Bioaccumulation of mercury in wood frog and spotted salamander eggs, larvae, and adults in Vermont vernal pools

Steven D. Faccio, Kate L. Buckman, Vivien Taylor, John D. Lloyd

**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 keystone ecosystems to maintaining viable populations of at-risk species. Due to their small size, ephemeral nature, spatial and hydrologic isolation, and relatively small watersheds, vernal pools can be affected by small-scale local conditions, such as land use and land cover type, as well as large-scale environmental perturbations, including acid precipitation (Pough 1976, Cook 1983) and atmospherically deposited mercury (Brooks at al. 2012, Loftin et al. 2012, Benoit et al. 2013).

Mercury (Hg) 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). In the Northeast, hotspots are often associated with forested regions with an abundance of wetlands, which facilitate the conversion of Hg to the more toxic and bioavailable methylmercury (MeHg) (Driscoll et al. 2007). Landscape plays a critical role in Hg accumulation, methylation and mobilization in forest floors. Mixed landscapes of forest cover and agricultural use are considered to have the highest Hg methylation efficiency (Krabbenhoft 1999), and land disturbance such as timber harvesting can increase MeHg and Hg export (Driscoll et al. 2007). 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 leach it to the forest floor in throughfall and litterfall (Lindberg et al. 1995, Rea et al. 1996). 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. And third, aquatic systems with low primary productivity (such as vernal pools) enhance Hg concentration in biota (Chen et al. 2005). In addition, hydrologic and biogeochemical properties of vernal pools provide ideal conditions for production of the sulfate-reducing bacteria responsible for Hg methylation (Wiener et al. 2003). These 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), suggesting that vernal pools are potential hotspots for MeHg production and bioaccumulation in fauna, particularly in higher trophic level taxa such as amphibian larvae, which may then transport Hg to terrestrial systems via metamorphosis.

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 methylation efficiency of pool water also increased during the spring, with mean methylation efficiencies reaching 43% to 58%. Methylation efficiencies 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). This could have negative implications for amphibian larvae and metamorph survival (Unrine et al. 2004) in pools surrounded by coniferous forests, as well as for increased Hg export into the terrestrial environment.

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 Hg, water chemistry (pH, DOC, aluminum), 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 burdens. And finally, because Hg bioaccumulates and is not easily eliminated from the body (Bergeron et al. 2010), we hypothesized that longer-lived spotted salamander adults would have higher Hg 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) (Table 1). All pools were located in east-central Vermont (N43°53’, N43°42’; W72°27’, W72° 16’), with four pools located in south-eastern Orange Co., and two in northeastern Windsor Co. 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 samples for Hg analysis were collected using clean Hg sampling techniques, and were filtered to 0.2 µm, preserved in 0.5% HCl, and stored in the dark prior to analysis. Independent water samples at each pool were collected for total suspended solids and ancillary water chemistry analyses, and temperature, pH and oxidation reduction potential were measured at the site. Water sampling was initiated at all pools immediately following ice-out in April, 2015, when water levels were at or near peak. Sampling at four pools 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. At two pools (one coniferous, one deciduous), samples were collected ca. every 7-10 days to more carefully monitor temporal trends of Hg species and water chemistry.

Amphibian Sampling

Following spring immigration to pools for breeding, adult wood frogs and spotted salamanders (n=4/pool/spp.) were captured between 20 Apr and 12 May, 2015 using dip nets or funnel traps that were partially submerged 16-24 hours 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 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 were anesthetized prior to amputation using topical lidocaine, and the remaining digit 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 minutes) 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.

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 to 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 examined under a dissecting scope, inspected for developmental abnormalities, Gosner stage (Gosner 1960) established for wood frogs, and then frozen. At each pool, four early- and late-stage 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

All inorganic mercury (IHg) and MeHg measurements were conducted at the Dartmouth Trace Element Analysis Core Facility. MeHg analysis was done by species-specific isotope dilution using an automated MERX-M interfaced with an Element 2 ICP-Mass Spectrometer, and THg analysis was done using cold vapor atomic fluorescence.

Statistical Analyses

We used t-tests to examine differences in pool basin morphology and water chemistry metrics by forest cover type. Relationships between pool physiochemical attributes and MeHg concentrations were examined using Spearman rank-order correlations. Bioaccumulation factors (BAF) were calculated for pools as the ratio of the concentration of MeHg in amphibian eggs and larvae to the concentration of MeHg in pool water. The effect of forest cover type on BAF within species and life stages were examined using t-tests, or Mann-Whitney U-tests if data failed to normalize. Significant differences were assigned for *P*≤0.05.

