# SALAMANDER RESPONSE TO CHANGES IN FOREST STRUCTURE CAUSED BY HEMLOCK WOOLLY ADELGID AND ELONGATE HEMLOCK SCALE INFESTATION

### ELIZABETH COLE

University of Illinois at Urbana-Champaign, Urbana, IL 61801 USA

# MENTOR SCIENTISTS: RADKA WILDOVA AND JONATHAN ROSENTHAL<sup>1</sup> <sup>1</sup>Ecological Research Institute, Kingston, NY 12401 USA

Abstract. Hemlock woolly adelgid (HWA; Adelges tsugae) and elongate hemlock scale (EHS; Fiorinia externa) infestations are causing increases in eastern hemlock (Tsuga canadensis) mortality rates throughout the eastern United States. In many instances, black birch (Betula lenta) replaces fallen hemlock, which can potentially alter forest structure and forest floor characteristics. Salamanders are extremely sensitive to changes in the environment and may be affected by the loss and replacement of eastern hemlock. This study compared environmental variables between hemlock plots and birch thickets to see how any differences were translated into differences in the salamander communities. Transects were surveyed in six different hemlock-dominated sites in a hemlock northern-hardwood forest. At each site, two transects were placed under hemlock trees and two in birch thickets that had established in large gaps opened after large hemlock trees died. In each transect, soil and leaf litter measurements were taken, canopy openness was assessed, and salamander and invertebrate abundances were recorded. Salamander and invertebrate abundances were greater under the hemlock trees than in birch thickets, although the difference was not significant, and no environmental variables showed significant differences between plot types. Salamander abundance was significantly positively correlated with leaf litter depth, moisture, average temperature, and invertebrate abundance. It may be that canopy openness and associated environmental variables have a greater influence on salamander habitat selection than the dominant tree species in plot.

#### INTRODUCTION

Forests are increasingly experiencing attacks by nonnative insects whose introduction has been facilitated by global trade (Lovett et al. 2016). In the United States, eastern hemlock trees (*Tsuga canadensis*) are slowly dying from infestations of hemlock woolly adelgid (HWA; *Adelges tsugae*) and elongate hemlock scale (EHS; *Fiorinia externa*), two species of insects native to Asia (Miller-Pierce and Preisser 2012). Although small, these insects can cause large-scale devastation in a relatively short amount of time, and tree mortality can range from zero to one hundred percent over the course of several years (Orwig and Foster 1998). As trees die, there is a decrease in crown density and increase in transparency and dieback (Eschtruth et al. 2006). Loss and alteration of hemlock is concerning because hemlock trees largely control forest microclimate and soil characteristics that other communities rely on for survival (Orwig and Foster 1998). Thus, understanding how forests are indirectly affected by hemlock loss caused by HWA and EHS is important for maintaining a healthy and diverse forest environment.

Hemlock loss can greatly alter forest floor characteristics and forest structure. Eschtruth et al. found that as hemlock health declines because of HWA infestation, understory light availability significantly increases (2006). As light availability increases, leaf litter cover decreases, while understory tree density, species richness, and percent cover of vegetation increases (Eschtruth et al. 2006). In many instances,

black birch replaces fallen hemlock. Orwig and Foster (1998) found that 76% of all plant seedlings growing in declining hemlock stands were black birch, which has significantly higher transpiration rates than hemlock (Daley et al. 2007). While an increase in understory tree density and percent cover of vegetation should shade and cool the forest floor, keeping the soil and leaf litter layer from drying out, it is uncertain how black birch's high water usage will affect forest floor characteristics over time.

