## Landscape influence on vernal pool mercury levels, cycling, and bioaccumulation

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- 1) Vernal pools are important sites for production of methylmercury, the bioavailable and toxic form of mercury, in forest ecosystems.
- 2) Methylmercury bioaccumulates in amphibians and invertebrates that inhabit vernal pools, and may be transferred to predators in forest foodwebs.

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http://www.nsrcforest.org

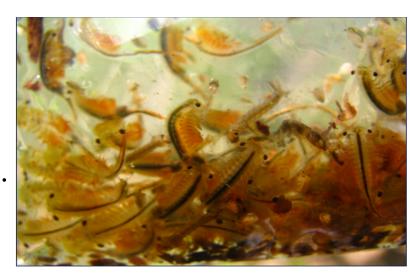
### **Project Summary**

The objective of this work was to examine the effects of landscape characteristics and water chemistry parameters on the levels of methylmercury in vernal pools, and to assess uptake of methylmercury into breeding amphibians and the terrestrial environment. Vernal pools are unique ecosystems in Northeastern forests that provide critical habitat for a diverse range of invertebrates as well as several species of frogs and salamanders, which utilize pools for breeding and larval development. Vernal pools are abundant in the Northern Forest and are potential "hotspots" for mercury accumulation and transformation to the bioavailable and toxic form, methylmercury. Methylmercury is a potent neurotoxin that bioaccumulates through foodwebs and can reach critical levels in sensitive wildlife populations. To fulfill our objectives, we measured methylmercury levels and other chemical parameters in 21 vernal pools across a mixture of hardwood and softwood forests and assessed mercury levels in invertebrates and amphibians at different life stages. Elevated levels of methylmercury were observed in these temporary wetland environments, relative to other natural bodies of water, and concentrations in the water column increased throughout the growing season. Pools supported efficient conversion of mercury to methylmercury, with proportions of methyl: total mercury reaching up to 40%. Canopy type had an influence on pool chemistry (pH, organic carbon), but could not be linked directly to methylmercury levels. However, methylmercury did associate with the concentrations of total mercury in the pool and with the levels of dissolved oxygen, suggesting mercury inputs (from atmospheric deposition and soil runoff and dissolution), as well as levels of anoxia in pools, are drivers of methylmercury levels. Spotted salamander tadpoles and adults had higher levels of mercury than woodfrogs. For larvae, this is likely due to the more predatory feeding habits of this species, while for adults it is likely due to the significantly longer life span of salamanders. Predatory beetles, as well as fairy shrimp, which are exclusively found in ephemeral wetlands, accumulated more mercury than detrital-feeding caddisflies and mosquito larvae.

This work emphasizes the sensitive nature of these forest environments and the species they support. Inputs of mercury, which can be augmented by land disturbances and climate change-related increases in runoff, can be efficiently methylated and taken up into pool fauna, where it can reach critical effects levels in sensitive species and be transferred to terrestrial foodwebs.

## Background and Justification

- Vernal pools are unique ecosystems that support sensitive wildlife species, some of which only inhabit these ephemeral environments, in part due to the lack of fish, which can be predators. Keystone species of vernal pools include spotted salamanders, wood frogs, fingernail clams and fairy shrimp. Invertebrates such as caddisflies, dragonfly larvae, and beetles also inhabit the pools.
- The temporal and small-scale nature of these environments has also made them difficult to study, and a better understanding of stresses on these environments is needed to protect them.



Fairy shrimp – unique to ephemeral pools



Predacious insects

## Background and Justification cont'd

• Biological mercury hotspots are areas in landscapes which disproportionally elevate mercury in the biota. Conditions that promote mercury "hotspots" include forested land cover and anoxic zones in wetlands, which are commonly associated with vernal pool environments.



Vernal pools are unique environments for studying mercury methylation:

- Prone to mercury methylation, like other wetlands
- Small in size influenced by catchment effects on a small landscape scale
- Temporary allow investigation of MeHg dynamics

## Background and Justification cont'd

- The Vermont Vernal Pool
   Mapping Project has mapped and field-verified over 600 pools
- Landscape and environmental factors (canopy type, area and hydroperiod) have been collected at each pool
- Egg mass surveys for amphibians were conducted providing information on species present at each site
- This data base provides the framework for studying mercury bioaccumulation in vernal pools across a range of landscape and environmental conditions.