Some sort of multivariate models were used to confuse the hell out of the senior author.

We used generalized linear mixed models to examine causes of variation in methylmercury loads in eggs, larvae, and adults. 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 methylmercury in eggs and larvae might reflect conditions in the breeding pool, especially levels of methylmercury in the water, whereas we had no reason to expect a correlation between methylmercury in adults and methylmercury levels in the water in breeding pools because of the short duration of time that adults spend in the breeding pool.

For both analyses, we treated the pool (n = 6) as a random effect to account for the nested nature of our sampling, in which methylmercury levels were estimated from multiple individuals from the same pool. For eggs and larvae, we considered a suite of models constructed from four variables 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 (primarily insectivorous) might contribute to differences in methylmercury accumulation; habitat around the pool, because pools surrounded by coniferous forest may experience higher rates of methylation; life stage (egg, early-stage larvae, or late-stage larvae) because later stages of development have both more time to accumulate methlymercury and perhaps more exposure to methylmercury through consumption of prey items; and finally levels of methylmercury in the water, as this is presumably the primary means by which individuals accumulate methylmercury. 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 methylmercury 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, habitat, life stage, and water methylmercury, 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 habitat. We did not consider methylmercury in the water of the vernal pool as a predictor of methylmercury in adults because we assumed that adults had a short duration of exposure to the pool and that it was thus unlikely that methylmercury in adults could be linked plausibly to methylmercury in the water. This left us with three possible models: the combined effect of species and habitat plus two models that considered each variable in isolation.

We fit each model using the lmer function in R package lme4 (Bates et al. 2014). 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 (AIC­­c), because of the uncertainty in determining the effective sample size for mixed-effects models (Burnham and Anderson 2002). However, when we ranked models according to AIC­­c, 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 (Burnham and Anderson 2002, Cade 2015); however, our findings were the same whether relying on model-averaged coefficients or model-averaged predictions as the basis of inference about methylmercury 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 R2 values estimated by the r.squaredGLMM function in the R package MuMIn (Barton 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 pool size (area, perimeter), 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 (mean coniferous = 5.16, deciduous = 6.21), greater DOC (mean coniferous = 17.46 mg/L, deciduous = 11.90 mg/L), lower conductivity (mean coniferous = 17.53 µS/cm, deciduous = 37.13 µS/cm), and greater aluminum concentrations (mean coniferous = 152.45 µg/L, deciduous = 61.62 µg/L) (Table 2). Additionally, coniferous pool water had greater concentrations of THg (mean coniferous = 5.56 ng/L, deciduous = 3.05 ng/L) and MeHg (mean coniferous = 1.19 ng/L, deciduous = 0.56 ng/L) compared to deciduous pool water (Table 2). Concentrations of MeHg in coniferous pool water showed significant positive correlations to DOC (r2 = 0.785, *P*<0.001) and aluminum (r2 = 0.669, *P*<0.001), and correlated negatively with pH (r2 = -0.618, *P*<0.001). Temporally, during the inundation period (April to July), increases in water MeHg coincided with increases in temperature and decreases in dissolved oxygen (Fig. 1).

Eggs and larvae of Wood Frog and Spotted Salamander accumulated methylmercury at different rates, such that models without the interaction term between species and life stage received no support in the model-selection process (Table 1). The global model provided a good fit to the data (R2 = 0.92). Although each of the four predictor variables appeared among the top-ranked models, model-averaged regression coefficients for both habitat (*b*deciduous = 0.16, 95% CI = -0.04 – 0.35) and water methylmercury (*b* ***=*** *-0.04, 95% CI = -0.21 – 0.13*) were not distinguishable from zero.

Table 1. 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, USA in 2015 and 2016. Only the models receiving some support (i.e., with an AIC weight > 0) from the data are shown.

|  |  |  |  |
| --- | --- | --- | --- |
| Model | Ka | ΔAICb,c | *wid* |
| Species \* life stage + habitat | 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 + habitat + water methylmercury | 10 | 2.00e | 0.15 |
| a *K* is the no. of parameters estimated by the model.  b ΔAICis 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 (*wi*) reflects the relative support for each model.  e The next best-supported model had ΔAIC = 60.62 and *wi* = 0. | | | |

Indeed, methylmercury loads in eggs and larvae were best explained by the effect of species, life stage, and the interaction between these two variables (Figure 1). Levels of methylmercury in eggs of both species were low but increased substantially among both early- and late-stage larvae. The increase was more pronounced among Spotted Salamanders, which had higher levels of methylmercury at both larval stages than did Wood Frogs. Surprisingly, predicted methylmercury loads in adults of both species were 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]).