Forest salamanders are extremely sensitive to changes in the environment and may be impacted by the loss and replacement of hemlock trees. Salamanders prefer cool, moist environments with a thick leaf layer and plenty of rocks and logs for cover (Pough et al. 1987; Heatwole 1962). As hemlocks die, there is an increase in plant cover, plant density, and logs from dead trees, which could provide the necessary temperature and moisture levels to support healthy salamander populations (Heatwole 1962). However, high water use by black birch may negatively affect salamanders because birch has the potential to alter the forest water balance (Daley et al. 2007). Additionally, salamanders are very sensitive to disturbances in the environment. Welsh and Hodgson (2013) found that salamander abundance was greater and overall health better in older forests due to the differences in structural complexity and stability. In particular, older forests tend to have more stable moisture levels and temperatures, making them better suited for salamander communities will be affected by a shift from hemlock-dominated forest to newly establishing black birch stands, this issue has not been previously investigated.

In this study, I looked at differences in environmental variables between hemlock plots and birch thickets, and how those differences affected the salamander community. Based on the known ecological effects of hemlock loss (Eschtruth et al. 2006) and environmental impacts of black birch (Daley et al. 2007), I hypothesized that hemlock and birch sites would have significantly different environmental conditions, and that salamanders would prefer hemlock sites to birch thickets. The number of salamanders detected was assumed to be indicative of the relative population levels in the plot types.

# METHODS

### Study Site

This study was conducted in the Northern Shawangunk Mountains at Mohonk Preserve, located in New Paltz, NY (41.74722° N, -74.20972° E). The preserve is composed of chestnut oak forests, hemlock forests, and pitch pine woodlands, and is home to 27 rare species of plants and animals.

# Experimental Design

Forest floor characteristics were measured in both hemlock- and birch-dominated areas. Six hemlock plots were selected from twenty-one pre-established, permanent one-acre plots that had at least 50% basal area of hemlock and some level of HWA infestation. The center of each hemlock plot had been marked with rebar. Birch thickets located nearest to the hemlock plots were used as the six birch-dominated areas for this study. Within each hemlock plot and birch thicket, two 1 x 12 meter transects running parallel to the mountain slope were created. Transects in hemlock plots were generally located five meters from the center of the plot on each side of the rebar. If there was birch thicket encroaching on the hemlock plot, transects were placed where the birch was not present, but always at least five meters apart. Birch transects were also placed in the center of the thicket, always with at least five meters between transects.

All data except for temperature were taken at the beginning, middle, and end of each transect. Data collected included soil and leaf samples to measure moisture, canopy openness, salamander abundance, diversity, and weight, and invertebrate abundance and diversity. Temperature was recorded by using iButtons (remote temperature data loggers,  $\pm 1$  °C) that were set to log temperature every two hours for a total of two weeks. iButtons were placed in a small Ziploc bag to protect them from the elements, and were placed between the two transects in each plot type at the six-meter mark, just below the leaf litter layer and above the soil layer. Daily average, maximum, and minimum temperatures were used in the analysis.

Using a gloved hand, soil and leaf litter samples were collected for each transect and placed in single Ziploc bags for their respective transect and variable type. Each bag was labeled to indicate the source transect, plot type, and plot number, and was shaken to ensure an even mixture. All samples were stored in a refrigerator at the Cary Institute of Ecosystem Studies in Millbrook, New York until testing. Leaf litter depth was found by measuring from the top soil layer to the top of the leaf layer with a ruler. In a lab at the Cary Institute of Ecosystem Studies, soil and leaf moisture were found by following the methods of Pough et al. (1987). Five grams of each sample were placed in pre-weighed tins and then dried for 48 hours at 60° C. After drying, the tins were weighed again, and percent moisture was calculated by dividing grams of water by grams of dried soil and then multiplying by 100. To determine soil pH, ten grams of soil and 20 ml of deionized water were placed in a jar to create a slurry. If the soil was very dry, more water was added 5ml at a time until it reached a slurry consistency. The pH of the soil water solution was then measured using a meter. The pH test results were averaged between the two transects for each plot and plot type.

Canopy opening was analyzed with the Gap Light Analysis Mobile App created by Lubomír Tichý (2014). Using a Blue Vivo Air LTE smartphone, photographs of the canopy were taken directly above beginning, middle, and end of each transect, and the percentage of open canopy was calculated for each picture using the app. Percentages were averaged from three measurements for each transect.

