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Bioaccumulation of methylmercury in wood frogs and spotted salamanders in Vermont vernal pools

Steven D. Faccio[1] · Kate L. Buckman[2] · John D. Lloyd[1] · Amanda N. Curtis[2,3] · Vivien F. Taylor[4]

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Abstract

Mercury (Hg) has accumulated in forested landscapes in the Northeastern U.S., and hotspots with enhanced deposition have been identified throughout the region. Due to a variety of favorable landscape characteristics, including relatively high dissolved organic carbon (DOC), fluctuating water levels, and low pH and dissolved oxygen, vernal pools provide ideal conditions for the conversion of Hg to its more toxic and bioavailable form, methylmercury (MeHg). Yet little is known about the concentrations, speciation, and bioavailability of Hg in vernal pools, or its bioaccumulation in vernal pool fauna and potential export into terrestrial systems. We investigated the role of forest cover type on the bioaccumulation of MeHg in wood frog (*Lithobates sylvatica*) and spotted salamander (*Ambystoma maculatum*) eggs, larvae, and adults, and investigated relationships among MeHg and water chemistry (pH, DOC). Water samples from pools located in coniferous stands had greater concentrations of THg and MeHg compared to deciduous pool water, and showed significant positive correlation to DOC ($r = 0.683$, $P < 0.001$) and correlated negatively with pH ($r = -0.613$, $P < 0.001$). Methylmercury levels in amphibian embryos were similar between the two species (*L. sylvatica* mean = 5.4 ng/g dw; *A. maculatum* mean = 3.5 ng/g dw). Concentrations of MeHg increased substantially in larvae, and were significantly greater in *A. maculatum* (mean = 237.6 ng/g ± 18.5 SE) than *L. sylvatica* larvae (62.5 ng/g ± 5.7 SE). Forest cover type did not explain variation in MeHg concentration among amphibian embryos or larvae. Methylmercury levels in adult tissue samples were significantly greater in *A. maculatum* (mean = 79.9 ng/g ± 8.9 SE) compared to *L. sylvatica* (mean = 47.7 ng/g ± 9.7 SE). This research demonstrates that vernal pools are important hotspots where amphibians bioaccumulate MeHg, which may then be transferred to terrestrial ecosystems. The abundance of amphibian larvae suggests they could be important bioindicators for monitoring MeHg loading and bioavailability.

Keywords Vernal pools · Methylmercury · Amphibians · *Lithobates sylvatica* · *Ambystoma maculatum*

Introduction

Vernal pools are temporary to semi-permanent, isolated wetlands occurring in shallow depressions that typically fill during the spring and/or fall and dry during summer or in drought years (Calhoun and deMaynadier 2009). In the Northeastern and North-central United States and adjacent Canada, vernal pools are relatively widespread and abundant in forested landscapes (Colburn 2004; Van Meter et al. 2008; Faccio et al. 2016), and provide critical breeding habitat for amphibians, such as wood frogs (*Lithobates sylvatica*) and *Ambystomid* salamanders (Semlitsch and Skelly 2009), as well as numerous invertebrate taxa adapted to temporary waters (Colburn et al. 2009). Globally, amphibians are among the most imperiled vertebrate groups, due to widespread population declines and species extinctions (Wake and Vredenburg 2008; Adams et al. 2013). In the Northeastern United States, 70% of vernal pool-breeding amphibians are considered moderate- to high-priority species of regional conservation concern (NEPARC 2010), underscoring the importance of these

✉ Steven D. Faccio
sfaccio@vtecostudies.org

[1] Vermont Center for Ecostudies, Norwich, VT, USA

[2] Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

[3] Program in Ecology, Evolution and Conservation Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[4] Department of Earth Science, Dartmouth College, Hanover, NH, USA

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keystone ecosystems to maintaining viable populations of at-risk species.

Vernal pools may also be hotspots for accumulation of mercury (Hg), and its more bioavailable and toxic form, methylmercury (MeHg), which can bioaccumulate through food webs reaching levels that cause reproductive and neurological effects in top predators (Scheuhammer et al. 2007). Mercury contamination via atmospheric deposition, originating primarily from coal-fired power plants and industrial incinerators, is widespread in the Northeast (Miller et al. 2005), and hotspots with enhanced deposition and biological uptake have been identified throughout the region (Evers et al. 2007). These hotspots are often associated with forested areas with an abundance of wetlands, which facilitate the conversion of Hg to MeHg by anaerobic bacteria (Benoit et al. 2003; Driscoll et al. 2007).

Landscape plays a critical role in Hg accumulation, methylation and mobilization in forest floors. A variety of landscape characteristics common to vernal pools are associated with enhancing Hg deposition. First, vernal pools are typically found embedded in a forest matrix where leaves in the canopy scavenge Hg from the atmosphere and subsequently transfer it to forest soil in throughfall and litterfall (Zheng et al. 2016). Second, Hg transport from the forest floor is greatest along shallow hydrologic flowpaths (Grigal 2002; Galloway and Branfireun 2004), such as surface waters that typically fill most vernal pools. Lastly, hydrologic and biogeochemical properties of vernal pools provide ideal conditions for the sulfate-reducing bacteria responsible for Hg methylation (Wiener et al. 2003). These conditions include water level fluctuations and periodic wetting, low dissolved oxygen, high dissolved organic carbon (DOC), and low pH, all of which increase methylation efficiency (Benoit et al. 2003). This suggests that vernal pools are potential hotspots for MeHg production and bioaccumulation in fauna, particularly among higher trophic level taxa such as amphibian larvae, which may then transport Hg to terrestrial systems via metamorphosis and dispersal to the surrounding forest. The wide abundance of amphibian larvae across vernal pools in the Northeast may also make them valuable bioindicators for monitoring MeHg loading, bioavailability, and ecological impacts in these sensitive environments (Evers et al. 2016).

Few studies have investigated Hg in vernal pools (Brooks et al. 2012; Loftin et al. 2012, Benoit et al. 2013, Davis 2013) and only Brooks et al. (2012) and Loftin et al. (2012) evaluated Hg concentrations in amphibians, with both studies sampling developing wood frogs. We found no studies reporting on Hg concentrations in adult wood frogs or vernal pool-breeding *Ambystomid* salamanders at any life stage. In Maine vernal pools, total Hg (THg) concentrations in wood frog larvae were correlated with THg in pool water, which increased during April to June when amphibian embryos and larvae were developing (Loftin et al. 2012). In New York and Vermont pools, Davis (2013) found that THg, MeHg, and the percent Hg present as MeHg (% MeHg) in pool water also increased during the spring, reaching 43 to 58%. Methylation efficiencies, estimated as % MeHg in water, exceeding 10% have been linked to elevated levels of MeHg in biota (Krabbenhoft et al. 1999), suggesting that amphibian metamorphs may export significant MeHg into surrounding terrestrial systems, especially given the significant amount of biomass that emerging amphibians contribute into forested uplands surrounding vernal pools (Windmiller 1996; Berven 2009). In addition, water from pools in coniferous forests were found to have higher THg than those surrounded by hardwoods (Loftin et al. 2012), which is consistent with higher throughfall and Hg capture by coniferous trees (Risch et al. 2012).

As part of a broader study to evaluate the role of landscape characteristics and land-use on the production and transfer of MeHg in Vermont vernal pools, this study focused on the bioaccumulation of MeHg in wood frog and spotted salamander (*Ambystoma maculatum*) eggs, larvae, and adults, and investigated relationships among MeHg and Hg, water chemistry (pH, DOC), and forest cover type surrounding pools. We hypothesized that MeHg in amphibian larvae would increase as they developed, and that predatory salamander larvae, which feed at a higher trophic level, would have higher concentrations of MeHg than omnivorous wood frog larvae due to biomagnification. We also hypothesized that water in pools surrounded by conifers would have higher Hg concentrations compared to those in deciduous stands, and that this would correlate with amphibian Hg tissue concentration. And finally, because MeHg bioaccumulates and is not easily eliminated from the body (Bergeron et al. 2010a), we hypothesized that longer-lived spotted salamander adults would have higher MeHg concentrations compared to shorter-lived wood frog adults.

