communities and hydrological

data (B. Connery, National Park Service, ANP, Bar Harbor, ME, pers. comm.; Cunningham et al. 2007; Gahl and Calhoun 2010; Kolozsvary 2003) and Hg litterfall, throughfall, and snow chemistry data (Johnson et al. 2007, Nelson et al. 2008, Sheehan et al. 2006). ANP covers approximately half (122 km²) of Mount Desert Island (MDI) and is at the southern limit of the spruce-fir/northern hardwoods zone (Westfeld et al. 1956) in the Fundy Coastal and Interior section of the Laurentian Mixed Forest (Bailey et al. 1994). Uplands are dominated by thin, granitic soils (Chapman 1970, Gilman et al. 1988), whereas organic soils are common in wetlands (Calhoun et al. 1994). Palustrine wetlands are concentrated in the eastern half of MDI, while ponds and lakes cover 4% of the island. A fire ignited in Bar Harbor in 1947 burned coniferous forests on the east side of the Park. Post-fire forests are dominated by deciduous species (*Betula* spp. [birch], *Acer* spp. [maple], *Populus* spp. [aspen]), while conifers (*Picea* spp. [spruce], *Tsuga canadensis* (L.) Carr [Eastern Hemlock], *Abies balsamea* (L.) P. Mill [Balsam Fir], *Pinus* spp. [pine]) dominate the unburned regions of the Park (Schauffler et al. 2007). Two of

Table 1. Characteristics of seasonal pools sampled during April–October 2008, Acadia National Park, ME.

Characteristic	B1	B2	U1	U2
Pool type	Upland depression	Upland depression	Upland depression	Forested wetland complex
Forest vegetation ^A	Red Maple, Gray Birch, Red Oak	Red Oak, Red Maple, Gray Birch, White Pine	White Spruce, White Pine, Tamarack, Gray Birch	Red Spruce, Black Spruce, Tamarack, White Pine, Red Maple
Landscape burn history	Burned	Burned	Unburned	Unburned
Within-pool litter composition ^B ; decay condition	Birch, maple, sedge spp. leaves; moderate	Oak leaves; minor	Spruce needles, gravel, sand; minor	Sphagnum, spruce needles, sedge spp.; peat (advanced decay)
Sediment % organic matter ^C	54.2 ± 2.67	53.8 ± 17.1	4.8 ± 1.2	81.3 ± 3.0
Pool pH range	5.56–5.93	4.41–4.93	5.57–6.11	4.03–4.33
Dissolved organic carbon (mg/L)	1.6–12.0	1.2–3.7	1.8–7.0	12.8–39.8
Acid neutralizing capacity (µeq/L)	112–254	1.29–2.61	57.6–130	117–42.1
Dissolved aluminum (µg/L)	40–165	114–157	13.9–157	207–806
Sulfate (µeq/L)	58–90	37–49	29–52	27–62
Chloride (µeq/L)	81–99	73–90	107–185	83–138
Calcium (mg/L)	4.08–6.75	0.26–0.31	1.11–1.97	0.22–0.51

^A*Quercus rubra* L. (Red Oak), *Acer rubrum* L. (Red Maple), *Betula populifolia* Marsh (Gray Birch), *Picea rubens* Sarg. (Red Spruce), *P. mariana* (P. Mill) B.S.P. (Black Spruce), *P. glauca* (Moench) Voss (White Spruce), *Pinus strobus* L. (White Pine), *Larix laricina* (Du Roi) K. Koch (Tamarack).

^B*Carex* spp. L. (sedge spp.), *Sphagnum* (sphagnum).

^C*n* = 3, mean ± SD; *n* = 2 for U2.

our study pools (B1, B2) occur in areas burned in 1947, and two study pools (U1, U2) were located in the unburned region, providing a contrast in forest cover type (deciduous vs. coniferous) and burn history.

Sample collection

The first sample collection from three pools (B1, U1, U2) was on 7 April 2008, whereas pool B2 was first sampled on 11 April. All pools dried by late June. Rainfall during 26 September–2 October refilled pools to water levels similar to those recorded in mid-April. Our final collection of environmental samples for THg and chemical analyses was on 2 October 2008, after the first pool-filling rain following mid-summer (June) drying.

We sampled pool water (within 10 cm of water surface), litter, and sediment (top 6 cm) soon after ice-out to establish initial chemical conditions. We collected one 500-mL pool-water sample in HDPE bottles for major ion and DOC analysis (after rinsing the collection bottle 3 times with pool water) and one 100-mL pool-water sample in a syringe for closed-cell pH determination. Water sampling was conducted following methods used in US Environmental Protection Agency (US EPA) long-term monitoring programs at ANP (Kahl et al. 2004). We collected 5 grab samples of wetland (water-saturated) litter and combined them into one sample to represent each pool's litter. Similarly, we collected upland litter within 3 m of the pool perimeter. We collected two sediment samples with a 5-cm-diameter pre-cleaned plastic tube pushed into the pool bottom and removed with the sediment plug retained intact. We extruded the sediment in 2-cm increments, retaining the water above the first 2 cm with that sample given the flocculent nature of the substrate surface. Two sediment samples from each collection were analyzed individually for THg and reported as a mean for that collection date and pool. Additional sediment samples ($n = 3$) were collected with these methods from each pool on 14 September 2010 for determination of ash-free dry mass in the top 2 cm. Samples to be analyzed for THg or MeHg were placed on dry ice immediately after collection and frozen to $-80\text{ }^{\circ}\text{C}$ within four hours until analyzed. Water samples for general chemical and THg analysis were stored in an ice-filled cooler and filtered, preserved, and refrigerated within 4 hours of collection.