Salamander populations were surveyed using the techniques of Gustafson et al. (2001). Throughout each transect, the leaf litter layer was pulled back and examined for salamanders, and any rocks and logs were overturned. For each salamander found, species type was recorded and weight was calculated following the methods of Moore and Wyman (2010), such that salamanders were placed in a clear plastic Ziploc bag that was lightly sprayed with spring water, and weighed using a 10g Pesola spring scale. Gloves were changed after handling each salamander to prevent the spread of disease. Invertebrate type and abundance were also noted while surveying for salamanders.

# Statistical Tests

Mann-Whitney U tests were used to compare salamander abundance and environmental variables, as assumptions of parametric testing could not be met. All tests were analyzed using R (R Core Team) Principal component analysis was used to find correlations between salamander response variables and environmental variables using CANOCO 5.0 (ter Braak and Šmilauer 2012). Significance was determined at p<0.05.

### RESULTS

Salamander abundance (Table 2) was greater in hemlock plots than in birch thickets, although the difference was not significant (Fig. 1A, u= 25.5 p=0.247). A total of fifteen salamanders were found in hemlock plots, while seven were found in birch thickets. Total invertebrate abundance was also greater in hemlock plots (Table 2), with this difference being nearly significant (Fig. 1B, u= 30, p=0.064). Millipedes, the most abundant group in the invertebrate survey, had especially high abundance in hemlock plots, although it was not significantly different from birch (Table 2, u=27.5 p=0.148). All other environmental variables were not significantly different between hemlock plots and birch thickets (Table 1).

Salamander abundance was significantly and positively correlated with litter depth, litter moisture, or invertebrate abundance, and was significantly negatively correlated with average temperature (Figs. 2, 5). Salamander abundance was not correlated with soil pH or soil moisture or with canopy openness. Salamander weight was nearly significantly negatively correlated with average temperature, and nearly significantly positively correlated with leaf litter depth and invertebrate number (Fig. 3). Weight was not correlated with soil pH or moisture, leaf litter moisture, or canopy openness. Millipede abundance was not correlated with any of the measured environmental variables (Fig. 4,5).

Hemlock plots had greater variation in environmental variables and the salamander community than did the birch plots (Fig. 5). Hemlock plots (16, 10, and 9; see Fig. 5) either had cool, deep, moist leaf litter, or warm, shallow, and drier leaf litter (plots 14, 17, and 18; see Fig. 5). Birch thickets had less variation in environmental variables and tended to be warmer, with dry leaf litter (Fig. 5).

### DISCUSSION

The prediction that hemlock and birch environmental variables would differ significantly was not supported, as all environmental variables between plot types were similar. Hemlock plots varied widely in environmental conditions, as closed canopy plots were cool and moist, and open canopy plots were warm and dry. Thus, the unfavorable conditions associated with birch thickets may have occurred with the opening of the hemlock canopy before birch thicket grew. Because birch thickets are located on or near recently formed gaps in hemlock plots, environmental conditions of the thickets may still be similar to declining hemlock plots and may not be directly result from the birch itself.

In contrast to the results of the present study, prior studies have found that hemlock- and birch-dominated plots have significant differences in moisture and leaf litter. Daley et al. (2007) conducted a study at the Harvard Forest that measured the differences between hemlock and birch transpiration rates. Using whole-tree transpiration measurement techniques, they found that black birch had 1.6 times greater daily transpiration rates than hemlock, which amounts to 90 mm more water transpired per year by birch stands (Daley et al. 2007). The increase in water use would result in seasonal drying during the growing season, which would ultimately translate to a drier forest environment (Daley et al. 2007). Although my analysis shows that black birch tended to have drier leaf litter than some hemlock plots, the difference was not significant, in contrast to Daley's study. This could be because I used different techniques to measure moisture. In a different study, Cobb (2010) compared leaf litter characteristics between hemlock and birch sites in New England forests. He found that black birch leaf litter would be more shallow in birch plots.