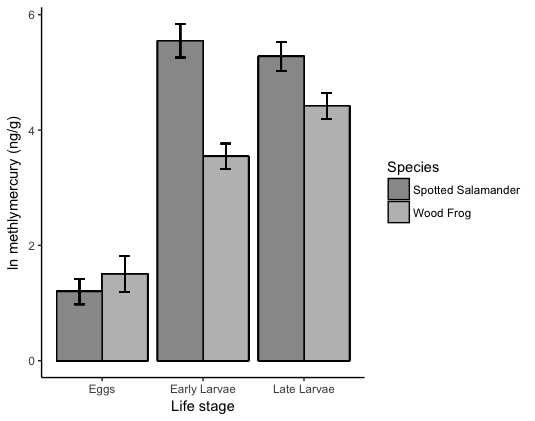


Figure 1. Predicted values (and 95% confidence intervals) of methylmercury (shown on the log scale for ease of comparing across all life stages) in eggs and larval stages of Spotted Salamander and Wood Frog collected in vernal pools in Vermont, USA in 2015 and 2016. Predicted values were averaged across all models that included effects of life stage and species.

Variation in methylmercury levels among adults was explained by species, with Wood Frogs having substantially lower levels of methylmercury than Spotted Salamanders (*b*Wood Frog  = -0.66, 95% CI = -0.99 - -0.34) (Fig. 2). Habitat had no discernible effect on methylmercury levels in adult salamanders or frogs (*b*deciduous = -0.02, 95% CI = -0.21 – 0.17). Most of the observed variance in methylmercury loads among adults remained unexplained by the global model (R2 = 0.27).

Table 2. 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, USA in 2015 and 2016.

|  |  |  |  |
| --- | --- | --- | --- |
| Model | Ka | ΔAICb,c | *wid* |
| Species | 4 | 0.00 | 0.71 |
| Species + habitat | 5 | 1.81 | 0.29 |
| Habitat | 4 | 108.46 | 0.00 |
| a *K* is the no. of parameters estimated by the model.  b ΔAICis 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 (*wi*) reflects the relative support for each model. | | | |

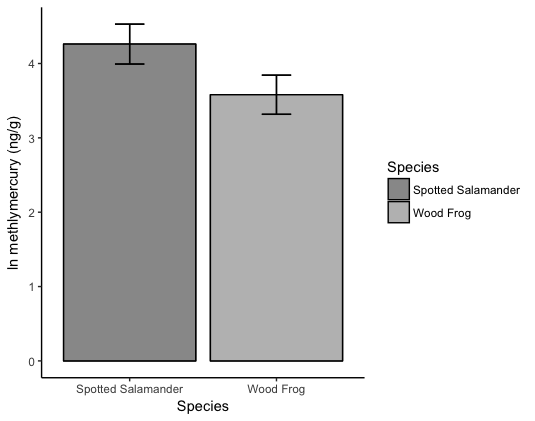


Figure 2. Predicted values (and 95% confidence intervals) of methylmercury (shown on the log scale for ease of comparing across all life stages) among adult Spotted Salamander and Wood Frog collected in vernal pools in Vermont, USA in 2015 and 2016.

Methylmercury concentrations in amphibian embryos were relatively low compared to larval and adult burdens (Table 3; Fig. 2), but were 3 to 4 orders of magnitude greater than water MeHg levels, suggesting that female amphibians may depurate some of their Hg burdens during oviposition. There were no differences in embryo MeHg concentrations between species or habitats. Within larvae, MeHg levels varied among species, larval stage, and as a function of water MeHg. Both early and late stage spotted salamander larvae had significantly greater levels of MeHg compared to wood frog larval stages (early stage larvae, *t*-stat = 8.97, *df* = 11.39, *P* <0.001; late stage larvae, *t*-stat = 4.72, *df* = 16.73, *P* <0.001) (Table 3; Fig. 2). Within species, MeHg decreased from early to late stage larvae in salamanders, but increased in wood frogs (Table 3; Fig. 2). 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), but our sample sizes were quite small.