Wood Frog embryos and larvae were collected from each pool on several dates. The animals were placed in individual plastic containers filled with pool water until returned to the lab, where they were photographed, evaluated for Gosner stage (GS; Gosner 1960) and abnormalities (with a stereomicroscope at 10X), euthanized with tricane methanesulfonate (MS222), and frozen. We collected one developing Wood Frog embryo from up to 8 separate egg masses (only one embryo removed from each egg mass) in each pool on each visit. We first collected embryos on 18 April in B1 ($n = 8$; GS 10–17), B2 ($n = 8$; GS 9), and U1 ($n = 8$; GS 7–13) and on 25 April in U2 ($n = 8$; GS 3–16), and we collected a second sample of embryos from U1 on 25 April ($n = 7$; GS 18–23). Our subsequent collections were timed to capture larvae in late developmental stages

but just before pools dried. Tadpoles were collected from U1 on 16 May ($n = 8$ tadpoles; GS 26), from U1 ($n = 8$; GS 24–33) and U2 ($n = 8$; GS 29) on 6 June, and from B1 ($n = 8$; GS 34–37) on 19 June; pool B2 dried before tadpoles could be collected. No juveniles were collected, because all pools dried before tadpoles completed metamorphosis.

We strictly adhered to clean Hg-collection protocols to prevent Hg contamination of field samples (see Nelson et al. 2008). Powder-free gloves were used when collecting all sample types, and sampling equipment and sample containers were teflon, glass, stainless steel, or plastic that had been tested for Hg prior to field use and transported to the field in clean plastic bags. All sample-collection equipment was acid washed between sampling trips, and sediment sample-collection equipment was rinsed with ultrapure water between samples. Samples were double-bagged in the field to minimize contamination.

Chemical analyses

Water chemistry analyses (THg, Ca, Mg, K, Na, Al, Cl, NO_3 , SO_4 , DOC, closed-cell pH, ANC, specific conductance) were conducted at the University of Maine Sawyer Environmental Chemistry Research Lab (UMSECRL), Orono, ME (methods detailed in Kahl et al. 2007 and Navratil et al. 2010). Analytical methods for determination of analytes in liquid samples were as follows: Ca, Mg, Na, K, and total Al were measured by inductively coupled plasma optical emission spectroscopy (ICP). Sulfate (SO_4), NO_3 , and Cl were measured by ion chromatography. ANC was determined by Gran titration, and closed-system pH was measured by collecting samples underwater with a syringe and injecting samples directly into an electrode cell (Hillman et al. 1986). Closed-system pH, which is not exposed to air with ambient CO_2 concentrations, reflects the in-situ pH experienced by biota within the waterbody. DOC was quantified with persulfate oxidation and infrared detection. Determination of ash-free dry mass followed ASTM D 2974 (2007).

Water samples were preserved with 1% v/v 0.2N bromine monochloride, and analyzed for THg by dual amalgamation, cold vapor atomic fluorescence spectrometry (EPA method 1631E) using a Tekran 2600 MDS in a clean room (US EPA 2002). The method reporting limit (MRL) was 0.5 ng/L, and the method detection limit (MDL) was 0.04 ng/L. Sediment, litter, and Wood Frog samples were stored frozen at -20°C , and THg in sediment, litter, and Wood Frogs (wet weight) was analyzed for THg by thermal decomposition, amalgamation, atomic absorption spectrometry (EPA method 7473) using a Milestone Direct Mercury Analyzer (DMA-80) with EPA method 7473 at the UMSECRL (US EPA 1994). The MRL was 1 ng absolute mass of Hg, and the MDL was 0.032 ng. The MRL and MDL for the DMA are given in mass rather than concentration in calibration, and concentration limits are determined by mass of sample analyzed. THg and MeHg were analyzed in a subset of tadpoles by Brooks Rand Lab (Brooks Rand Lab, Seattle, WA) using EPA Method 1631 Appendix (for THg) and EPA Method 1630 modification (for MeHg) (US EPA 2002). The MRL was 3.00 ng/g (MeHg) and 1.00 ng/g (THg), and the MDL was 1.00 ng/g (MeHg) and 0.40 ng/g (THg). All values are reported on a wet-weight basis, except for upland litter, which was air dried.

Data analyses

Our analyses focused on identifying spatio-temporal patterns among pools in water chemistry and THg concentrations in the litter, sediment, water, and developing Wood Frogs. We identified correlations between THg in water, upland litter, wetland litter, wetland sediment, water chemistry analytes, and Wood Frog THg with Spearman rank-order correlations. We compared sediment, water, upland and wetland litter, water chemistry analytes, and Wood Frog THg among pools with Kruskal-Wallis tests, a non-parametric analysis analogous to a one-way analysis of variance. We used an $\alpha \leq 0.05$ to determine significance in these analyses. Statistical analyses were conducted in R version 2.11.1 (The R Foundation for Statistical Computing).

