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Source: Copeia, 2013(2):254-261. 2013.

Published By: The American Society of Ichthyologists and Herpetologists

DOI: <http://dx.doi.org/10.1643/CE-12-051>

URL: <http://www.bioone.org/doi/full/10.1643/CE-12-051>

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# Effect of Sub-Canopy on Habitat Selection in the Blue-spotted Salamander (*Ambystoma laterale-jeffersonianum* unisexual complex)

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**Elucidating the mechanisms that influence spatial distribution patterns is vital to understanding how populations persist. We examined distribution in one of the most common salamanders in southeastern Michigan, the Blue-spotted Salamander (*Ambystoma laterale-jeffersonianum* unisexual complex). Three major tree species dominate the sub-canopy in the study site and form monospecific patches, which may have an effect on the distribution of terrestrial phases of local amphibian species. To examine this, we tested whether adult salamanders are significantly associated negatively or positively with patches of particular tree species. We then examined two potential causal factors: habitat quality, using leaf litter macroinvertebrate biomass as a proxy, and behavioral site choice in the Blue-spotted Salamander. We found that this species is positively associated with patches of red maple and negatively associated with patches of black cherry. We also found that, when presented with a choice, salamanders choose red maple leaf litter over black cherry leaf litter. In the absence of differences in physical leaf litter characteristics between patches of red maple and black cherry, we suggest that the Blue-spotted Salamander may be utilizing chemical cues to select habitat. Our findings imply that salamander populations may be affected not only by habitat loss, but also by changes in forest composition. These results provide evidence for a more complex model than the traditional amphibian metapopulation concept, where even fully forested habitat may form a matrix of optimal and sub-optimal, or even intolerable, patches.**

**E**LUCIDATING the underlying processes that govern amphibian migration and distribution is key to determining how populations persist. Identifying mechanisms that contribute to limited amphibian distribution may allow us to predict metapopulation extinction risks, which have conservation and management implications (Smith and Green, 2005). The cues that influence amphibian habitat selection and use could indicate performance constraints and help predict future migration barriers (Rittenhouse and Semlitsch, 2006).

The majority of amphibians are biphasic, which has led to habitat classifications that describe the terrestrial stage in terms of coarse land cover divisions. Thus, although it has been well established that terrestrial forms of amphibians prefer forest habitat (Rittenhouse et al., 2004; Rittenhouse and Semlitsch, 2006; Greenwald et al., 2009), the finer distinctions within terrestrial habitats are only beginning to be understood. At the scale of the individual, amphibian refugia have been associated with certain forest microhabitat characteristics, such as leaf litter abundance, presence of coarse woody debris, and presence of small mammal burrows (Faccio, 2003). At the population or community level, multiple habitat quality factors, including moisture, humidity, and temperature are often cited as major drivers of amphibian distribution. However, Rittenhouse et al. (2004) argue that amphibians may be unable to detect differences in moisture and temperature at the fine scale at which semi-permanent sites are selected. In addition, the majority of salamanders disperse at night when visual cues may not be perceptible, or during rainy or overcast days when soil is saturated with moisture (Semlitsch, 1985; Madison, 1997). Therefore, in studies of habitat selection, it is necessary to examine cues at multiple scales, and to incorporate behavioral aspects of site selection in describing ideal habitats and dispersal routes (Joly et al., 2008).

Large-scale influences on amphibian spatial distribution include forest composition and proximity to bodies of water (Burne and Griffin, 2005; Renaldo et al., 2011). Coniferous forests are actively avoided in favor of deciduous forests by salamanders (Renaldo et al., 2011), but heterogeneity within deciduous forests may influence distribution throughout the habitat. Forests are often patchy (Condit et al., 2000; Allen and Vandermeer, unpubl.), meaning the habitat matrix through which amphibians disperse is not uniform. Qualities of the habitat matrix may influence both distribution and dispersal, and potentially isolate metapopulations. This would give rise to discrete clusters of associated species, including salamanders and other amphibians that rely on woody debris for refugia and overwintering sites (Hyde and Simons, 2004). For example, differences in forest disturbance, such as at edges of forests or between forest patches of different maturity, present different levels of permeability for migrating terrestrial amphibians (Gibbs, 1998; Homan et al., 2008). Beyond larger-scale terrestrial habitat characteristics, the effects of heterogeneity within forests on amphibian distribution remain relatively unexplored.