Adult spotted salamanders had higher MeHg loads than adult wood frogs (Table 3; Fig. 3). Among adult spotted salamanders, tissue samples tended to have greater MeHg loads than blood, while wood frogs showed the opposite pattern (Fig. 3). Again, habitat did not explain variation in MeHg loads among adult samples.

References

Adams M.J., D.A.W. Miller, E. Muths, P.S. Corn, E.H.C. Grant, L.L. Bailey, G.M. Fellers, R.N. Fisher, W.J. Sadinski, H. Waddle, and S.C. Walls. 2013. Trends in Amphibian Occupancy in the United States. PLoS ONE 8(5): e64347. DOI:10.1371/journal.pone.0064347

Benoit, J.M., C.C. Gilmour, A. Heyes, R.P. Mason,and C.L. Miller. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In: Cai Y. and O.C. Braids (eds) Biogeochemistry of environmentally important trace elements. ACS Symposium Series 835: pp 262–297. DOI: 10.1021/bk-2003-0835.ch019.

Benoit, J.M., D.A. Cato, K.C. Denison, and A.E. Moreira. 2013. Seasonal mercury dynamics in a New England vernal pool. Wetlands 33:887–894.

Bergeron, C.M., C.M. Bodinof, J.M. Unrine, and W.A. Hopkins. 2010. Mercury accumulation along a contamination gradient and nondestructive indices of bioaccumulation in amphibians. Environmental Toxicology and Chemistry 29:980–988.

Berven, K.A. 2009. Density dependence in the terrestrial stage of Wood Frogs: Evidence from a 21-year population study. Copeia 2:328–338.

Brooks R.T., S.L. Eggert, K.H. Nislow, R.K. Kolka, C.Y. Chen, D.M. Ward. 2012. Preliminary assessment of mercury accumulation in Massachusetts and Minnesota seasonal forest pools. Wetlands 32:653–663.

Calhoun, A.J.K. and P.G. deMaynadier. 2009. Science and conservation of vernal pools in Northeastern North America. CRC Press, Boca Raton, FL. 363pp.

Chen C.Y., R.S. Stemberger, N.C. Kamman, B.M. Mayes, and C.L. Folt. 2005. Patterns of Hg bioaccumulation and transfer in aquatic food webs across multilake studies in the northeast US. Ecotoxicology 14: 135–147.

Colburn, E.A. 2004. Vernal pools: Natural history and conservation. McDonalds and Woodward Publishing Co., Blacksburg, VA.

Colburn, E.A., S.C. Weeks, S.K. Reed. 2009. Diversity and ecology of vernal pool invertebrates. In: Calhoun, A.J.K. and P.G. deMaynadier (eds) Science and conservation of vernal pools in northeastern North America. CRC Press, Boca Raton, FL, pp 105–126.

Cook R.P. 1983. Effects of acid precipitation on embryonic mortality of *Ambystoma* salamanders in the Connecticut Valley of Massachusetts. Biological Conservation 27:77–88.

Davis, E. 2013. Seasonal changes in mercury stocks and methylation ratios in vernal pools in the northeastern united states. M.S. Thesis, University of Vermont, Burlington, VT, 56pp.

Driscoll, C.T., H. Young-Ji, C.Y. Chen, D.C. Evers, K.F. Lambert, T.M. Holsen, N.C. Kamman, and R.K. Munson. 2007. Mercury contamination in forest and freshwater ecosystems in the northeastern United States. Bioscience 57:17–28.

Evers D.C., Y-J Han, C.T. Driscoll, N.C. Kamman, M.W. Goodale, K.F. Lambert, T.M. Holsen, C.Y. Chen, T.A. Clair, and T. Butler. 2007. Biological mercury hotspots in the northeastern United States and southeastern Canada. BioScience 57:29–43.