Results

Both spatial and temporal differences occurred in pool water chemistry. Pool-water DOC concentrations differed (Kruskal-Wallis Chi Square = 10.0425, $df = 3$, $P = 0.0182$) among pools, gradually increased during April–October in all pools (with slight decrease in B1 in October), and consistently were greatest in U2 (Fig. 1). The pH of pool water differed (Kruskal-Wallis

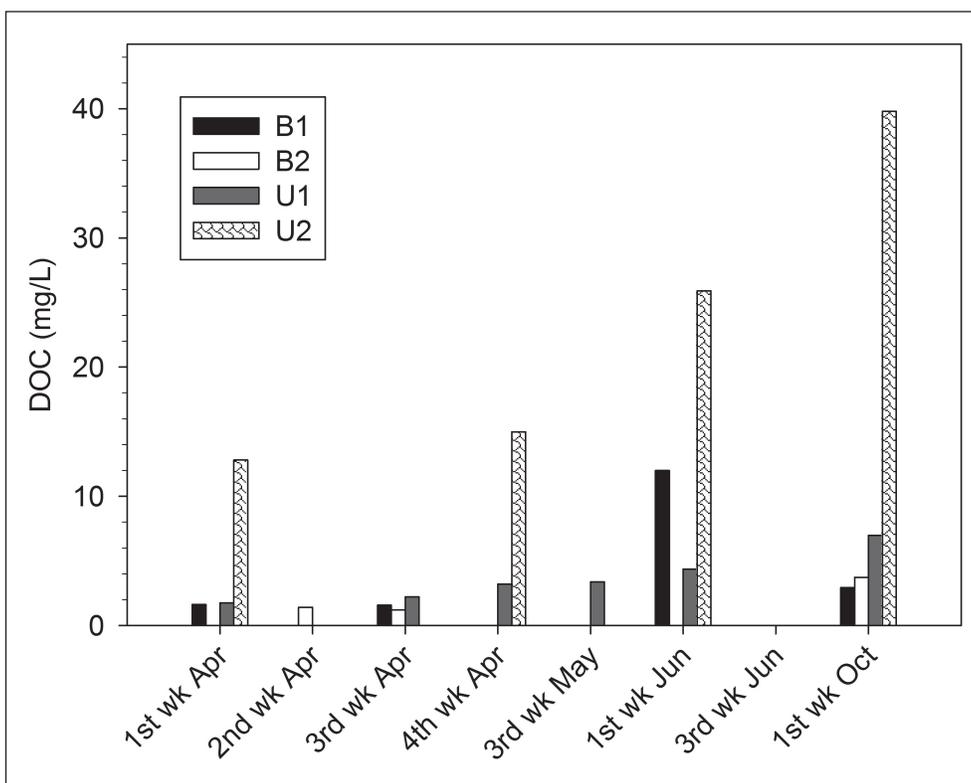


Figure 1. Dissolved organic carbon (DOC; mg/L) measured in water samples collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July. Each bar represents 1 sample.

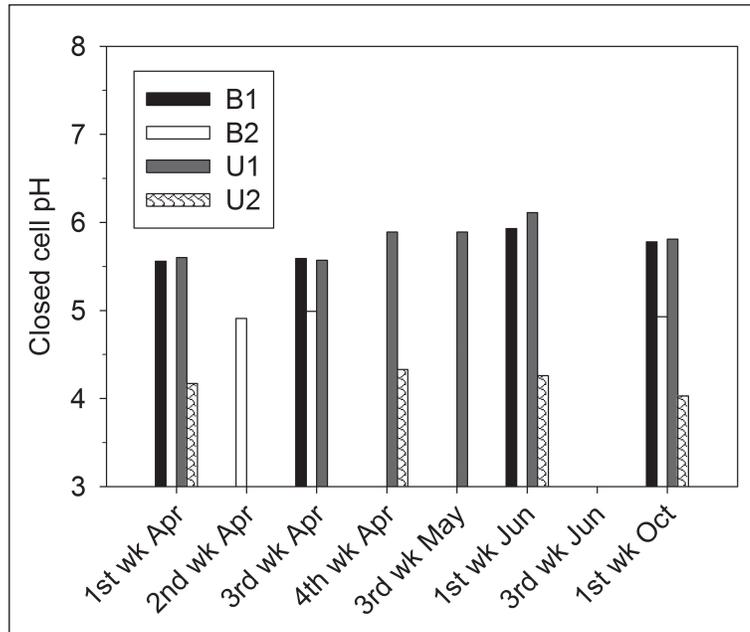


Figure 2. Closed-cell pH measured in water samples collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July. Each bar represents 1 sample.