Methods

Study sites

For this study, six vernal pools (three located in coniferous forests and three in deciduous forests) were selected from the Vermont Vernal Pool Mapping Project database (Faccio et al. 2013). Dominant forest cover type (%) was characterized within 100 m of each pool using leaf-off color infrared aerial photographs, while species composition of canopy trees was determined in the field (Table 1). All pools were located in east-central Vermont (N43°53′, N43°42′; W72°27′, W72°16′), with four pools located in southeastern Orange Co. and two in northeastern Windsor Co.

Table 1 Forest cover type, dominant canopy species, elevation, distance to nearest road or building, and maximum pool basin morphology at six vernal pools sampled in Vermont in 2015

Vernal pool	Forest cover type[a] (percent)	Dominant canopy Spp.[b]	Elevation (m)	Distance to nearest road or building (m)	Maximum		
					Depth (cm)	Perimeter (m)	Area (m^2)
KWN467	Deciduous (95%)	Sugar maple, American beech, yellow birch	457	290	120	114	606
SDF509	Deciduous (85%)	Sugar maple, yellow birch, white ash, eastern hemlock	479	1040	60	48	97
SDF791	Deciduous (90%)	Sugar maple, Am. beech, y. birch, eastern white pine	543	140	100	138	452
NEW110	Coniferous (85%)	Eastern hemlock, sugar maple, American beech	308	310	18	73	249
SDF516	Coniferous (80%)	Eastern hemlock, red spruce, yellow birch	430	185	45	73	287
SDF951	Coniferous (90%)	Red pine, sugar maple, white ash	448	145	65	70	337
Deciduous Mean (SE)			493.0 (25.8)	490.0 (278.4)	93.3 (17.6)	100.2 (26.9)	385.2 (150.7)
Coniferous Mean (SE)			395.3 (44.0)	213.3 (49.7)	42.7 (13.6)	71.6 (1.1)	291.3 (25.5)
t-test statistic			$t = 1.92$ $P = 0.15$	$t = 0.98$ $P = 0.43$	$t = 2.27$ $P = 0.08$	$t = 1.04$ $P = 0.40$	$t = 0.62$ $P = 0.60$

[a]Within 100 m of pool

[b]Sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), eastern hemlock (*Tsuga canadensis*), white ash (*Fraxinus americana*), red spruce (*Picea rubens*), red pine (*Pinus resinosa*), eastern white pine (*Pinus strobus*)

(Fig. 1). The largely forested, rural landscape is characterized by moderate hills with numerous wetlands and vernal pools. The forest in this region is actively logged second-growth dominated by mixed northern hardwoods of maple (*Acer* spp.), American beech (*Fagus grandifolia*), birch (*Betula* spp.), ash (*Fraxinus* spp.), eastern hemlock (*Tsuga canadensis*), and pine (*Pinus* spp.). All pools were located in interior forests ≥140 m from the nearest road or building.

Water sampling

Water sampling was initiated at all pools immediately following ice-out in April 2015, when water levels were at or near peak. Sampling continued monthly through July until pools were dry (all pools were dry by August), and then were sampled once after re-wetting in November 2015. Water samples were collected in 2L PTFE vials (Sarstedt), one per pool for MeHg/Hg analysis, with one duplicate taken every fifth sample. Samples were kept cool and in the dark until return to the laboratory (<6 h). An additional water sample at each pool was collected for total suspended solids (TSS) and ancillary water chemistry analyses, which included DOC and TSS. Temperature, pH (YSI datasonde), and oxidation reduction potential (Thermo Fisher portable ORP) were measured at the site using handheld probes. Water was collected from just below the surface, at least 1–2 m from the pool edge, while probe measurements were averaged across three locations within the pool. Surface water samples for Hg analysis were collected using clean Hg sampling techniques, and were filtered to 0.2 µm, preserved in 0.4% hydrochloric acid, and stored in the dark prior to analysis. Total suspended solids were filtered onto pre-weighed QFFs, noting volume of water filtered, and the filter subsequently dried in a 60 °C oven and reweighed to calculate TSS weight. An additional sample for DOC was filtered and collected into amber vials.

Amphibian sampling

Following spring immigration to pools for breeding, adult wood frogs and spotted salamanders were captured (n = 4/pool/spp.) between 20 April and 12 May 2015 using dip nets or funnel traps that were partially submerged 16–24 h prior. Salamanders (n = 8) at four pools were found by turning over logs around pool edge. After each individual was measured (snout-to-vent length, and total length), weighed to the nearest 0.1 g, and sexed (based on external characteristics), a tissue and blood sample was collected for MeHg and Hg analysis. Tissue samples from wood frogs consisted of a ca. 4–7 mm toe-clip, distal to the webbing, of the 4th (longest) toe from a single hind foot. Frog digits

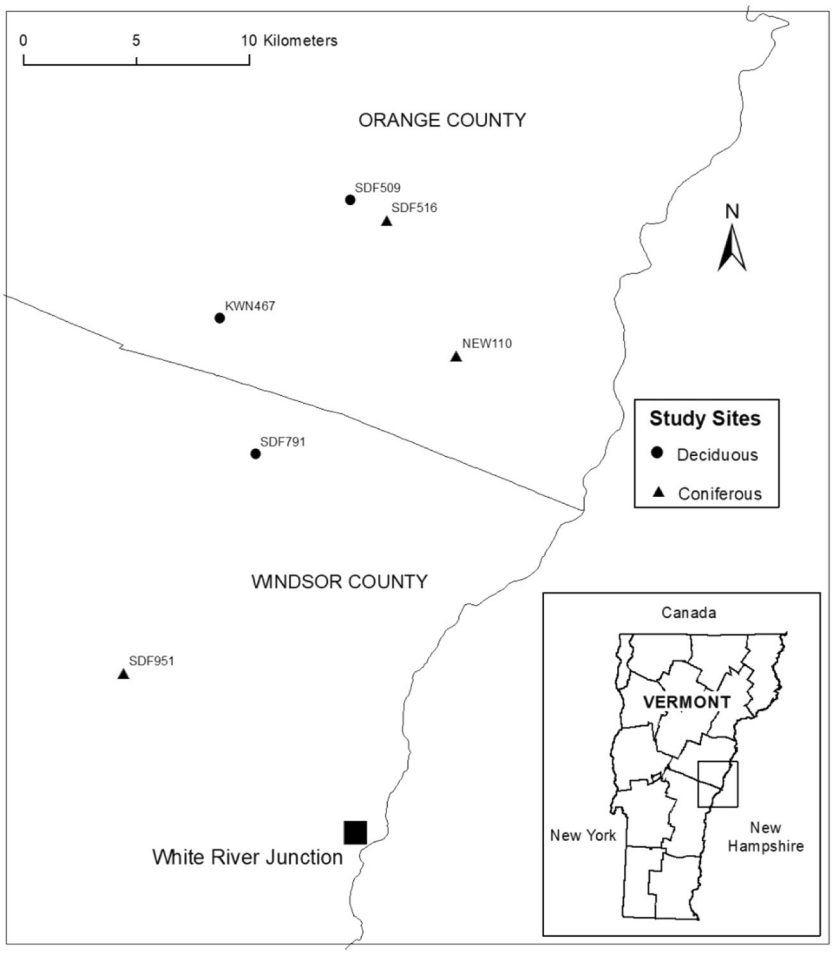

Fig. 1 Location of six vernal pool study sites by forest cover type in Orange and Windsor Counties, Vermont

were anesthetized prior to amputation using topical lidocaine, and the wound was treated with antibiotic cream afterward. From each frog, a 30–60 µl blood sample was collected in a 75 µl heparinized capillary tube by puncturing the facial vein with a 30 gauge needle, following the methods outlined in Forzan et al. (2012). Capillary tubes were sealed on both ends with Critocaps®. Tissue samples were collected from adult spotted salamanders by amputating ca. 1–2 cm of tail tip using either a scalpel or surgical scissors. Blood samples (<50 µl) were collected from tail wounds using heparinized capillary tubes. Salamander tails were anesthetized prior to amputation using topical lidocaine, and the wound was treated with antibiotic cream afterward. All tissue and blood sampling was conducted in the field, and animals were held for a short period (ca. 15–80 min) to ensure complete recovery before being released back to point of capture.