In this study, we examined spatial distribution in the Blue-spotted Salamander (*Ambystoma laterale-jeffersonianum* unisexual complex), one of the most common salamanders in southeastern Michigan. As in many temperate forests, the sub-canopy composition in the study site is shifting (Allen and Vandermeer, unpubl.), which we hypothesize will have an effect on the amphibian community. We examined to what extent salamander distribution is significantly influenced by particular understory tree species to detect any positive or negative associations. These associations would respectively imply preference or avoidance of monospecific understory patches. A prior study demonstrated that soil moisture does not significantly differ between these under-

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Submitted: 12 April 2012. Accepted: 19 November 2012. Associate Editor: J. W. Snodgrass.

© 2013 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CE-12-051

story patches in our study site (Allen, 2012), so we investigated the potential relationship between salamander aggregation and four other measures of habitat quality: depth, biomass, and moisture content of leaf litter, as well as litter macroinvertebrate biomass as an estimate of food abundance (Bolek, 1997). We then employed a behavioral experiment to determine whether salamanders use cues from the substrate to select the “preferred” habitat.

## MATERIALS AND METHODS

**Study site.**—The Edwin S. George Reserve (ESGR) is a 525-hectare preserve owned by the University of Michigan, and is located in Livingston County, Michigan. The Reserve is used for a variety of biological surveys and ecological studies, notably long-term studies of larval amphibians (Werner et al., 2007). The focal area for this study is known as the Big Woods Plot, and is located in the Northeast of the ESGR (42°27′44.234″N, 84°0′19.739″W). Recent studies of the spatial structure of this hardwood forest have revealed that individuals of a single tree species are spatially clustered into dense patches that are almost exclusively monospecific (Allen and Vandermeer, unpubl.). Historically, oak trees (*Quercus* spp.) dominated the overstory and understory canopies of the forest, but more recent fire suppression has shifted the forest sub-canopy to a predominantly shade tolerant composition (Abrams, 1992, 1998). This sub-canopy is dominated by witch-hazel (*Hamamelis virginiana*), black cherry (*Prunus serotina*), and red maple (*Acer rubrum*). The overstory of primarily oak and hickory is relatively uniform (Allen and Vandermeer, unpubl.), providing a constant background of leaf cover within which the highly non-uniform leaf input from the three understory species release their leaves.

**Study organism.**—We recorded all amphibian species observed in the initial survey (observational study), but focused our further experiment and analysis on the most common salamander observed, the Blue-spotted Salamander (*Ambystoma laterale-jeffersonianum* unisexual complex, Bogart and Klemens, 2008). This species complex is known to overwinter under logs or other woody debris (Rittenhouse et al., 2004), breed in the late spring (Regosin et al., 2005), and feed on small invertebrates (Bolek, 1997). The species is also known to be fossorial and exhibit limited dispersal (E. Werner, pers. comm.).

**Observational study.**—We surveyed the amphibian community in four 100 m<sup>2</sup> plots within the Big Woods (Fig. 1). The four-hectare area sampled was selected to compare the location of amphibians to the distribution of the three major understory tree species that occur in varying densities throughout the site. In a previous study, all trees larger than 3 cm diameter at breast height (DBH) were identified, tagged, geo-referenced, and measured (Allen et al., 2009). Tree location, via tag number, was used to estimate the location of every amphibian encountered. Within the Big Woods, there are two ponds (Fig. 1) in which Blue-spotted Salamanders are known to breed and undergo larval development (Werner et al., 2007). The surrounding forest contains many decomposing logs and forest floor litter that provide important habitat for terrestrial herpetofauna (Hyde and Simons, 2004).