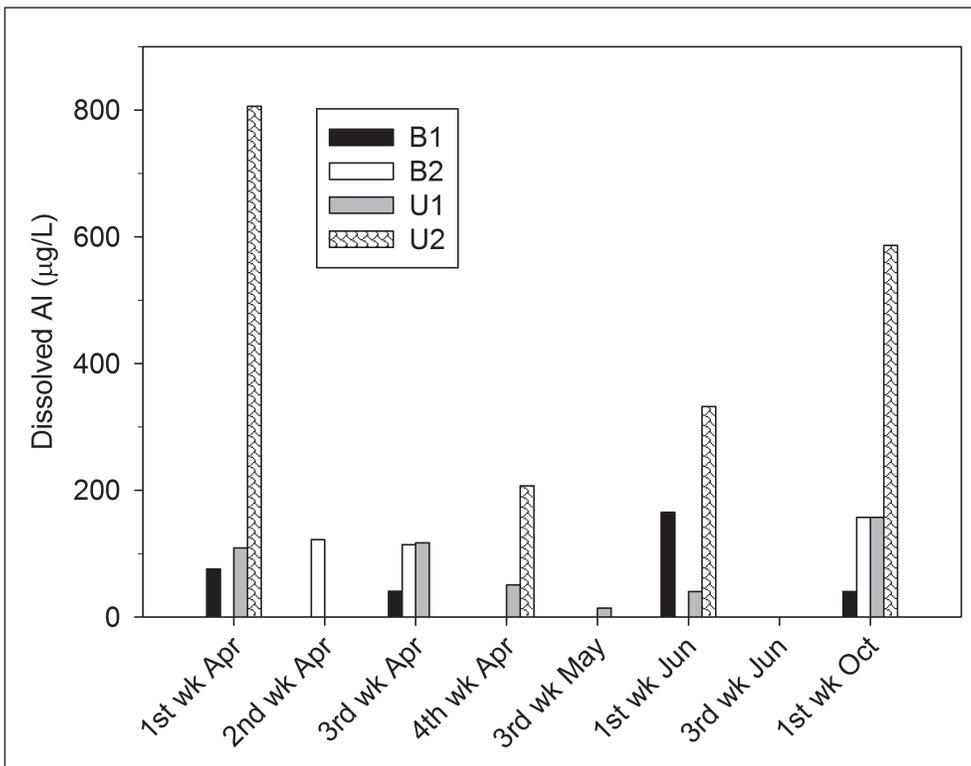


Figure 3. Dissolved aluminum ($\mu\text{g/L}$) measured in water samples collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July. Each bar represents 1 sample.

Chi Square = 12.7673, $df = 3$, $P = 0.0052$) among pools and consistently was lowest in U2 (Fig. 2). Dissolved aluminum (Al) concentrations differed (Kruskal-Wallis Chi Square = 9.932, $df = 3$, $P = 0.0192$) among pools and were greater in U2 than the other pools throughout the sample period (Fig. 3), with the greatest concentration (806 $\mu\text{g/L}$) measured in water collected from U2 while it was mostly ice-covered (7 April). The least dissolved Al concentration (207 $\mu\text{g/L}$) in U2 was measured during the first ice-free collection date (25 April). Pool-water DOC and dissolved aluminum concentration were correlated (Spearman rank-order correlation $r_{\text{adj}} = 0.62$, $P = 0.0081$, $n = 17$). Concentrations of THg in pool water differed among pools (Kruskal-Wallis Chi Square = 9.6384, $df = 3$, $P = 0.0219$), increased through the season (Fig. 4), were correlated with pool-water DOC (Spearman rank-order correlation $r_{\text{adj}} = 0.86$, $P = 0.0001$, $n = 17$), and were greater in pools in the unburned, softwood-dominated setting (U1, U2) than in the burned, deciduous setting (B1, B2). Concentrations of THg in water collected from U2 in October (17.4 ng/L) exceeded all records of THg measured in Acadia's streams (maxima for previous studies were 6.5–8.0 ng/L ; Nelson et al. 2007, Peckenham et al. 2007).

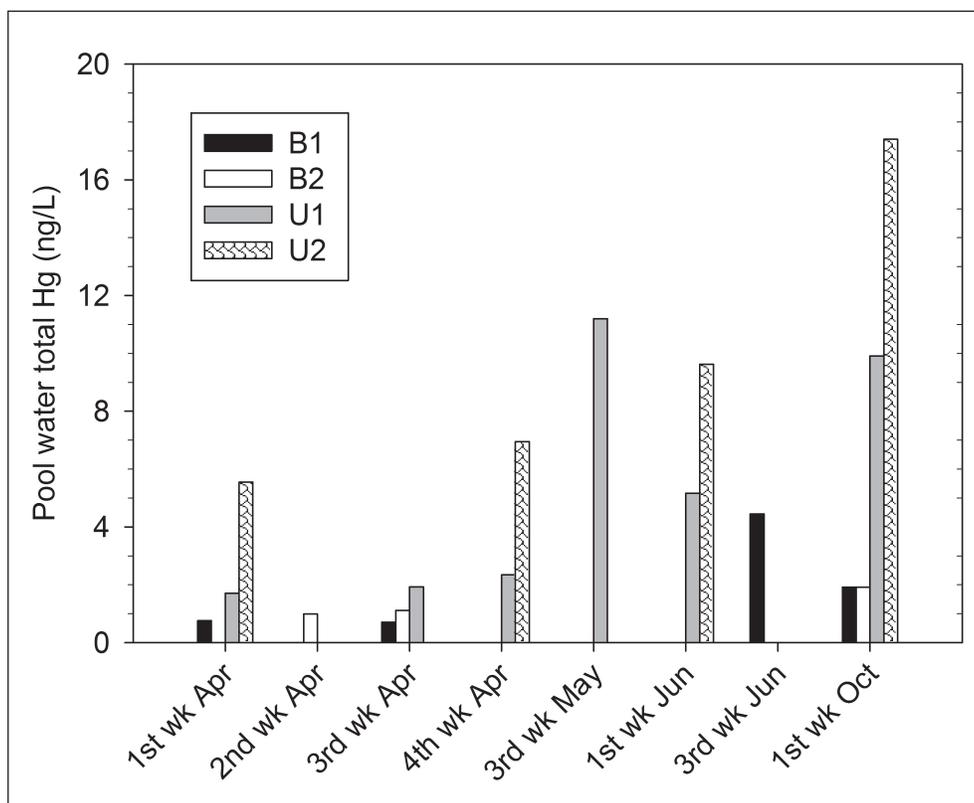


Figure 4. Total Hg (ng/L) measured in water samples collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July. Each bar represents 1 sample.