Wood frog and spotted salamander embryos were collected for Hg analysis between 9 and 12 May 2015. Wood frog egg samples were only collected at three pools (n = 4/pool; 12 samples total), while salamander samples were collected at all six pools (n = 4/pool; 24 samples total). A sample consisted of 10–15 embryos collected from a single egg mass; for a total of 40–60 embryos/species/pool. Samples were kept cool until return to the laboratory (<6 h) then frozen at −20 °C until analysis.

Wood frog and spotted salamander larvae were collected for Hg analysis between 12 May and 8 July, 2015. Using dip nets, larvae were collected during two sampling periods; the first (12 May to 6 June) was ca. 1-week post-hatching, the second (6–8 July) was ca. 5–8 weeks post-hatching. Larvae were placed in ziplock bags filled with pool water and returned to the lab where they were immediately frozen at −20 °C. Prior to Hg analysis, larvae were examined under a dissecting scope to inspect for developmental abnormalities and establish Gosner stage (Gosner 1960). Samples were then rinsed with ultra pure water, placed in trace metal clean glass vials, weighed, and freeze dried in preparation for Hg speciation analysis. Processing and analysis of egg and larval samples occurred during August-September 2015. At each pool, four early- (Gosner stage 22 to 25) and late-stage (Gosner stage 26–39) wood frog larvae were collected for a total of 48 samples. A total of 27 spotted salamander larvae were collected at five pools,

including early-stage larvae (n = 12) at three pools, and late-stage larvae (n = 15) at five pools.

We followed clean-Hg sampling protocols to prevent contamination of field samples. Sterile gloves were used when collecting field samples, and all samples were placed in sterile ziplock bags or plastic containers that had been acid-washed and transported to field sites in clean plastic bags. We also followed guidelines for decontamination of boots, nets, and other field equipment to minimize the risk of spreading Chytridiomycosis and Ranavirus between pools (NEPARC 2014).

Chemical analyses

Total Hg and MeHg measurements were conducted at the Dartmouth Trace Element Analysis Core Facility. Blood samples were transferred from capillary tubes to acid-washed, pre-weighed 2 ml centrifuge tubes. Samples were spiked with appropriate amounts of enriched isotope standards ($Me^{201}Hg$ and ^{199}Hg), then diluted with 0.5 ml double-distilled 2 M nitric acid (Fisher, Trace Element Grade), and heated to 90 °C on a heating block for 2 h (Rahman et al. 2014). Toe and tail clips, embryos and larvae were freeze dried and weighed into glass vials (IChem, Certified 300 series). Samples were spiked with enriched isotope standards, and diluted with 1–3 ml 4 M HNO_3, then heated to 60 °C overnight. Aliquots (0.1–0.5 ml) of blood and tissue extracts were transferred to 40 ml brown glass vials, and diluted with ultrapure water (>18 MΩ cm^{-1}; produced by PurelabPlus water purifier, US Filter, MA, USA). To each vial, 0.3 ml citrate buffer was added, and then samples were neutralized to pH 4.5 with potassium hydroxide. Finally, 40 µl ethylating reagent was added and vials were filled to the brim with water. Samples were analyzed for MeHg and inorganic Hg by species-specific isotope dilution gas chromatography–inductively coupled plasma mass spectrometry (GC-ICP-MS), using an automated MERX-M (Brooks Rand Instruments, Seattle, USA) interfaced with an Element 2 ICP-MS (Thermo, Bremen, GE) (Taylor et al. 2008, 2011). Note that THg and MeHg concentrations in embryos, larvae, and adult tissue samples are reported as dry weight (dw), while adult blood samples are reported as wet weight (ww). Aqueous MeHg and inorganic Hg standard recoveries for blood analyses were 113 ± 5 and 106 ± 6% (n = 2), respectively. Recoveries relative to certified reference values for NIST 2976 Mussel (National Institute of Standards and Technology; Gaithersburg, MD) were 110 ± 1% for MeHg (certified 28 ng/g; n = 4) and 113 ± 3% for THg (61 ng/g; n = 4).

Water samples for MeHg analysis were weighed into 40 ml brown glass vials, and spiked with $Me^{201}Hg$. Samples were buffered and pH adjusted, then ethylated, as described above. Water samples were analyzed by direct ethylation GC-ICP-MS (Jackson et al. 2009). Samples for THg analysis were weighed into 40 ml clear glass septa-lid vials (Brooks Rand Instruments), and digested chemically with bromine monochloride overnight. Samples were then neutralized with hydroxylamine hydrochloride and Hg was reduced to Hg^0 with stannous chloride. Water samples were analyzed by cold vapor atomic fluorescence spectrometry, in a method similar to EPA Method 1631 (EPA 2002), using a Merx-T automated system (Brooks Rand Instruments). The relative percent difference for duplicates samples was 7 ± 5% (n = 5) for MeHg, and 12 ± 6% (n = 5) for THg analyses. Recoveries of aqueous standards were 106 ± 2% (n = 3) and 101 ± 7% (n = 3) for MeHg and THg, respectively.

Dissolved organic carbon, on filtered and unfiltered samples, were analyzed by an Apollo 9000 Teledyne Tekmar with a Non-Dispersive Infra-Red detector. Duplicates were in good agreement (%RPD = 7 ± 5%), and spike recoveries were 96 ± 2%).

Statistical analyses

To examine differences in pool basin morphology and water chemistry metrics by forest cover type, and to examine differences in THg, MeHg, and % MeHg concentrations between species within life stages, we used t-tests, or where data failed to normalize, non-parametric Mann-Whitney tests. Pearson correlation coefficients were used to assess relationships between MeHg concentrations in adult amphibian blood and tissue samples, as well as between MeHg and water chemistry (DOC and pH). Significant differences were assigned for $P \leq 0.05$.

We used generalized linear mixed models to examine causes of variation in MeHg concentration in eggs, larvae, and adult tissue samples. We ran separate analyses for eggs and larvae, and for adults because we believed that the causes of variation likely differed between the juvenile and adult life stages. In particular, we expected that MeHg in eggs and larvae might reflect conditions in the breeding pool, especially levels of MeHg in the water, whereas we had no reason to expect a correlation between MeHg in adults and MeHg levels in the water because of the short duration of time that adults spend in breeding pools. Because sample sizes for adult blood (n = 35) were considerably smaller than for tissue (n = 49), we used tissue mercury as the measure for adult variation.

For both analyses, we treated the pool (n = 6) as a random effect to account for the nested nature of our sampling, in which MeHg levels were estimated from multiple individuals from the same pool. For eggs and larvae, we considered a suite of models constructed from four variables chosen *a priori* that we believed might reasonably influence levels of methylmercury in the amphibians: species, under

the expectation that dietary differences among larval wood frogs (primarily herbivorous) and larval spotted salamanders (predatory) might contribute to differences in methylmercury accumulation; forest type around the pool, because pools surrounded by coniferous forest may have higher levels of MeHg and differences in water chemistry (DOC, pH); life stage (egg, early-stage larvae, or late-stage larvae) because later stages of development have both more time to accumulate MeHg and perhaps more exposure to MeHg through consumption of prey items; and finally levels of MeHg in the water, as this is presumably a primary source of MeHg. MeHg concentration in the water was the average of collections up to and including the time point of animal collection. From this set of variables, we constructed a candidate set of models that included all combinations of the variables. In addition, we included three models with an interaction between species and life stage, allowing for the possibility that wood frogs and spotted salamanders might accumulate MeHg at different rates owing to differences in diet. This resulted in a total of 18 unique models: all subsets of the global model including species, forest type, life stage, and water MeHg, plus the three models from this group that included main effects of life stage and species with an added interaction term between species and life stage.

For adults, we used the same approach to construct a candidate set of models but included only two variables: species and forest cover type. We did not consider MeHg in the water of the vernal pool as a predictor of MeHg in adults because we assumed that adults had a short duration of exposure to the pool and that it was thus unlikely that MeHg in adults could be linked plausibly to MeHg in the water. This left us with three possible models: the combined effect of species and forest type plus two models that considered each variable in isolation.