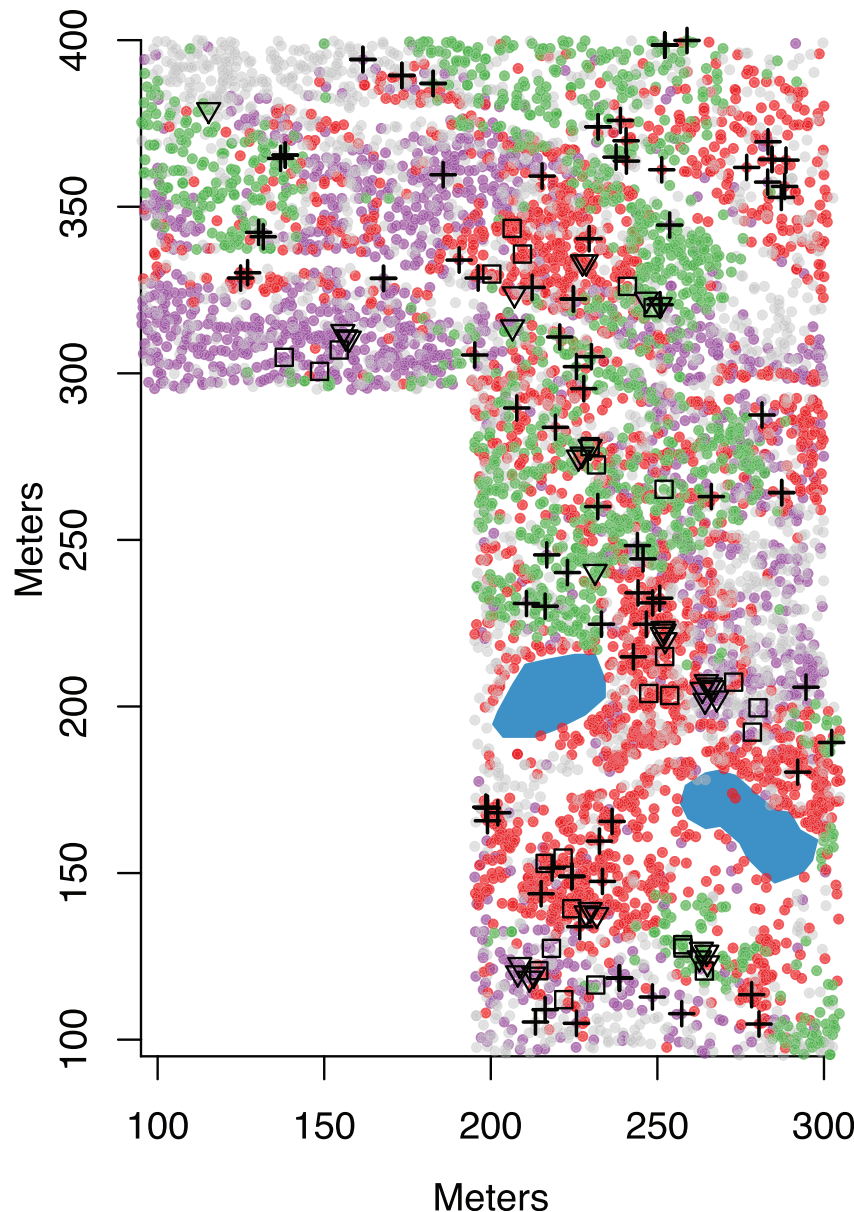
Eight surveyors sampled for approximately five hours on 25 September 2011. Sampling techniques included inspection

under natural cover objects along transects (Hyde and Simons, 2004) and opportunistic visual searching. Abundance under refugia was used as a proxy for species abundance, as ambystomatids are thought to primarily remain under cover objects during the day and migrate at night (Conant and Collins, 1998). Surveyors walked in parallel 12 m wide transects within each hectare, and examined all cover objects within the transect in order to census the entire plot. For each amphibian observed, the tag number of the nearest tree was noted and used as a proxy for location within a monospecific understory patch. Snout-vent length (SVL) was additionally recorded for each salamander captured. Individuals were not sexed because this area of Michigan is a zone of unisexual hybridization between *A. laterale* and *A. jeffersonianum* (Bogart and Klemens, 2008). Unisexual females have been found to make up the vast majority of a breeding aggregate (84% in Lowcock et al., 1991), and there is no significant sexual dimorphism within bisexual-unisexual complexes (Lowcock et al., 1992). Following measurement, surveyors replaced both the animal and the disturbed bark or log to minimize micro-habitat disturbance.