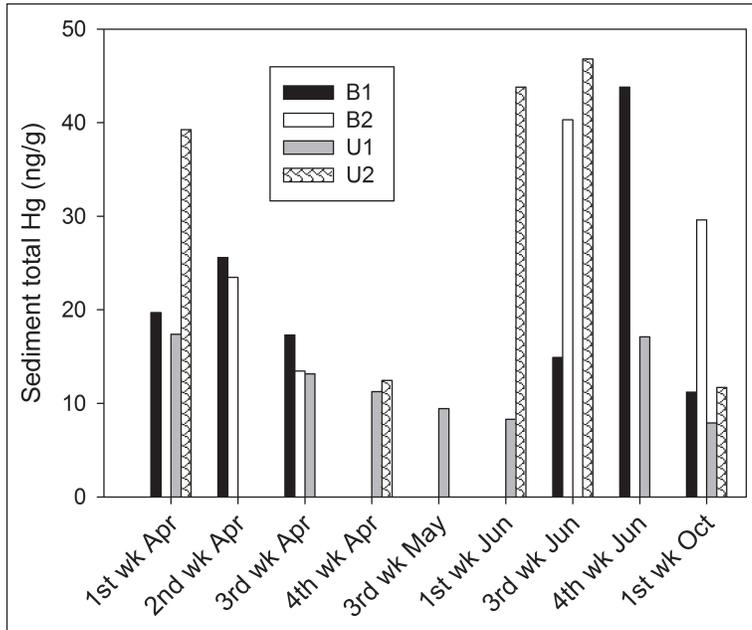


Figure 5. Total Hg (ng/g, wet weight) measured in 0–2 cm deep sediment samples collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. Each bar represents mean of 2 samples. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July.

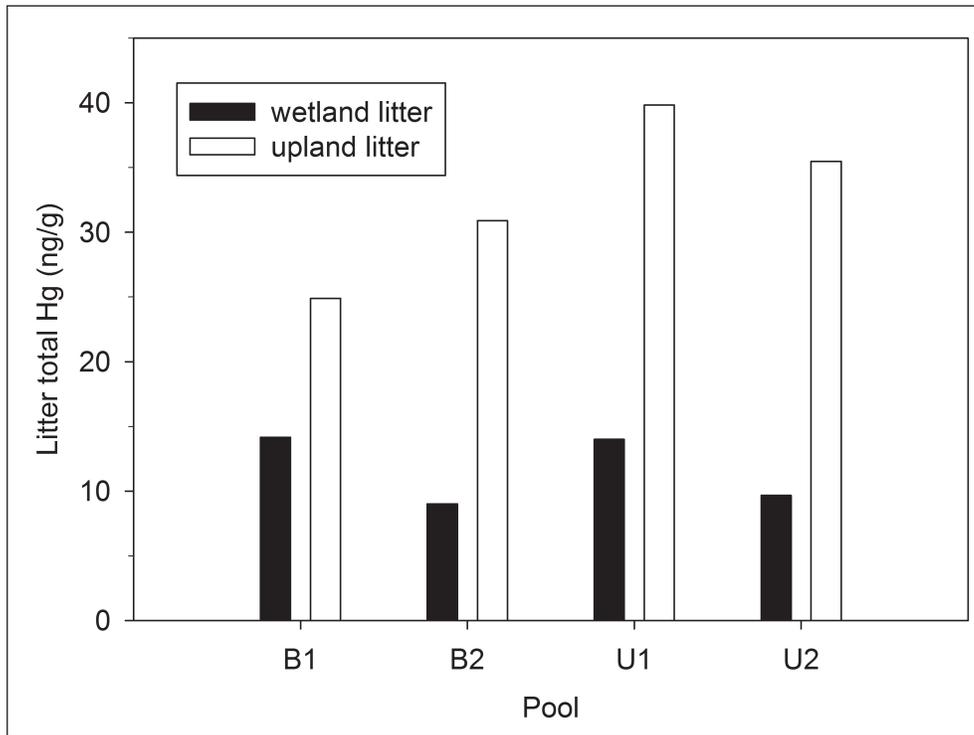


Figure 6. Total Hg (ng/g) measured in litter samples collected within seasonal pools and 3 m from the pool edge during April–October 2008 in Acadia National Park, ME. Litter subsamples were extracted from composited grab samples; coniferous and deciduous litter were analyzed separately and are reported as means of combined subsamples collected within the pool (wet weight) or upland (dry weight) across the study period.

Sediment THg concentration (mean for each pool and collection date) ranged from 7.9 to 46.8 ng/g among sites, although differences among sites were not significant, and these concentrations tended to be greatest late in June (Fig. 5). We detected no apparent associations between sediment THg (Fig. 5), landscape burn history, or pool setting in the landscape; however, patterns in sediment THg reflected sediment percent organic matter (Table 1). THg concentrations in wetland litter were not significantly different among pools (Fig. 6); however, THg in U1 and U2 upland litter was 25% greater than THg in upland litter from B1 and B2. THg concentrations in wetland litter were roughly half those in upland litter, a difference potentially reflecting our analysis approach (wet-analyzed wetland litter versus dry-analyzed upland litter) rather than true differences between THg concentrations in wetland and upland litter.