We fit each model using the lmer function in R package lme4 (Bates et al. 2015). We then used the R package AICcmodavg (Mazerolle 2017) to rank each model based on Akaike's Information Criteria (AIC). We chose to use AIC as the ranking variable, rather than the small-sample correction (AIC_c), because of the uncertainty in determining the effective sample size for mixed-effects models (Bolker et al. 2009). However, when we ranked models according to AIC_c, setting the effective sample size (using the nobs argument in AICcmodavg) to either the number of pools ($n = 6$) or the total number of observations, the results were qualitatively similar. We assessed the importance of each predictor variable based on the model-averaged regression coefficient and its unconditional 95% confidence interval. When averaging the coefficients, we used only the subset of models in which each term appeared because the inclusion of models with an interaction term precludes the use of shrinkage methods, which include all models in the averaging procedure (Mazerolle 2017). We note that disagreement exists among statisticians regarding the validity of generating inference from model-averaged coefficients (Bolker et al. 2009; Cade 2015); however, our findings were the same whether relying on model-averaged coefficients or model-averaged predictions as the basis of inference about MeHg levels in the amphibians. In the primary critique of using model-averaged coefficients as the basis of inference, Cade (2015: 2381) notes that "model averaging the predicted responses…can be used to indirectly explore model relationships". As such, we believe that the concordance between these two approaches suggests our findings are robust to any putative shortcoming of model-averaged regression coefficients. We assessed goodness-of-fit using R^2 values estimated by the r.squaredGLMM function in the R package MuMIn (Bartoń 2018). We used residual plots as described by Pinheiro and Bates (2000) to examine the validity of model assumptions.

Results

There were no differences in mean pool size (area, perimeter), maximum water depth, or pool elevation between forest cover types (Table 1). However, several water chemistry metrics differed significantly between pools in deciduous and coniferous stands: pools in coniferous stands had lower pH, greater DOC, and lower conductivity (Table 2). Additionally, coniferous pool water had greater concentrations of THg and MeHg compared to deciduous pool water (Table 2). Concentrations of MeHg in coniferous pool water showed significant positive correlations to DOC ($r = 0.683$, $P < 0.001$), and correlated negatively with pH ($r = -0.613$, $P < 0.001$). Temporally, during the inundation period (April through July), increases in water MeHg coincided with increases in temperature and decreases in dissolved oxygen (Fig. 2).

Mercury in amphibians

Eggs and larvae of wood frog and spotted salamander exhibited different MeHg concentrations (Table 3; Fig. 3b), such that models without the interaction term between species and life stage received no support in the model-selection process (Table 4). The global model (which included species, habitat, life stage, and MeHg in the water column) provided a good fit to the data ($R^2 = 0.92$). Although each of the four predictor variables appeared among the top-ranked models, model-averaged regression coefficients for both habitat ($b_{\text{habitat}} = 0.16$, 95% CI = −0.04 to 0.35) and water MeHg ($b_{\text{water MeHg}} = -0.04$, 95% CI = −0.21 to 0.13) were not distinguishable from zero.

Methylmercury concentrations in eggs and larvae were best explained by the effect of species, life stage, and the

Table 2 Mean (SE) vernal pool water chemistry and mercury (THg—total mercury, MeHg—methylmercury) concentrations by pool and forest cover type at six vernal pools sampled in Vermont, April-July and Nov., 2015

Vernal pool	Forest cover type[a] (percent)	Mean (SE)							
		pH	DOC (mg/L)	DO (mg/L)	Conductivity (µS/cm)	Temperature (°C)	THg (ng/L)	MeHg (ng/L)	%MeHg (%)
KWN467	Deciduous (95%)	6.05 (0.16)	23.33 (8.03)	4.87 (1.30)	54.43 (8.87)	9.82 (3.38)	3.36 (0.74)	0.43 (0.12)	12.39 (2.70)
SDF509	Deciduous (85%)	6.39 (0.18)	6.93 (1.00)	5.69 (1.01)	25.55 (4.71)	10.66 (1.68)	2.55 (0.48)	0.42 (0.13)	13.57 (3.41)
SDF791	Deciduous (90%)	6.31 (0.37)	10.19 (1.48)	3.35 (1.08)	30.68 (5.27)	11.37 (2.37)	3.76 (1.09)	0.98 (0.27)	27.35 (3.76)
NEW110	Coniferous (85%)	5.57 (0.16)	18.21 (3.45)	2.73 (0.88)	13.18(3.49)	10.60 (3.37)	6.63 (1.10)	1.63 (0.15)	26.37 (3.05)
SDF516	Coniferous (80%)	4.74 (0.11)	18.79 (2.61)	4.72 (0.67)	11.28 (3.43)	9.83 (1.82)	5.15 (0.72)	1.28 (0.27)	24.83 (6.15)
SDF951	Coniferous (90%)	6.00 (0.17)	15.15 (3.79)	3.58 (0.71)	26.36 (5.95)	14.53 (1.85)	5.33 (0.76)	0.56 (0.11)	10.81 (2.34)
Deciduous mean		**6.21 (0.14)**	**11.90 (2.50)**	4.93 (0.78)	**37.13 (4.53)**	10.66 (1.24)	**3.05 (0.40)**	**0.56 (0.11)**	16.72 (2.57)
Coniferous mean		**5.16 (0.15)**	**17.46 (1.96)**	4.01 (0.54)	**17.53 (3.36)**	11.02 (1.31)	**5.56 (0.49)**	**1.19 (0.17)**	21.71 (2.71)
t-test statistic		$t = 5.21$ $P < 0.001$	$t = 1.75$ $P = 0.044$	$t = 0.97$ $P = 0.338$	$t = 3.47$ $P = 0.002$	$t = 0.17$ $P = 0.862$	$t = 3.99$ $P < 0.001$	$t = 3.17$ $P = 0.003$	$t = 1.33$ $P = 0.190$

Results in boldface are significantly different at $P \leq 0.05$

[a]Within 100 m of pool

interaction between these two variables (Fig. 4). Levels of both THg and MeHg in eggs of both species were low but increased substantially among both early- and late-stage larvae. Early salamander larvae had significantly greater THg concentrations compared to early wood frog tadpoles ($t = 8.37$, $P < 0.001$), but THg increased three-fold in late-stage wood frog tadpoles, while it dropped slightly in older salamander larvae resulting in no significant difference between species for late-stage larvae THg concentration (Fig. 3a). Spotted salamander larvae had significantly greater concentrations of MeHg at both early- ($U = 85.6$, $P < 0.001$) and late-stages ($t = 4.7$, $P < 0.001$) than wood frogs (Fig. 3b). The proportion of total mercury that was comprised of methylmercury (e.g. %MeHg; MeHg/THg) was significantly higher among spotted salamander eggs ($t = 2.27$, $P = 0.03$), early larvae ($t = 12.84$, $P < 0.001$), and late larvae ($t = 19.56$, $P < 0.001$) compared to wood frogs (Fig. 3c). Although habitat did not explain variation in MeHg loads among samples of embryos or larvae, spotted salamander larvae and embryos of both species averaged higher MeHg in deciduous pools (Table 3), and this difference might become more pronounced with larger sample sizes.

Methylmercury concentrations in adult blood and tissue samples showed moderate correlation among spotted salamanders ($r = 0.541$, $P = 0.106$) and wood frogs ($r = 0.399$, $P = 0.060$), although our sample size for spotted salamander blood was quite small ($n = 10$) (Fig. 5). Among both species, %MeHg was significantly greater in blood samples than in tissue (wood frog, $t = 8.98$, $P < 0.001$; spotted salamander, $t = 5.24$, $P < 0.001$), while there was no difference in % MeHg in blood or tissue between species (Fig. 3c).

Predicted MeHg concentration in adult tissue from both species was substantially lower than predicted levels among late-stage larvae of both species (wood frog adults and late-stage larvae, respectively: 35.8 ng/g [95% CI = 27.6–46.7] versus 83.0 ng/g [66.4–103.8]; spotted salamander adults and late-stage larvae, respectively: 70.8 ng/g [54.1–92.8] versus 195.4 ng/g [151.5–252.0]). Variation in MeHg concentration among adult tissue was explained by species, with wood frogs having substantially lower concentration than spotted salamanders ($b_{\text{Wood Frog}} = -0.66$, 95% CI = −0.99 to −0.34) (Fig. 6). Habitat (forest type) had no discernible effect on MeHg levels in adult salamanders or frogs ($b_{\text{habitat}} = -0.02$, 95% CI = −0.21 to 0.17). Most of the observed variance in MeHg loads among adults remained unexplained by the global model ($R^2 = 0.27$) (Table 5).