**Habitat quality measurements.**—We collected leaf litter samples from patches of each major understory tree species to determine if there was a difference in physical habitat quality between sub-canopy patches. Litter was collected from three randomly chosen quadrats in each of three black cherry, red maple, and witch-hazel patches within the four-hectare study area to measure leaf litter biomass, depth, and water content, for a total of 27 samples. This method was then repeated in three separate quadrats in each of three patches of each species to collect macroinvertebrates, for a grand total of 54 samples (Fig. 1). Each sample was collected from within a 0.25 m<sup>2</sup> quadrat, with samples within each patch randomly clustered within 10 m of one another. Leaf litter depth was measured with transparent rulers to the nearest millimeter. Leaf litter was collected to the soil surface and oven dried at 65°C for 1.5 hours. The dry weight in milligrams was measured as the litter biomass, and the difference in weight before and after drying was used to calculate percent moisture content of the litter. To examine macroinvertebrate biomass, all leaf litter and the top humus layer within each designated quadrat was collected. In the laboratory, a 5 mm screen sieve was used to separate coarse and fine litter and debris. Each of these subsamples was then visually inspected for macroinvertebrates. Search time of each sample was limited to ten minutes to ensure equivalent sampling effort. Macroinvertebrates were dried and weighed to obtain biomass.

**Habitat choice experiment.**—Initial analyses revealed that the distribution of Blue-spotted Salamanders was significantly associated with black cherry and red maple. We set up experimental “leaf litter choice” terraria to test for a behavioral mechanism for apparent habitat preferences with regard to these two habitat types (Rittenhouse et al., 2004). Soil and leaf litter samples were collected from a red maple patch and a black cherry patch within the Big Woods Plot (Fig. 1). Samples were collected from three randomly selected 50 by 50 cm plots, no further than three meters apart, within each patch. These samples included a mix of leaves from the sub-canopy and overstory, so that the natural conditions encountered by salamanders would be replicated.

Five 38-liter (10-gallon) glass terraria were lined with an equal depth (three to five cm) of red maple leaf litter on one



**Fig. 1.** Distribution of Blue-spotted Salamanders and tree species within the sampled area. Blue-spotted Salamanders are marked with a "+" and understory trees with a dot: green are witch hazel (*Hamamelis virginiana*), red are red maple (*Acer rubrum*), purple are black cherry (*Prunus serotina*), and gray are all other understory tree species. The two blue polygons represent the ponds within the plot. Sampling quadrats for leaf litter measurements are indicated with boxes, and quadrats for macroinvertebrate collection are indicated with triangles. The treeless stripes are walking trails that have become overgrown in recent years.

half and black cherry leaf litter on the other half. The middle was lined with a strip of white filter paper (5 cm in width) which is an aversive substrate for salamanders (Rittenhouse et al., 2004). Individuals were placed on the center line of the filter paper, parallel to the boundaries of each section of leaf litter. Observations were recorded for ten minutes to eliminate the effect of initial leaf litter choice, which is likely an artifact of salamanders seeking a refuge quickly (Graeter et al., 2008). The terraria were rotated 180° between trials to eliminate sun direction or left/right effects, and filter paper was replaced between trials to eliminate potential influence of the previously tested salamander.

The experiment was conducted on 9 October 2011 between 1000 and 1300 h, when the ambient temperature permitted salamander movement. Although migration in

temperate salamanders is known to occur primarily at night (Rittenhouse et al., 2004), we were interested in habitat selection, and therefore selection of refugia. Because of this we deemed daytime to additionally provide an incentive for the salamanders to select a preferred refuge. Sixteen Blue-spotted Salamanders, collected from within the Big Woods, were individually tested for a behavioral habitat choice. Salamanders were collected from the adjacent area, and although we searched in both black cherry and red maple patches, we only found salamanders in red maple. Trials were done in the field such that animals were tested immediately after capture and released after the experimental trial. Observers took care not to disturb the salamanders during the experiment by remaining motionless and standing approximately 2 m from terraria after the start of each trial.