THg measured in Wood Frog embryos ($n = 15$) in GS 3–21 were below detection limits (<0.2 ng; 0 – 0.49 ppb; all concentrations in Wood Frogs reported as ppb wet weight); however, concentrations rapidly increased to 15.2 – 54.2 ppb in tadpoles ($n = 25$) within 2–4 weeks post-hatch (GS 24–36) (Fig. 7). THg concentrations in Wood Frogs were correlated with THg in pool water (Spearman rank-order correlation $r_{\text{adj}} = 0.74$, $P = 0.0366$, $n = 8$). Pool B2 dried before embryos hatched, and the remaining 3 pools dried before tadpoles metamorphosed. Final THg concentrations in Wood Frog tadpoles collected

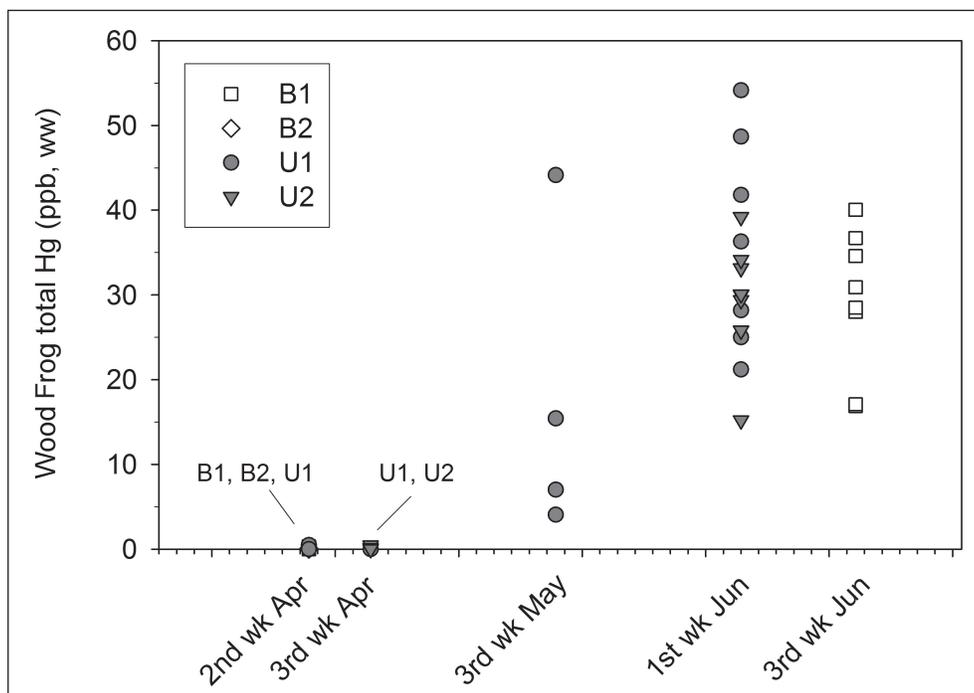


Figure 7. Total Hg (ng/g, wet weight) measured in Wood Frog embryos and tadpoles collected during April–October 2008 in seasonal woodland pools in Acadia National Park, ME. Symbols represent individual embryos or larvae. B2 dried by the first week in June, U1 and U2 dried by the third week in June, and B1 dried by early July.

when pools were nearly dry were similar among pools (Fig. 7), and ratio of MeHg to THg concentrations at pool drying ranged from 7–42% in pools surrounded by softwoods (U1,U2; 4 tadpoles) and 36% and 37% in pools in hardwood settings (B1, B2; 2 tadpoles).

Discussion

Wood Frog development

Our findings support our hypotheses that (a) developing Wood Frogs in ANP's seasonal pools bioaccumulate detectable concentrations of THg in the natal pool, and (b) elevated concentrations of THg in the water would persist throughout Wood Frog larval development, increasing the probability that THg is delivered into the adjacent terrestrial habitat with emigrating juvenile Wood Frogs. Wood Frogs oviposited in all study pools; however, only 3 of 4 pools retained water through embryonic development. Wood Frog tadpoles in those 3 remaining study ponds were within 12 weeks of completing metamorphosis when the pools dried. Although there is some support for our hypothesis that THg concentrations in Wood Frog larvae would track patterns of THg concentrations in the pool water that reflect the forest composition in the surrounding landscape, this result should be considered cautiously, owing to the small number of pools sampled in our study. Our study pools are in landscapes with different burn histories and forest compositions, and we were not able to apply a study design that replicated these conditions in addition to that of the gradient of long to short hydroperiod. We also repeatedly sampled the same pools to capture Hg temporal dynamics, creating pseudoreplication in our dataset. These factors are caveats in interpretation of our results (i.e., there is limited separation of variables describing pool type), yet our study suggests compelling patterns worth additional study.

Concentrations of THg in Wood Frogs were at or below detection limits (<0.02 ng wet wt, which translated to <0.5 ppb) from egg laying to final embryo collections (GS 21), indicating that maternal transfer is absent or minimal in the study area. This finding contrasts with that of Bergeron et al. (2010a), who reported maternal transfer of Hg in American Toads breeding in Virginia ponds contaminated with Hg. In our Wood Frog tadpoles, however, concentrations of THg were similar to those in adults and tadpoles of other frog species. In our study, THg had accumulated to 44.1 ppb in weeks-old Wood Frog tadpoles collected from U2 by the third week in May (GS 26), and THg ranged from 28.2–54.2 ppb across our seasonal pools by the first week in June (GS 24–36). These Hg concentrations overlap ranges of concentrations reported in 2–3 year old *L. clamitans* Latreille in Sonnini de Manoncourt and Latreille (Green Frog) (30–110 ppb) and *L. catesbeiana* Shaw (American Bullfrog) (42–75 ppb) tadpoles collected from permanently flooded ponds in nearby watersheds in ANP (Bank et al. 2005).