Discussion

Our results demonstrate that vernal pools provide highly suitable conditions for the conversion of Hg to MeHg and

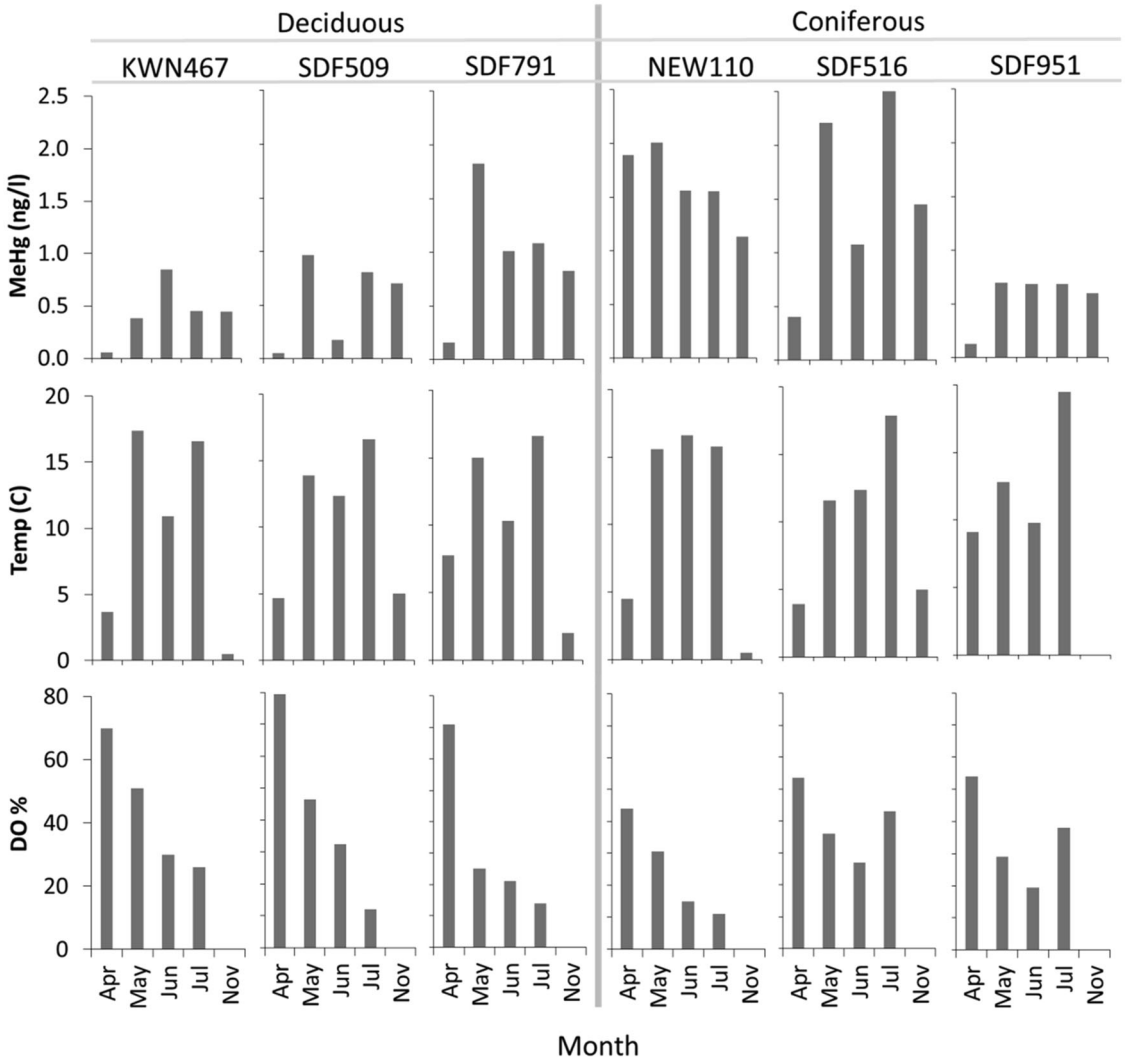

Fig. 2 Mean dissolved MeHg, temperature, and percent dissolved oxygen (DO) in pool water by month (April to July and November) and forest cover type for six vernal pools in Vermont, 2015. Changes in MeHg coincided with increases in water temperature and decreases in dissolved oxygen as pools dried. Percent DO was not sampled in November

bioaccumulation in amphibian larvae, which may then be exported to terrestrial systems via predation and metamorphosis. These findings support those of others (Brooks et al. 2012; Loftin et al. 2012; Benoit et al. 2013; Davis 2013), yet are the first to quantify Hg and MeHg concentrations in spotted salamander eggs, larvae, and adults, and in adult wood frogs.

Methylmercury in amphibian eggs and larvae

Methylmercury concentrations in amphibian embryos were relatively low compared to larval and adult burdens (Table 3). If converted to an estimated wet weight concentration (assuming 95% water content), MeHg concentrations in eggs were in the same order of magnitude as MeHg concentrations in the pool water. This suggests that maternal transfer of Hg during oviposition was absent or low, consistent with results from Loftin et al. (2012) for wood frogs in Maine. However, Bergeron et al. (2010b) reported that American toads (*Anaxyrus americanus*) transferred approximately 5% of their Hg burdens to their eggs, and that concentrations of THg and MeHg in eggs were positively correlated with concentrations in both maternal blood and whole-body, indicating that Hg depuration to eggs is related to Hg levels of the female.

Between embryo and larval stages, mean concentrations of MeHg increased by a factor of 11.5 for wood frogs and 67.9 for spotted salamanders, emphasizing the role of feeding on MeHg accumulation. Water column MeHg also increased across pools as temperature and anoxic conditions increased between embryo stages (early May) and larval stages (May–July), although conditions fluctuated over time and were not predictive of egg or larval tissue concentrations, likely due to the rapid temporal fluctuations.

Table 3 Mean (SE) methylmercury (MeHg) (ng/g dw) concentrations in amphibians by species, life stage, pool, and forest cover type at six vernal pools sampled in Vermont in 2015

		Mean MeHg (ng/g) (SE)								
	Forest cover type[a] (percent cover)	Spotted Salamander					Wood frog			
Vernal pool		Embryos	Early larvae	Late larvae	Adult tissue	Adult blood[b]	Embryos	Early larvae	Late larvae	Adult tissue	Adult blood[b]
KWN467	Deciduous (95%)	4.62 (1.84)	348.24 (15.51)	276.54 (10.64)	97.57 (9.70)	104.96 (42.96)	6.25 (1.03)	30.18 (1.28)	40.96 (3.76)	24.32 (5.26)	75.90 (28.19)
SDF509	Deciduous (85%)	3.51 (0.53)	nd	320.64 (31.84)	64.11 (17.09)	23.19 (10.67)	nd	32.83 (0.88)	79.15 (3.73)	24.41 (1.97)	28.89 (2.87)
SDF791	Deciduous (90%)	4.19 (0.76)	293.94 (8.27)	119.75 (13.03)	113.75 (32.90)	57.34 (na)	6.25 (1.03)	61.33 (4.68)	56.62 (9.44)	64.13 (26.76)	57.19 (5.27)
NEW110	Coniferous (85%)	2.98 (0.38)	nd[c]	nd	79.22 (27.70)	70.88 (20.04)	6.35 (2.09)	15.35 (2.49)	96.21 (8.31)	75.26 (32.67)	48.12 (7.41)
SDF516	Coniferous (80%)	2.21 (0.38)	nd	108.11 (13.49)	44.46 (4.90)	63.72 (na[d])	3.46 (1.52)	51.59 (4.01)	146.36 (18.19)	75.25 (41.53)	57.30 (0.65)
SDF951	Coniferous (90%)	3.64 (0.52)	153.89 (1.41)	148.11 (na[d])	88.78 (15.08)	54.65 (17.88)	nd	29.79 (1.87)	109.32 (3.94)	28.73 (2.73)	30.85 (9.70)
Deciduous mean	4.11 (0.64)	265.36 (26.45)	243.35 (28.17)	89.02 (13.80)	62.73 (23.04)		6.25 (1.03)	41.45 (4.50)	58.91 (5.74)	36.60 (9.26)	50.08 (8.57)
Coniferous mean	2.94 (0.29)	153.89 (1.41)	121.44 (15.44)	70.82 (11.20)	48.78 (11.15)		4.90 (1.32)	32.25 (4.75)	117.30 (8.88)	59.74 (17.27)	44.34 (5.29)

Note that adult blood concentrations are wet wt.