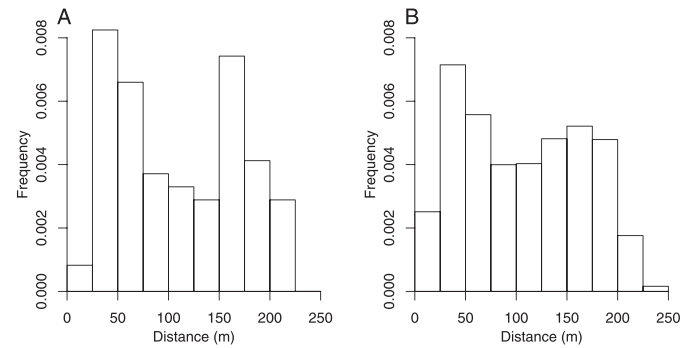
**Data analysis.**—We used resampling analyses to test whether the spatial distribution of Blue-spotted Salamanders was affected by either distance to closest pond or local tree species. To test for the effect of distance to closest pond we created a model (R v. 2.11.1) in which the same number of Blue-spotted Salamanders we found in our survey were placed randomly within a digitized version of the survey plot 10,000 times. For each of these 10,000 random placements we calculated the mean distance, across all ‘salamanders,’ to the nearest pond edge. We compared the distribution of predicted mean distances to the observed mean distance from Blue-spotted Salamanders to closest pond edge, to see whether the observed salamanders were on average closer to or farther from the ponds than expected if they were randomly distributed. We then compared the frequency distribution of observed distances to the frequency distribution of randomly created distances using a two-sample Kolmogorov-Smirnov test. Thus, even if the means were not different we could detect whether the observed distribution of distances was different from the distribution of distances of randomly placed salamanders.

We used modified Ripley’s K analyses (Ripley, 1981) to determine whether Blue-spotted Salamanders were more likely to be found around specific trees, and if so, at what scale. A distribution of expected results was again generated from 10,000 random ‘salamander’ placements. Using the actual distribution of witch-hazel trees (identified *post hoc* according to the tag number recorded as a proxy for amphibian location) we counted the number of random ‘salamanders’ found in 1-meter wide rings of radii ranging from 1 to 25 meters. We then averaged over all of the witch-hazel trees. We employed the same method to quantify actual Blue-spotted Salamanders around individual witch-hazel trees. This allowed us to evaluate whether there were more or fewer than predicted if the salamanders were distributed randomly (see Jolles et al., 2002 for a similar application of Ripley’s K). We repeated this analysis for red maple and black cherry.

Leaf litter, macroinvertebrate, and behavioral choice data were analyzed in SPSS (IBM, v. 20, Chicago, 2011), and SVL data were analyzed in R (v. 2.11.1). One-way ANOVAs were used to compare mean differences in leaf litter characteristics and macroinvertebrate biomass across the three tree species, as well as differences in mean salamander SVL according to closest tree species. Assumptions of ANOVA were verified using Levene’s test for equality of error variances and Q-Q plots for normality of the residuals. Tukey’s HSD was used for *post-hoc* pairwise comparisons. Behavioral choice data were analyzed using chi-square tests.

## RESULTS

**Observational study.**—Within the four hectares sampled at the E.S. George Reserve, 277 amphibians were found, including four species of salamanders and three species of frogs. In total, 97 Blue-spotted Salamanders were observed during the survey. The Blue-spotted Salamanders observed were, on average, 110.0 meters from the edge of the closest pond. This did not significantly differ from the means of the random distribution of ‘salamanders’ generated in our model, 95% of which fell within 94.4 and 117.0 meters ( $P = 0.304$ ). When the distance from pond frequency distributions were compared (Fig. 2), observed salamander distribution was not significantly different from the random



**Fig. 2.** Histograms of Blue-spotted Salamander distance to nearest pond. (A) Frequency of distances of observed salamanders to nearest pond. (B) Frequency of distances of an equal number of ‘salamanders’ randomly placed within the survey plot 10,000 times.