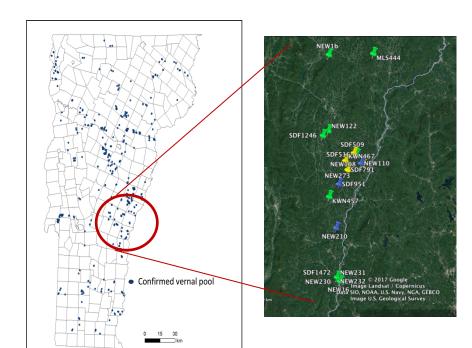


Fig. 1 – Map of confirmed vernal pools from the Vermont Vernal Pool Mapping Project. Pools were mapped by color infrared photo interpretation, and field-verified.

## Methods

#### Study design:

- 21 pools selected for landscape characteristics (hardwood vs softwood canopy); controlled for elevation (1100-1400 ft)
- Temporal sampling of 6 pools (4 samples over the hydroperiod, incl. fall rewetting); 2 pools were sampled intensively (every 10 days) during the wetting period.



• Water samples were collected at each pool and time point; underlying leaves and sediment were collected at the 6 temporally sampled pools. Amphibians (spotted salamanders and wood frogs) at different life stages (embryos, larvae, adults) and invertebrates were sampled at the 6 temporal pools.

## Methods – cont'd

- Blood and tissue samples were collected from adult frogs and salamanders for methylmercury and total mercury analysis.
- Embryos were collected in early spring, followed by early stage (May) and late stage (June) larvae.





## Methods – cont'd

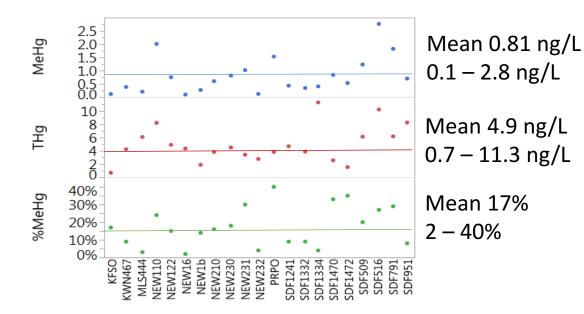
• Water samples were analyzed for methyl and total mercury. Ancillary water quality parameters were also measured (including dissolved organic carbon, pH, dissolved oxygen, sulfate).



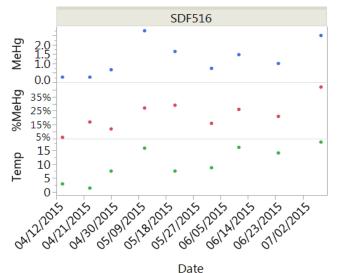
• Leaf, sediment and biota samples were analyzed for methyl and total mercury.

## Results/Project outcomes

- Methylmercury (MeHg) concentrations were captured across pools; the mean %MeHg was 17% (of total Hg), indicating these environments promote efficient methylation of mercury.
- Methylmercury increased during the growing season, following patterns in temperature change.

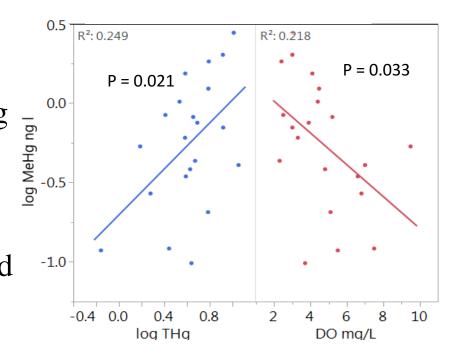


Vernal pools, May, n=21; Dissolved concentrations



Temporal data from a single pool.

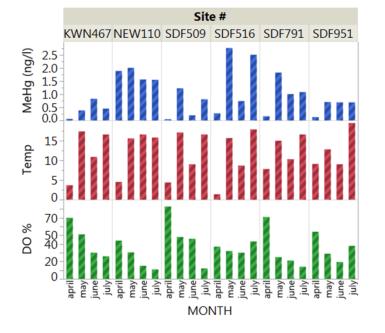
- Levels of methylmercury in pools
  were weakly associated with total
  mercury concentrations, and with
  dissolved oxygen content, indicating
  mercury in pool water is available
  for methylation.
- Total mercury was weakly correlated with dissolved organic carbon content, which was higher in pools with coniferous canopies.
   Methylmercury levels were not related to canopy type.
- Anoxia is a driver of methylmercury levels, but dissolved oxygen was not related to other chemical or landscape parameters.

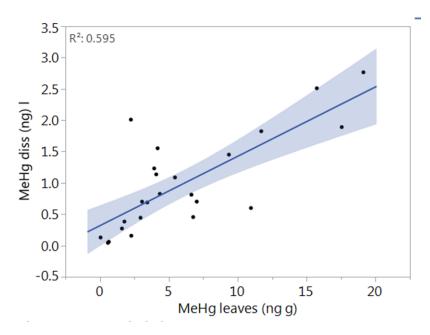


Levels of methylmercury (MeHg) in vernal pools relative to total mercury (THg) and dissolved oxygen (DO).