[a]Within 100 m of pool

[b]Wet weight

[c]nd—no data, no specimens collected

[d]na—not applicable, single data point, no SE

Although we found significantly higher levels of MeHg in water samples from pools located in coniferous stands, forest cover type did not explain variation in MeHg concentration among samples of amphibian embryos or larvae. This may be due to our small sample sizes, or the complexities of MeHg bioavailability, which can be affected by differences in water chemistry, such as DOC concentration and quality (Herrero Ortega et al. 2018), hydrology, physical attributes, landscape characteristics, and land-use around individual pools (Benoit et al. 2003; Wiener et al. 2003). In addition to water chemistry, other factors can affect the accumulation of MeHg in amphibian larvae, including diet and feeding niche (Eisler 2006) and whether or not there was maternal transfer of Hg to eggs (Bergeron et al. 2011). Higher levels of MeHg in salamander larvae are consistent with their predatory feeding habits relative to wood frogs. Among wood frog larvae, mean concentrations of MeHg in this study were twice as high as those reported for larvae from Massachusetts pools, and 0.5 times higher than Minnesota pools at a similar life stage (Brooks et al. 2012). THg concentrations in wood frog larvae reported from pools at Acadia National Park, Maine ranged from 15.2–54.2 ng/g ww (Loftin et al. 2012), which, assuming a moisture content of 80%, would equal 76–271 ng/g dw, similar to results in our study (range = 25.4–454.9 ng/g dw). Wood frog and spotted salamanders showed the opposite pattern of MeHg bioaccumulation between early- and late-stage larvae. Wood frog showed the expected pattern of increasing MeHg over time, while early-stage spotted salamander larvae had higher concentrations of MeHg compared to late-stage larvae (Figs. 3b and 4). This could be due to the relatively small sample size for spotted salamander larvae (n = 27) compared to wood frog (n = 48), faster growth rates of the salamander larvae causing dilution of tissue concentrations of MeHg, a shift in diet of late-stage salamander larvae, or to physiological changes as larvae approach metamorphosis.

Spotted salamander eggs and larvae had significantly higher percent MeHg compared to wood frogs of the same life stage (Fig. 3c). Although eggs of both species were collected on the same date at each pool, we do not know the date of oviposition, nor did we attempt to age embryos. Therefore, it is possible that salamander eggs were in the water longer and had more time over which to accumulate MeHg. It is also possible that maternal transfer of Hg to eggs is greater from female salamanders than wood frogs; a slightly lower THg and MeHg concentration but significantly higher %MeHg in salamander eggs is suggestive of this, but further study is warranted to conclusively identify drivers of differences in %MeHg in egg masses. Among larvae, we hypothesize that dietary differences between predatory salamanders and herbivorous wood frogs accounted for the difference in %MeHg, since predators

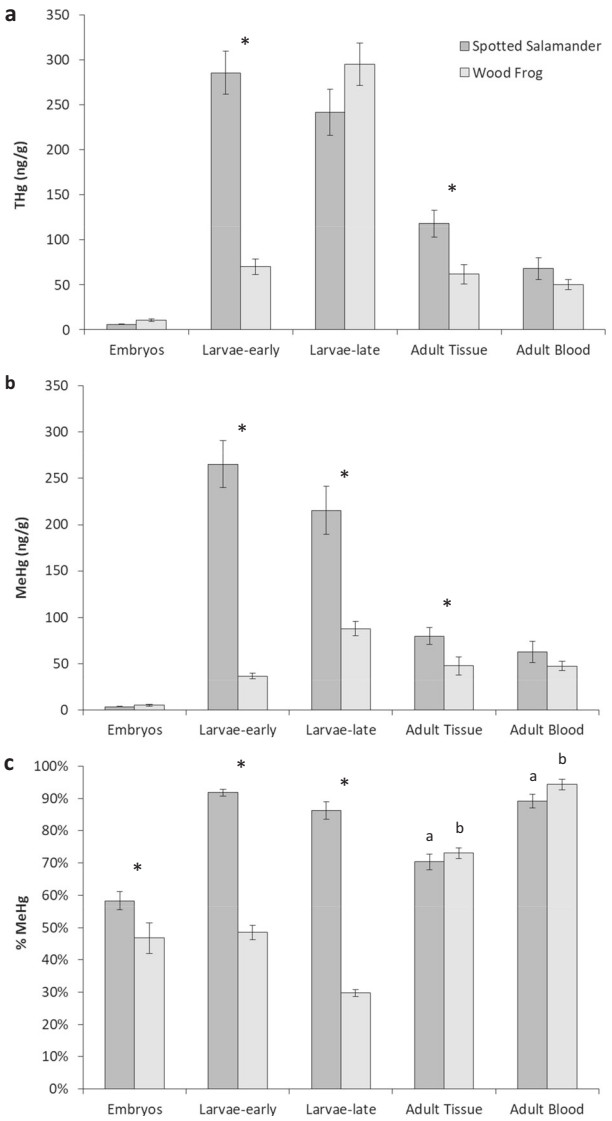

Fig. 3 **a** THg concentrations (ng/g; mean ± 1 SE), **b** MeHg (ng/g; mean ± 1 SE), and **c** %MeHg (%; mean ± 1 SE) in spotted salamander (SPSA) and wood frog (WOFR) eggs (dry wt), larvae (dry wt), and adult tissue (dry wt) and blood (wet wt) from Vermont vernal pools, 2015. Asterisks indicate significant difference between species within life stage; letters indicate significant difference between tissue and blood within species

Table 4 Akaike's information criterion values for candidate models explaining variation in methylmercury loads in eggs and larvae of wood frogs and spotted salamanders collected in vernal pools in Vermont in 2015

Model	K[a]	ΔAIC[b, c]	w_i [d]
Species * life stage + forest cover type	9	0.00	0.40
Species * life stage	8	0.60	0.30
Species * life stage + water methylmercury	9	1.97	0.15
Species * life stage + forest cover type + water methylmercury	10	2.00 [e]	0.15

Only the models receiving some support (i.e., with an AIC weight > 0.001) from the data are shown

[a]*K* is the no. of parameters estimated by the model

[b]ΔAIC is the difference between a given model and the best model (the model with the lowest AIC score)

[c]The lowest AIC score was 174.94

[d]AIC wt (w_i) reflects the relative support for each model

[e]The next best-supported model had ΔAIC = 60.62 and $w_i = 0$

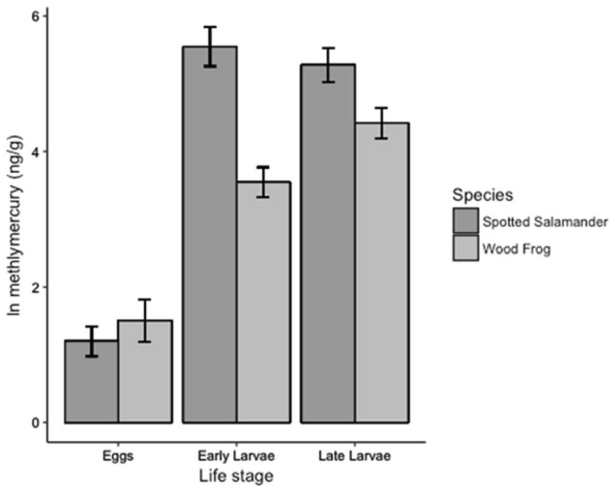

Fig. 4 Predicted values (and 95% confidence intervals) of methylmercury (shown on the log scale for ease of comparing across all life stages) in eggs and larval stages of spotted salamander and wood frog collected in vernal pools in Vermont, 2015. Predicted values were averaged across all models that included effects of life stage and species

may be more efficient at assimilating MeHg due to partitioning of MeHg in their prey (Wada et al. 2011). It is interesting to note however, that THg increased almost three-fold between early- and late-stage wood frog tadpoles, while %MeHg declined about 20% (Fig. 3a, c). The observed increase in THg was largely due to high concentrations in late-stage larvae collected at two pools located in coniferous stands. Late-stage larvae from pools SDF516 and SDF951 had mean THg concentrations of 424 ng/g (range = 363–562 ng/g), while larvae from the four other pools had mean THg concentrations of 231 ng/g (range = 106–352 ng/g). Still, the ~30% MeHg that we found in late-stage wood frog larvae was consistent with the 25% MeHg that Wada et al. (2011) measured among wood frog tadpoles at Gosner stage 42 that were fed low Hg diets.