We analyzed MeHg and THg in only a subset ($n = 6$) of our collected Wood Frog larvae. MeHg comprised 6.6–42.0% of THg in Wood Frog tadpoles collected

when pools were nearly dry in our study and 7.6–40.0% of THg in Green Frog and Bullfrog tadpoles collected in permanent water bodies in nearby watersheds (Bank et al. 2007b). We did not detect any malformations or indications that the developing Wood Frog tadpoles were physically compromised by the Hg they had accumulated in their tissues, and we do not know if these Hg body burdens compromise Wood Frog fitness upon metamorphosis.

Wood Frog tadpoles are opportunistic predators and will consume aquatic invertebrates as well as embryos and larvae of sympatric amphibian species during this period of rapid growth within seasonal pools (Baldwin and Calhoun 2002, Petranka et al. 1994, Sours et al. 2007). Although pool water remained clear through June, and algal accumulation appeared minimal, bacterial and fungal biofilms growing on sediments and leaves may have contained Hg and been grazed by the Wood Frog tadpoles (Unrine et al. 2005, 2007), which may, in addition to THg in pool water, explain the concentrations of THg detected in their tissues.

Total mercury concentrations in the pool environment

Hg concentrations at any location are affected by landscape characteristics and atmospheric conditions spanning local to regional scales. Because of their small size and forested character, seasonal woodland pools may receive elevated inputs of dry deposition initially captured by the forest canopy compared with, for example, lakes with large surface areas without forest canopy. In addition to throughfall from the forest canopy, Hg is delivered directly into terrestrial systems through overland flow, precipitation, and litterfall (Grigal 2002). The relationships we report between THg concentrations and environmental conditions at our study sites may reflect the variety of conditions within the study pools and the surrounding landscapes in which they are embedded. Owing to our small sample size, we cannot be certain that observed differences in THg concentrations among pools are determined primarily by landscape-scale patterns or local conditions such as observed in wetland ecosystems in Nova Scotia, Canada (Rencz et al. 2003) and in southeastern US Carolina bays (Unrine et al. 2005).

Conifers capture Hg more efficiently than deciduous species and also generally deliver more Hg in throughfall (Demers et al. 2007, Johnson et al. 2007), and as expected we found that pools embedded in softwood-dominated (coniferous) landscapes (U1, U2) contained greater concentrations of THg in pool water than pools embedded in hardwood landscapes (B1, B2). The reduced Hg concentrations in B1 and B2 sediments also may reflect reemission and mobilization of Hg in the 1947 fire that burned the watersheds of these pools (Amirbahman et al. 2004). Mean THg concentration was 7.18 ± 1.57 ng/L (mean \pm SD; $n = 10$) in water from pools embedded in the softwood-dominated landscape (U1, U2) and 1.69 ± 1.31 ng/L ($n = 7$) at pools embedded in the hardwood-dominated landscape (B1, B2). This result agrees with previous studies that estimated Hg deposition of throughfall at softwood forested sites

(resembling U1 and U2) in ANP as 34.3 ± 22.2 ng/m²/day, whereas, deposition of Hg in hardwood sites (near B1 and B2) that burned in 1947 was about 18% less (28.1 ± 19.5 ng/m²/day) (Johnson et al. 2007).

Litterfall

Litterfall is a major pathway for distributing Hg to the forest floor and throughout watersheds (Grigal 2002). Hg accumulated on deciduous leaves during the growing season is deposited in greater mass in less time than from conifer leaves, especially during autumn dehiscence when Hg concentrations in leaves are at their maximum (Grigal et al. 2000, Lindberg 1996, Rea et al. 1996). Seasonal woodland pools embedded in a forested landscape capture this litterfall annually, often coincident with autumn storms that refill these wetlands. We expected THg in coniferous litter to be greater than in deciduous litter, as previously reported in ANP by Sheehan et al. (2006). We found this expected forest or landscape composition-related difference in Hg concentrations of upland litter but not of wetland litter. THg concentrations in upland litter were slightly greater in the pools (U1, U2) in the conifer-dominated, unburned area of ANP than in upland litter from pools (B1, B2) in the burned area. THg concentrations in deciduous and coniferous litter collected within our study pools, however, generally were similar among pools (Fig. 6). THg estimates in our wetland litter samples are wet weights, whereas THg estimates in our upland samples are dry weights, restricting our comparisons to those among pools (rather than between upland and wetland litter), and reflecting the wetland litter-associated THg to which a tadpole would be exposed.

Sediment

Contrary to our expectation, pool sediment THg concentrations did not reflect watershed burn history. We expected that THg concentrations in sediments collected from unburned sites U1 and U2 would exceed those collected in burned sites B1 and B2 owing to release of Hg with burning in the contributing watersheds of B1 and B2, similar to observed effects of the 1947 fire on ANP soils (Amirbahman et al. 2004). Instead, the lowest THg concentrations were found in U1 sediments, which were a sand-gravel mixture. It may be that the differences we observed in sediment THg among pools is owing to within-pool sediment type and percent organic matter. Sediments collected from the other pools (U2, B1, B2) contained less sand and gravel and more organic matter, were predominantly decaying leaves, and consistently contained greater THg concentrations than U1 (Fig. 5, Table 1).