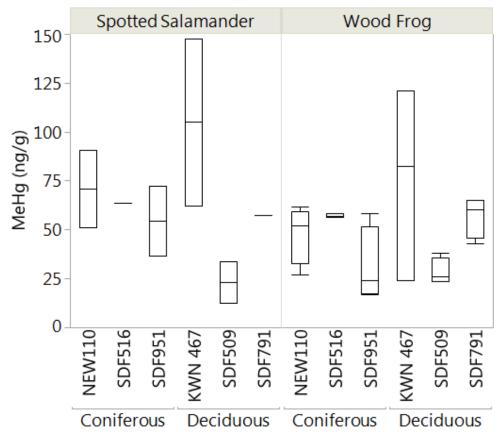
• Temporally, steep increases in MeHg in pools (n=6 pools) coincided with decreases in dissolved oxygen and increases in temperature over the growing period (April to July).

• Increases in %methylmercury (relative to total mercury) were evident in both the water column and submerged leaf litter, with strong correlations between these compartments.





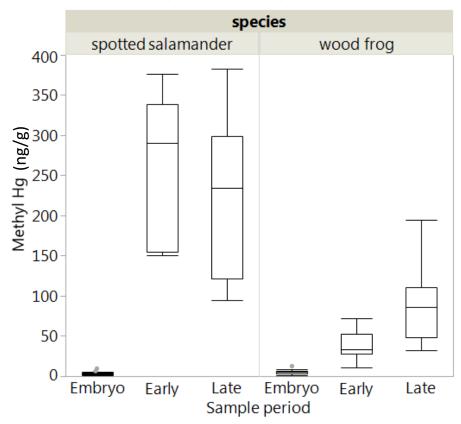
#### Adult amphibians (blood):





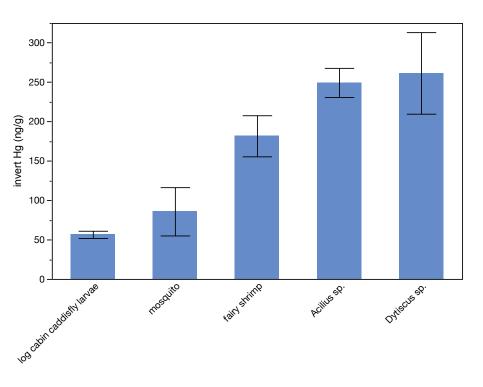
- Levels of methylmercury in spotted salamander and wood frog blood varied between pools, with a consistent pattern for both species.
- No effect was evident between hardwood and softwood environments.
- Salamanders had higher MeHg in blood than wood frogs, likely due to being longer lived and metabolically active during the winter.

#### Amphibian embryos and larvae:



- Levels of methylmercury in embryos were low.
- Methylmercury body burdens increased substantially at the larval stages; salamander larvae had higher mercury tissue concentration than wood frogs.
- had higher mean mercury than late stage larvae, whereas frog mercury burden increased with time. This pattern may be related to changes in diet, or to rapid growth in salamander larvae diluting methylmercury burdens.

#### **Invertebrates**



species ordered by invert Hg (ng/g) (ascending)

Mean methylmercury (MeHg) levels in caddisfly, mosquito and fairy shrimp and mean total mercury (THg) of beetles • average THg in invertebrates increased from caddisfly larvae (shredders) to predatory beetles and may represent a significant conduit for Hg transfer to terrestrial food webs.



#### Outreach

• Citizen scientists were engaged in the project during the course of fieldwork. They assisted with identifying pool characteristics, invertebrate collections, and conducting amphibian egg mass surveys. 10 citizen scientists, including a high school teacher, were involved in the project.



• An undergraduate student, worked on the project for 2 semesters, and presented a poster on her findings at the Wetterhahn Undergraduate Science Symposium. She identified the different species of invertebrates, prepared them for methylmercury analysis, and analyzed samples for total mercury.

#### Outreach cont'd

- Steve Faccio gave an invited talk at the Vermont Monitoring Cooperative Conference 2016.
- Articles about the project were written in Seven Days, a weekly Vermont newspaper, and the Norwich Times.



- Amanda Curtis presented a poster on mercury body burdens in amphibians at the North Atlantic Chapter of the Society for Environmental Toxicology and Chemistry.
- Vivien Taylor presented a talk and Kate Buckman gave a poster on the project at the International Conference on Mercury as a Global Pollutant in Rhode Island, 2017.
- Steve Faccio presented a poster on this work at the Northeast Partners in Amphibian and Reptile Conservation.
- Information about the project continues to be available through the Vermont Center for Ecostudies' Vermont Vernal Pool Mapping Project.