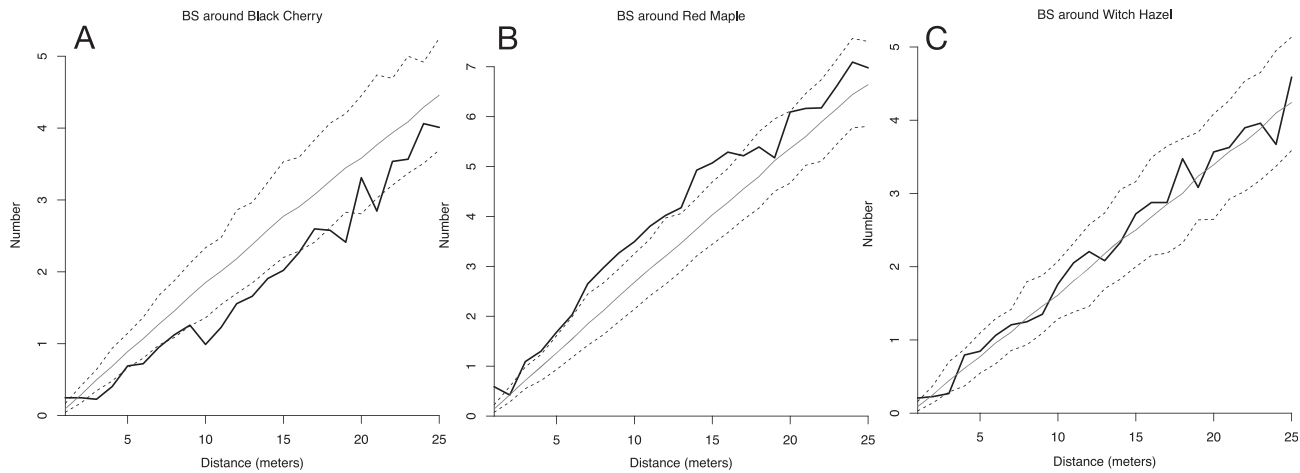
model ‘salamander’ distribution (two-sample Kolmogorov-Smirnov test,  $D = 0.1174$ ,  $P = 0.1437$ ).

However, the distribution of Blue-spotted Salamanders was strongly affected by understory tree species. Significantly fewer salamanders were found 2 to 20 meters from black cherry trees than expected if the salamanders were distributed randomly across the study site (Fig. 3A). Conversely, significantly more salamanders were observed 2 to 15 meters from red maple than predicted from a random distribution of ‘salamanders’ (Fig. 3B). The observed distribution of the salamanders around witch-hazel trees was not significantly different from random (Fig. 3C). Mean salamander SVL was not different across the three tree species ( $F_{2,24} = 1.044$ ,  $P = 0.356$ ).

**Habitat quality measurements.**—No significant differences were found in leaf litter biomass ( $F_{2,24} = 1.620$ ,  $P = 0.219$ ), depth ( $F_{2,24} = 0.327$ ,  $P = 0.725$ ), or percent moisture content ( $F_{2,24} = 2.484$ ,  $P = 0.105$ ) across the three patch types. Mean macroinvertebrate biomass was significantly lower in black cherry than in witch-hazel patches ( $F_{2,24} = 8.684$ ,  $P = 0.001$ ). On average, witch-hazel contained the highest arthropod biomass among the three species ( $0.1218 \text{ g} \pm 0.04731 \text{ SE}$ ), followed by red maple ( $0.0482 \text{ g} \pm 0.1588 \text{ SE}$ ) and then black cherry ( $0.0193 \text{ g} \pm 0.00445 \text{ SE}$ ). No significant difference was found between witch-hazel and red maple, or between red maple and black cherry macroinvertebrate biomass.