This is similar to the findings of the present study, which found that healthier hemlock plots have thicker and moister leaf litter than most of the birch plots; however, this difference was not significant.

The hypothesis that salamanders prefer hemlock over birch thickets was not supported. While more salamanders were found in hemlock plots, the difference was not significant, which may have been due to the variability of the canopy openings and associated environmental conditions within hemlock and birch plots. Closed canopy hemlock plots had more favorable environments for salamanders as they were cooler and moister, while open canopy hemlock plots and birch thickets had a less favorable environment for salamanders, as they were warmer and drier. Thus, canopy openness and associated environmental variables may have had a greater influence on salamander habitat selection than the dominant tree species in plot.

Previous research has shown that forest salamanders, specifically red-backed, are found in greater abundance in areas with smaller canopy gaps, less frequent disturbance, and greater litter moisture and depth (Reidel 2008, O'Shea in press). A study by O'Shea (in press) found that red-backed salamander abundance decreased with increasing canopy gap. These findings are similar to those of the present study, as hemlock sites with smaller canopy openings were found to be cooler and moister, which could account for the greater salamander abundance seen at those sites. Likewise, Reidel (2008) found that environmental conditions such as vegetation cover and soil moisture have a greater influence on salamander abundance than habitat type (e.g., forest edge versus forest interior). Thus, environmental conditions could explain salamander presence regardless of the dominant species present. These findings are similar to the present study, as less-disturbed hemlock sites and birch thickets were warmer, drier, and salamander abundance, while declining hemlock sites and birch thickets were warmer, drier, and had fewer salamanders. Therefore, environmental conditions could have had a greater impact on salamander abundance than the dominant species type (birch or hemlock).

The major limitation of the study was the lack of hemlock plots with nearby birch thickets. With only six plots each for hemlock and birch thickets, the sample size was relatively small. A future study could use more plots to compare differences between hemlock and birch sites. Other future studies may look at structural differences between plot types, as hemlock and birch had different stem densities and log coverage, both of which influence salamander abundance (Heatwole 1962). Additionally, it is important to examine the stability of black birch moisture levels and temperature, as salamanders will likely not live in areas with an unstable environment.

Hemlock woolly adelgid and elongate hemlock scale have the potential to alter forest ecosystems. This study showed that the loss and replacement of hemlock may potentially negatively impact forest salamander communities, as shifts in forest structure may alter leaf litter moisture, depth, and forest floor temperature. Future studies combined with management techniques will have to be implemented to ensure changes in forest structure do not significantly alter communities of organisms dwelling within the forest.

### ACKNOWLEDGMENTS

Funding for this project was provided by the National Science Foundation through the Research for Undergraduate Program. A special thanks to Radka Wildova and Jonathan Rosenthal for mentoring me throughout the project, the Cary Institute of Ecosystem Studies for their support and assistance, and Andrew Nunn for help with surveying salamanders.