# Implications and applications in the Northern Forest region

- Mercury in vernal pools is efficiently converted to methylmercury, its bioavailable and toxic form. Inputs of mercury to vernal pools are likely from canopy inputs, as submerged leaf litter was correlated with water mercury levels. The canopy receives mercury from atmospheric deposition of mercury from long range transport. Reduction of mercury requires legislation to reduce mercury emissions, and sensitive ecosystems, like vernal pools, are important metrics for demonstrating and monitoring emissions impacts.
- Land disturbances due to anthropogenic pressure, and runoff due to climate change-related increases in precipitation in the Northeast, have been linked with mobilizing mercury from forest soils. Because mercury is efficiently methylated in these vernal pool environments, reduction of total mercury to the pools by improved land-use practices could reduce the impact on the foodweb. Limiting timber harvesting and development in vernal pool watersheds could reduce mercury inputs to these environments.

- Amphibian species that undergo larval development in vernal pools may accumulate elevated body burdens of methylmercury from these environments, and may represent a significant source of methylmercury transfer to terrestrial systems following metamorphosis.
- Resident invertebrate species also export
   Hg to the terrestrial food web
- This is the first report of methylmercury levels in the spotted salamander, and demonstrates their susceptibility to accumulating mercury.
- Protecting these sensitive forest habitats requires monitoring of methylmercury levels; salamander tadpoles may be a useful biosentinel for assessing mercury in forest environments.



## Future directions

- In this work vernal pools were studied across a gradient of forest canopy types. Other landscape parameters, particularly land-use and land disturbance, are expected to have a driving effect on methylmercury accumulation in these environments. Future studies of land use impacts on mercury levels in vernal pools would have an important implication for land use management over small spatial scales.
- Amphibians are sensitive to aquatic pollutants including mercury.
   More information is needed on the drivers of mercury uptake in amphibians across different ecosystem types. Future work looking at mercury loading in a broader suite of frog and salamander species and age classes, and assessing the controls on mercury accumulation, would provide a valuable contribution to the protection of these unique fauna.

## List of products

#### **Presentations:**

Vivien Taylor, oral presentation: Landscape and chemical influences on mercury cycling and bioavailability in vernal pools. International Conference on Mercury as a Global Pollutant. Providence, Rhode Island, 2017.

Kate Buckman, poster presentation: Methylmercury bioaccumulation in vernal pool invertebrates. International Conference on Mercury as a Global Pollutant. Providence, Rhode Island, 2017.

Steve Faccio, invited oral presentation: Bioaccumulation and Trophic Transfer of Methylmercury in Wood Frogs and Spotted Salamanders in Vermont Vernal Pools. Vermont Monitoring Cooperative Conference, Burlington, Vermont 2016.

Vivien Taylor, poster presentation: Spatial and temporal trends in methylmercury in Vermont Vernal Pools. Vermont Monitoring Cooperative Conference, Burlington, Vermont 2015.

#### Presentations cont'd:

Vivien Taylor, poster presentation: Mercury cycling in Vermont Vernal Pools. Vermont Monitoring Cooperative Conference, Burlington, Vermont 2014.

Amanda Curtis, poster presentation: Mercury body burdens in amphibians at different stages of life cycle in Vermont vernal pools. North Atlantic Chapter of the Society for Environmental Toxicology and Chemistry, Amherst, MA 2016.

Steve Faccio, poster presentation: Mercury body burdens in wood frogs and spotted salamanders in Vermont vernal pools. Northeast Parners in Amphibian and Reptile Conservation. Rhode Island. 2015.

#### Workshop contribution:

Vivien Taylor, analytical chemistry workshop presentation: Measuring mercury and methylmercury in vernal pools. International Conference on Mercury as a Global Pollutant. Jeju, Korea, 2015.

#### Papers in prep:

<u>Vivien Taylor</u>, Kate Buckman, Steve Faccio. Mercury cycling in Vermont Vernal Pools. For submission to Biogeochemistry, May 2018.

<u>Kate Buckman</u>, Steve Faccio, Vivien Taylor. Mercury bioaccumulation in vernal pool invertebrates. For submission to Environmental Toxicology and Chemistry, June 2018.

Steve Faccio, Kate Buckman, Amanda Curtis, Vivien Taylor. Bioaccumulation and trophic transfer of methylmercury in wood frogs and spotted salamanders. For submission to Ecotoxicology, March 2018.