Prior to refill, pool sediments likely incorporate Hg from decomposing litter, as well as from Hg translocated to the litter from the underlying soil (Demers et al. 2007). Concentrations of THg are greatest in the upper 1–2 cm of upland soil (Schluter et al. 1995) and in water in contact with upper soil horizons, where concentrations of DOC also are greatest (Fleck 1999, Grigal 2002, Shanley et al. 2005). Episodic release of Hg in high-flow events is

correlated with releases of particulate organic carbon from soils, particularly the O-horizon, which contains most of the soil Hg burden (Grigal 2002, Hurley et al. 1998, Shanley et al. 2005).

We observed an increase in sediment THg in all pools during the June drawdown, then a decrease in sediment THg in all pools upon refill in October (Fig. 5). When inundated, topographic depressions with seasonally wet soils such as seasonal woodland pools could provide conditions leading to significant Hg reduction and methylation owing to increased sulfate concentrations and activity of sulfate-reducing bacteria (Grigal 2003). Our observed decrease in sediment THg at pool re-fill in October may reflect dilution of Hg in pool sediments by storm run-off. Although pool B2 sediment THg concentration decreased upon refill in October, THg concentration in this pool remained greater than that at April ice-out (Fig. 5); this pool had the earliest dry date of the sampled pools. Future research should include speciation of sediment Hg and focus on dynamics of sulfate-reducing bacteria, largely responsible for Hg methylation, in pool sediments as pools fill and dry.

Temporal dynamics of pool chemical environments

We expected peak THg concentrations in pool water with spring snowmelt; however, we found maximum THg concentrations occurred during May, June, or October (Fig. 4). We attribute this pattern to Hg mobilized from the pool sediments with drying and rewetting and with throughfall and litterfall inputs occurring over several weeks or months of summer dry deposition. Terrestrial systems distribute atmospherically deposited Hg into embedded aquatic systems such as woodland pools via throughfall and runoff (Krabbenhoft et al. 1995, Lee et al. 1994, Lorey and Driscoll 1999) that carries Hg associated with dissolved and particulate organic material (Grigal 2003, Rencz et al. 2003, Schuster et al. 2008). During spring snowmelt, Hg carried with dissolved and particulate organic matter from accumulated litter and the soil organic layer is re-suspended in meltwater infiltrating the upper soil horizons with thawing (Hurley et al. 1998, Nelson et al. 2008, Shanley et al. 2002). As the snowpack melts from below, the meltwater combines with Hg released from the melting soil frost layer, resulting in a peak release of Hg to streams and other water bodies immediately preceding peak snowmelt discharge (Schuster et al. 2008) that often occurs with early spring rain. Scherbatskoy et al. (1998) reported that nearly half the annual Hg flux in a Vermont stream occurred in a single day of peak snowmelt. Both THg (Fig. 4) and DOC (Fig. 1) concentrations measured in our study's pool water generally increased during April–June, suggesting concentration of solutes with dry-down. Autumn storms may mobilize Hg into these pools; concentrations of DOC and THg were high for all but pool B1 in the early October, pool-refilling storm.

Our data reflect the expected trends in chemical covariates with Hg: high concentrations of DOC, low pH, and low ANC correlated with elevated THg concentrations in biota, including lake fish (Chen et al. 2005, Driscoll et al. 2007).

We found that pools with high DOC and low pH had greater THg in water, greater dissolved Al in water (Palmer et al. 2005), and greater THg concentrations in Wood Frogs. Greatest total dissolved Al concentrations were measured in U2 in April (806 $\mu\text{g/L}$, $\text{pH} = 4.17$), exceeding the LC_{50} (750 $\mu\text{g/L}$ at $\text{pH} = 4.8$; Sparling et al. 1997) for Wood Frogs. LC_{50} values may not be predictive of Al toxicity, which is affected by water chemistry.

Implications for future research

The spatial variation of Hg concentrations among pools and within-pool characteristics suggest that local conditions are important in determining THg accumulation (Grigal 2002, 2003; Rencz et al. 2003, Unrine et al. 2005). Although pool water demonstrated predicted patterns of THg (greater in softwood-embedded sites with high DOC and low pH), THg measured in other components (sediment, litter, Wood Frogs) did not exhibit this pattern. Pools selected for future study should include replicates of the variety of local pool conditions, such as sediment type, forest species dominance, and hydroperiod range including pools that dry before metamorphosis, those that hold water through metamorphosis in years with average precipitation, and pools that dry only occasionally, to reveal the role of these conditions in determining THg and MeHg dynamics throughout the pool drawdown and refilling cycle. The proportion of accumulated THg that is MeHg in Wood Frogs near metamorphosis in our study pools was in the range of that reported by Bank et al. (2005) for 2–3 year old Green Frog and Bullfrog tadpoles that had not yet metamorphosed. It is unknown when the Green Frog and Bullfrog tadpoles accumulated the Hg in their tissues; however, the THg and MeHg detected in our Wood Frog tadpoles accumulated during the 6–8 weeks between egg-laying and metamorphosis, indicating accumulation was quite rapid. Future research should quantify the ratio of THg to MeHg in developing embryos, tadpoles, and juveniles emigrating from natal ponds to better understand transport of this contaminant from seasonal pools into the surrounding environment and potential for uptake into the terrestrial food web.

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