Faccio, S. D.; M. Lew-Smith, and A. Worthley. Vermont Vernal Pool Mapping Project 2009–2012. Final Report to the Natural Heritage Information Project of the Vermont Department of Fish and Wildlife. 40pp. http://vtecostudies.org/wp-content/uploads/2014/08/vce-vernal-pool-mapping-final-report.pdf

Faccio, S.D., S.W. MacFaden, J.D. Lambert, J. O’Neil-Dunne, and K.P. McFarland. 2016. The North Atlantic Vernal Pool Data Cooperative: 2016 revision. Unpublished report submitted to the North Atlantic Landscape Conservation Cooperative. 86pp. http://vtecostudies.org/wp-content/uploads/2016/07/NALCC\_VPDC\_Final-Report.V2.July2016\_HiRez.pdf

Forzan, M.J., R.V. Vanderstichel, C.T. Ogbuah, J.R. Barta, and T.G. Smith. 2012. Blood collection from the facial (maxillary)/musculo-cutaneous vein in true frogs (Family *Ranidae*). Journal of Wildlife Diseases 48:176–180.

Galloway M.E. and .B.A Branfireun. 2004. Mercury dynamics of a temperate forested wetland. Science of the Total Environment 325:239–254.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.

Grigal D.F. 2002. Inputs and outputs of mercury from terrestrial watersheds: A review. Environmental Review 10: 1–39.

Krabbenhoft, D.P., J.G. Wiener, W.G. Brumbaugh, M.L. Olsen, J.F. DeWild, and T.J. Sabin. 1999. A National Pilot Study of Mercury Contamination of Aquatic Ecosystems along Multiple Gradients. U.S. Geological Survey Toxic Substances Hydrology Program, Proceedings of the Technical Meeting, Charleston, South Carolina, March 8-12, 1999, 147pp.

Lindberg S.E., K-H. Kim, T.P. Meyers, and J.G. Owens. 1995. A micrometeorological gradient approach for quantifying air/surface exchange of mercury vapor: Tests over contaminated soils. Environmental Science and Technology 29: 126–135.

Loftin, C.S., A.J.K. Calhoun, S.J. Nelson, A.A. Elskus, and K. Simon. 2012. Mercury bioaccumulation in wood frogs developing in seasonal pools. Northeastern Naturalist 19:579–600.

Miller E.K., A. VanArsdate, G.J. Keeler, A. Chalmers, L. Poissant, N.C. Kamman, and R. Brulotte. 2005. Estimation and mapping of wet and dry mercury deposition across northeastern North America. Ecotoxicology 14:53–70.

NEPARC. 2010. Northeast Amphibian and Reptile Species of Regional Responsibility and Conservation Concern. Northeast Partners in Amphibian and Reptile Conservation (NEPARC). Publication 2010-1. http://northeastparc.org/wp-content/uploads/2016/08/NEPARC\_NEspeciesofresponsibility.pdf

NEPARC. 2014. Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus. NEPARC Publication 2014-02. http://www.northeastparc.org/products/pdfs/NEPARC\_Pub\_2014-02\_Disinfection\_Protocol.pdf

Pough F.H. 1976. Acid precipitation and embryonic mortality of spotted salamanders, *Ambystoma maculatum*. Science 192:68–70.

Rea A.W., G.J. Keeler, and T. Scherbatskoy. 1996. The deposition of mercury in throughfall and litterfall in the Lake Champlain watershed—a short-term study. Atmospheric Environment 30:3257–3263.

Risch M.R., J.F. DeWild, D.P. Krabbenhoft, R.K. Kolka, L. Zhang. 2012. Litterfall mercury dry deposition in the eastern USA. Environmental Pollution 161:284–290.

Semlitsch, R.D. and D.K. Skelly. 2009. Ecology and conservation of pool-breeding amphibians. In: Calhoun, A.J.K. and P.G. deMaynadier (eds) Science and conservation of vernal pools in northeastern North America. CRC Press, Boca Raton, FL, pp 127–147.

Unrine, J.M., C.H. Jagoe, W.A. Hopkins, and H.A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environmental Toxicology and Chemistry 23:2964–2970.

Van Meter R., L.L. Bailey, E.H. Campbell Grant. 2008. Methods for estimating the amount of vernal pools in the northeastern United States. Wetlands 28:585–593.

Wake, D.B., and V.T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Science USA 105:11466–11473.

Wiener J.G., D.P. Krabbenhoft, G.H. Heinz, and A.M. Scheuhammer. 2003. Ecotoxicology of mercury. In: Hoffman D.J., B.A. Rattner, G.A. Burton, and J. Cairns (eds) Handbook of ecotoxicology. Lewis Publishers, New York, NY.

Windmiller, B.W. 1996. The pond, the forest, and the city: Spotted salamander ecology and conservation in a human-dominated landscape. Ph.D. Dissertation. Tufts University, Medford, MA.