#### LITERATURE CITED

- Cobb, R. 2010. Species shift drives decomposition rates following invasion by hemlock woolly adelgid. Oikos **119**:1291-1298.
- Daley, M.J., N.G. Phillips, C. Pettijohn, and J.L. Hadley. 2007. Water use by eastern hemlock (*Tsuga canadensis*) and black birch (*Betula lenta*): implications of effects of the hemlock woolly adelgid. Canadian Journal of Forest Res 37:2031-2040.
- Eschtruth, A.K., N.L. Cleavitt, J.J. Battles, R.A. Evans, and T.J. Fahey. 2006. Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. Canadian Journal of Forest Res **36**:1435-1450.
- Gustafson, E.J., N.L. Murphy, and T.R. Crow. 2001. Using a GIS model to assess terrestrial salamander response to alternative forest management plans. Journal of Environmental Management **63**:281-292.
- Heatwole, H. 1962. Environmental factors influencing local distribution and activity of the salamander, *Plethodon cinereus*. Ecology **43**:460-472.
- Lovett, G.M, M. Weiss, A.M. Liebhold, T.P. Holmes, B. Leung, K.F. Lambert, D.A. Orwig, F.T. Cambell, J. Rosenthal, D.G. McCullough, R. Wildova, M.P. Ayres, C.D. Canham, D.R. Foster, S.L. LaDeau, and T. Weldy. 2016. Nonnative forest insects and pathogens in the United States: impacts and policy options. Ecological Applications 26:1437-1455.
- Miller-Pierce, M.R. and E.L. Preisser. 2012. Asymmetric priority effects influence the success of invasive forest insects. Ecological Entomology 37:350-358.
- Moore, J.D. and R.L. Wyman. 2010. Eastern red-backed salamanders (*Plethodon cinereus*) in a highly acid forest soil. The American Midland Naturalist **163**:95-103.
- Orwig, D.A., and D.R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Society **125**:60-73.
- O'Shea, D. In press. Factors affecting the distribution and abundance of salamanders at the Huyck Preserve.
- Pough F.H., E.M. Smith, D.H. Rhodesi, and A. Colla. 1987. The abundance of salamanders in forest stands with different histories of disturbance. Forest Ecology and Management **20**:1-9.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Riedel, B. L., K.R. Russell, W.M. Ford, K.P. O'Neill, and H.W. Godwin. 2008. Habitat relationships of eastern red-backed salamanders (Plethodon cinereus) in Appalachian agroforestry and grazing systems. Agriculture, Ecosystems & Environment 124:229-236.
- ter Braak, C.J.F. and P. Šmilauer. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca.
- Tichý, L. 2014. Gap light analysis mobile app [mobile phone application]. Retrieved June 30, 2016.
- Welsh, H.H. and G.R. Hodgson. 2013. Woodland salamanders as metrics of forest ecosystem recovery: a case study from California's redwoods. Ecosphere **4**:1-25.
- Wyman, R.L, and D.S. Hawksley-Lescault. 1987. Soil acidity affects distribution, behavior, and physiology of the salamander *Plethodon cinereus*. Ecology **68**:1819-1827.

### APPENDIX

	Birch		Hemlock		Mann- Whitney U test value	Significance (p=0.05)
Environmental variable	mean	SD	mean	SD		
soil pH	3.98	0.43	3.98	0.79	14	p=0.589
soil moisture (%)	120	34.67	112	38.81	19	p=0.937
leaf moisture (%)	97	47.62	100	48.61	19	p=0.937
leaf thickness (cm)	3.15	0.99	3.66	1.33	23	p=0.485
canopy open (%)	5	3.34	4	1.48	17.5	p=1.0
max temperature (°C)	29.9	5.34	29	4.21	18.5	p=1.0
mean temperature (°C)	20.63	0.56	20.76	1.05	18	p=1.0
temperature range (°C)	15.17	6.0	14	4.84	17	p=0.936

TABLE 1. Means and standard deviations of environmental variables for all birch and hemlock sites.

**TABLE 2.** Means, standard deviations, and total numbers of invertebrates and salamanders for all birch and hemlock sites.

	Birch			Hemlock			Mann- Whitney U test value	Significance (p=0.05)
	total	mean	SD	total	mean	SD		
Millipede abundance	70	5.83	7.19	159	13.25	11.78	27.5	p=0.148
Invertebrate abundance	124	20.7	12.38	267	44.5	22.7	30	p=0.064
Salamander abundance	7	1.2	1.3	15	2.5	2.07	25.5	p=0.247



**FIGURE 1.** A) Comparison of hemlock and birch total salamander abundances; B) Comparison of hemlock and birch total invertebrate abundances



**FIGURE 2.** Correlation of total salamander abundance (all hemlock and birch plots together) with invertebrate abundance and environmental variables.



**FIGURE 3.** Correlation of salamander weight with environmental variables (all hemlock and birch plots together).



**FIGURE 4.** Total millipede abundance correlations with environmental variables (all hemlock and birch plots together).



**FIGURE 5.** PCA (Principal component analysis) of salamander and invertebrate (only millipedes are shown) communities and their correlation with environmental variables (projected passively onto the ordination space).