It is unknown whether the MeHg concentrations measured in amphibian larvae, especially among spotted salamanders, could have sublethal effects on fitness traits such as reduced body size, increased tail resorption time, and reduced locomotor performance. Marbled salamander (*Ambystoma opacum*) larvae experienced 50% mortality with THg levels of 103 ng/g (Sparling et al. 2000), approximately 2.5 times lower than the THg concentrations of ~260 ng/g that we found in spotted salamander larvae. In

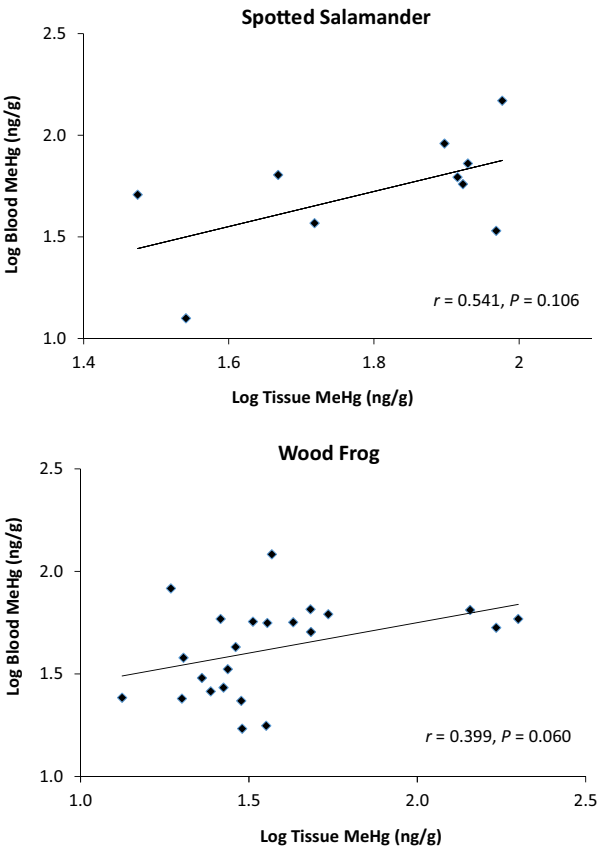

Fig. 5 Relationship between MeHg concentrations in blood and tissue samples among adult spotted salamander (top) and wood frog (bottom) collected in vernal pools in Vermont, 2015

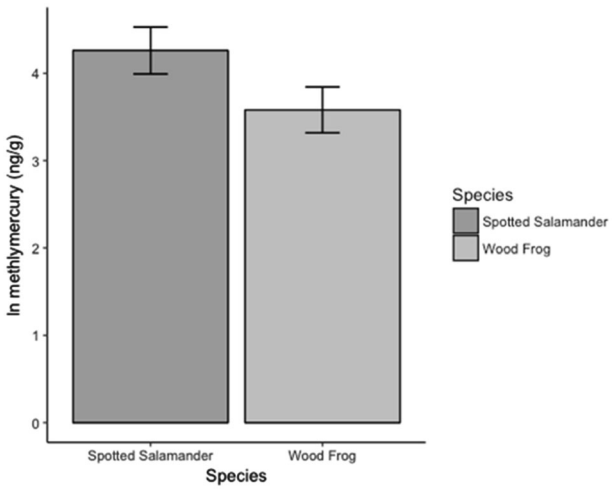

Fig. 6 Predicted values (and 95% confidence intervals) of methylmercury among adult spotted salamander and wood frog tissue collected in vernal pools in Vermont, 2015

a laboratory study investigating the effects of dietary Hg on developing wood frogs, Wada et al. (2011) did not observe any adverse effects on larval development, size at metamorphosis, survival, or hopping performance at MeHg concentrations 2–3 times those that we detected in wood frogs. However, differences in dietary selenium concentrations between tadpoles raised in a controlled environment versus those developing in natural systems could alter Hg toxicity (Ralston et al. 2007). In addition, external environmental stressors present in wild populations, including synergistic effects of other metals (e.g., Al), may exacerbate effects of MeHg exposure. While the existing literature on Hg toxicity thresholds among amphibians is limited, it does appear that interspecific sensitivity to Hg is highly variable. Southern leopard frog tadpoles (*Lithobates sphenocephalus*) suffered lower survival, decreased tail resorption rates, and lower metamorphic success at MeHg concentrations of ~28 ng/g dw (Unrine et al. 2004), while American toad tadpoles showed impaired growth at MeHg concentrations of ~500 ng/g dw (Bergeron et al. 2011). Additionally, considerable genetic variation in sensitivity to toxicants can occur within amphibian populations (Bridges and Semlitsch 2001), which could potentially confound observed differences between species. More research is needed to better understand the effect levels of MeHg among amphibians, especially for *Ambystomid* salamander species, as well as the regional variability between populations.

Table 5 Akaike's information criterion values for candidate models explaining variation in methylmercury loads in adult wood frogs and spotted salamanders collected in vernal pools in Vermont in 2015

Model	K[a]	ΔAIC[b, c]	w_i [d]
Species	4	0.00	0.71
Species + forest cover type	5	1.81	0.29
Forest cover type	4	108.46	0.00

[a]*K* is the no. of parameters estimated by the model

[b]ΔAIC is the difference between a given model and the best model (the model with the lowest AIC score)

[c]The lowest AIC score was 94.99

[d]AIC wt (w_i) reflects the relative support for each model

Methylmercury in adult amphibians

This study utilized two non-destructive sampling techniques to quantify Hg exposure in adult amphibians. For both species, MeHg concentrations between tissue (tail clips and toe clips) and blood samples showed only moderate correlation. The lack of strong correlation could be due to the samples being collected immediately following overwintering and before any feeding occurred, which would explain the lower mean MeHg concentrations in blood relative to tissue. MeHg in blood is comprised of both a transient component which reflects recent dietary intake, and a more stable component related to life-time accumulation (Day et al. 2005). Given that both species in our study

were captured within days of emerging from winter dormancy and do not forage during spring migration and breeding (Smallwood 1928; Wells and Bevier 1997), it is unlikely that they accumulated any recent dietary Hg. Therefore, early spring may represent the lowest blood Hg levels of the year among spring-breeding amphibians that do not begin foraging until after the breeding season, and may be more representative of life-time Hg accumulation. It is also possible that blood Hg concentrations could decline during extended periods of heat and drought when terrestrial amphibians estivate. Studying stranded loggerhead sea turtles (*Caretta caretta*), Day et al. (2005) found significantly lower blood Hg levels in a severely emaciated individual with no fat stores compared to other stranded loggerheads, suggesting that a cessation of dietary input of prey resulted in a decline in blood Hg concentration. Therefore, it is important to know the precise timing of blood collection and whether animals recently emerged from hibernation or estivation when comparing blood Hg concentrations between studies.

It is also possible that our low sample size for salamander blood could have been responsible for the weak relationship. Pfleeger et al. (2016) demonstrated that tail clips from four aquatic salamander species, including two *Ambystomid* species, accurately predicted whole-body THg concentrations. Although this suggests that tail clips may be a more reliable sample to use in future studies, Pfleeger et al. (2016) did not sample terrestrial species, which may partition Hg differently than aquatic salamanders. However, since blood samples can usually be obtained during the process of amputating tail tips, future studies should consider collecting both sample types to increase our understanding of the relationship between blood and tail clips. We found that using a scalpel to amputate tail tips produced sufficient bleeding for sample collection compared to using scissors, which was more likely to crush blood vessels and reduce or eliminate bleeding.

The weak relationship that we found between wood frog blood and toe clips may have been due to the small size of the single toe clip we collected from each frog, and because so much of the toe consists of bone, which accumulates Hg at a different rate than muscle and whole body (Goldstein et al. 1996). Although Pfleeger et al. (2016) found that the outer three posterior toes had the strongest correlations to whole body Hg (which includes the fourth toe that was sampled in this study), they only sampled a single species (*Rana cascadae*) which is more aquatic than wood frogs. Additionally, the relationship between toe clips and whole body Hg could vary significantly between frog species. We found that collecting blood from the facial vein using the technique described in Forzan et al. (2012) was easily carried out in the field, produced reliable samples, and had no discernable impact to adult frogs. We highly recommend this method over toe clipping when possible.

Among adult amphibians, blood had significantly higher %MeHg in both species (spotted salamander mean = 89.2 ± 2.2%; wood frog mean = 94.3 ± 1.7%) compared to tail-clip (mean = 70 ± 2.4%) and toe (mean = 73 ± 1.6%) samples (Fig. 3c). Bergeron et al. (2010b) found that American toad blood had the highest %MeHg of any tissues sampled (73%), which they attributed to recent dietary uptake. As discussed above, it seems unlikely that the high proportion of MeHg that we measured in adult amphibian blood was due to recent dietary intake since neither species is known to feed during immigration to breeding pools or during breeding. Wood frogs metabolize glycogen reserves in their abdominal muscles during the breeding period (Wells and Bevier 1997), while spotted salamanders presumably metabolize fat reserves stored in their tails and abdomen (Fitzpatrick 1976). It is possible that metabolizing fat and/or muscle following winter dormancy could mobilize MeHg stored in these tissues, thereby elevating MeHg in the blood (Day et al. 2005).

Following metamorphosis, juveniles and adults of both species are vagile and primarily occupy terrestrial habitats (Faccio 2003; Baldwin et al. 2006). Even though Hg bioaccumulates and is not easily eliminated from the body, the lower concentrations that we observed in adults relative to larvae are invariably due to dilution over time as they feed in a terrestrial-based food web. Although data are limited, adults of both species appear to prey on similar small invertebrates, including spiders (Order Araneae), beetles (Order Coleoptera), centipedes (Class Chilopoda), lepidoptera larvae, slugs (Order Stylommatophora), and snails (Order Stylommatophora) (Smallwood 1928; Gilhen 1984). If that is the case, and assuming that both species eliminate MeHg from the body at similar rates, it suggests that the significantly greater MeHg concentrations that we detected in adult spotted salamanders compared to adult wood frogs may be due to the fact that salamander metamorphs started off terrestrial life with higher Hg burdens because they accumulate Hg more rapidly as larvae than wood frog tadpoles. The higher MeHg concentrations in salamanders may also be related to a higher ingestion rate, although the feeding rates of these species are not known. It is also likely that the *Ambystoma* sampled in our study were older than the wood frogs, as salamanders are the longer-lived species, and thus would have more time over which to accumulate Hg. Further research is needed to determine the time frame over which Hg levels decline following metamorphosis. We hypothesize that Hg concentrations remain relatively stable during the first few weeks or months following metamorphosis, then steadily decline due to biodilution during the juvenile stage as amphibians grow to adult size (1–2 years for wood frogs; 3–4 years for spotted salamanders), eventually reaching equilibrium that is dependent on Hg intake from prey (Carrier et al. 2001). Given the

large amount of biomass (Windmiller 1996; Berven 2009) and nutrients (Capps et al. 2015) that are exported from vernal pools following a successful amphibian breeding season, and the wide range of predators that may consume juvenile amphibians (Mitchell et al. 2009), vernal pools have the potential to export a significant amount of MeHg from the aquatic system to the terrestrial food web.

There are no published studies that investigated Hg concentrations or toxicity thresholds for adult *Ambystomid* salamanders or wood frogs. Among adult Anurans, data on mercury concentrations are lacking, hence there were few relevant studies with which to compare our results. MeHg in American toad blood from an uncontaminated site in Virginia (Bergeron et al. 2010b) was about three times higher than wood frog blood in the present study. Among Caudata, Burke et al. (2010) showed that behavioral responses of adult two-lined salamanders (*Eurycea bislineata*) with mean THg concentrations of ~4500 ng/g dw from a Hg contaminated site had lower speeds of locomotion, diminished responsiveness, and reduced ability to capture prey compared to an uncontaminated reference site. THg concentrations in red-backed salamanders (*Plethodon cinereus*) have been reported from three different geographic areas in the eastern U.S. Whole-body concentrations of THg from red-backed salamanders in Virginia (Bergeron et al. 2010a) and the Catskill Mountains, NY (Townsend and Driscoll 2013) were similar to THg concentrations in tail-clips from salamanders in the Green Mountains, Vermont (Rimmer et al. 2010) (mean values were 100; 127, 110 ng/g dw, respectively). These results were similar to mean THg in adult *A. maculatum* tail-clips in this study (118 ± 14.8 ng/g dw). Although *P. cinereus* is terrestrial throughout its entire life cycle, it occupies similar habitats as adult *A. maculatum* and co-occurring populations would likely be exposed to similar dietary Hg concentrations through atmospheric deposition. Still, we would expect adult spotted salamanders to bioaccumulate more Hg than red-backed salamanders due to increased exposure during their aquatic larval stage (Todd et al. 2012) and longer life span (Flageole and Leclair 1992). Although Hg samples from salamander tail-clips have been shown to correlate strongly with whole-body Hg, it is not a 1:1 relationship and can vary substantially between species (Pfleeger et al. 2016). Therefore, comparison of tail and whole-body Hg concentrations between- and within-species must be interpreted cautiously.

Conclusions

This research demonstrates that vernal pools are important hotspots of MeHg bioaccumulation, and biota from the pools may be vectors of MeHg to the terrestrial ecosystem. Water from pools located in conifer-dominated forest stands had higher THg and MeHg concentrations, higher DOC, and lower pH compared to pools in deciduous forest stands. Although forest cover type did not explain the variation in Hg concentrations among amphibian eggs or larvae, our sample sizes were quite small. We found that spotted salamander larvae rapidly accumulated MeHg (concentrations 1.5 to 2 orders of magnitude greater than their eggs), while wood frog larvae accumulated MeHg more moderately (one order of magnitude greater than their eggs), suggesting that predators that feed on amphibian larvae in these ephemeral systems may be exposed to relatively high concentrations of MeHg. These predators may include a variety of aquatic beetles (Dytiscidae, Hydrophilidae, and Gyrinidae), backswimmers (Notonectidae), water scorpion (Nepidae), Odonate larvae, *Ambystoma* larvae, eastern newt (*Notophthalmus viridescens*), snakes, and birds (including wading birds and shorebirds) (personal communication, M. Burne and J. Kubel). The abundance of larvae combined with the high concentrations of MeHg they bioaccumulate suggest they could potentially be important bioindicators for monitoring MeHg loading and bioavailability in these sensitive ecosystems. Although MeHg concentrations were 2 to 3 times lower in adult amphibians compared to their aquatic larvae, spotted salamander adults had higher MeHg levels than wood frog adults. It is unknown how rapidly mercury levels decline following metamorphosis, but since mercury is slow to flush from the body we suspect that it declines slowly due to dilution as amphibians grow to adult size. This work suggests that vernal pool amphibians may be a significant source of Hg to terrestrial predators, including frogs, snakes, birds, and small- to medium-sized mammals. Future research that quantifies mercury concentrations among amphibian metamorphs and juveniles will help elucidate cycling and trophic transfer of mercury from aquatic to terrestrial systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This research was conducted under a protocol approved by Dartmouth College's Institutional Animal Care and Use Committee.

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