**Habitat choice experiment.**—Salamanders selected red maple leaf litter at the end of ten minutes in 12 out of 16 trials. This was significantly different from random refuge selection ( $\chi^2_1 = 4.000$ ,  $P = 0.0455$ ). Observed behavior of individual salamanders revealed what seemed to be an avoidance of the black cherry litter. On three occasions an individual was seen initially entering the black cherry litter, but in all three cases in less than one minute the salamander returned to the filter paper to later move into the red maple litter. The reverse, first entering red maple litter and then moving to black cherry, was observed only once.

Because only filter paper was changed between trials, we tested whether there was a significant effect of previous choice on subsequent refuge selection. We performed a chi-square test on the second through fourth trials (since terraria were cleaned and housed no other salamanders before the first trial) and found no significant effect of previous choice ( $\chi^2_1 = 0.818$ ,  $P = 0.366$ ).



**Fig. 3.** In each of the three subfigures, solid black lines show mean number of actual Blue-spotted Salamanders found within 1-meter 'donuts' of various radii of different focal tree species within the study plot. Using 10,000 random placements of the same number of 'salamanders' we constructed an expectation due to random distribution, with 95% confidence interval, which are shown by solid and dashed gray lines, respectively. (A) The focal tree is black cherry. There were significantly fewer salamanders found 2 to 20 meters from black cherry trees than expected. (B) The focal tree is red maple. There were significantly more salamanders observed within 0 and 15 meters of red maple trees than expected. (C) The focal tree is witch-hazel. The distribution of salamanders is no different from random with respect to distance to witch-hazel trees.

## DISCUSSION

**Spatial distribution patterns.**—The Blue-spotted Salamander was observed less than expected from a random distribution within black cherry patches and more than expected from a random distribution within red maple patches (Fig. 3A, B). Within the witch-hazel patches the number of salamanders was not significantly different from what would be expected from a random distribution (Fig. 3C). These results suggest that this species either avoids black cherry, prefers red maple, or both. In our survey data, we found no significant differences in mean salamander SVL among the three major understory tree species, suggesting that salamanders are selecting similar habitat regardless of age/size class.

Because the Blue-spotted Salamander has a fully aquatic larval stage (Conant and Collins, 1998) we might expect a higher density of recently emerged juveniles and/or breeding adults in areas surrounding ponds. The observational study results could therefore be an artifact of an increased occurrence of red maple around the ponds in the study plot. However, salamanders were not found to be more significantly aggregated around the ponds than predicted by a random distribution, which eliminates pond distance as a confounding variable for the distribution with respect to sub-canopy. The three trails found in the study site (Fig. 1) have the potential to influence salamander distribution as well. However, salamanders were observed under refugia within one meter of these paths, so we assumed that these are unlikely to have had a significant effect on dispersal.

**Behavioral site choice.**—The choice experiment indicated a behavioral mechanism in which Blue-spotted Salamanders select red maple substrate over black cherry substrate. Because the same leaf litter was used in subsequent behavioral choice tests, there is the possibility that salamanders followed chemical cues from previous individuals in selecting substrate. Salamanders have been widely documented to use olfactory cues for orientation (Shoop, 1965; Stenhouse, 1983; McGregor and Teska, 1989) and avoidance of predators (Elliott et al., 1993; Marvin et al., 1994; Chivers et al., 1996); however, this has not been

documented in the Blue-spotted Salamander. Further, Graves and Quinn (2000) showed that chemical cues used by conspecifics in Red-backed Salamanders (*Plethodon cinereus*) are very short-lived, persisting in the environment for only five minutes. If this is generalizable across species, the time spent releasing salamanders and changing filter paper between trials would have been sufficient for chemical cues to dissipate. Nevertheless, the presence of conspecific cues in the terraria remains a potential caveat of our experiment, though our results do not support the idea that previously tested salamanders influenced choice in subsequent trials.

The results of the choice experiment imply active selection of particular refugia by Blue-spotted Salamanders, corroborating the spatial patterns found in our observational study. However, because we did not offer salamanders a choice between each habitat type and a neutral substrate, it remains unclear whether red maple is always a preferred habitat, or black cherry is always avoided. Further choice tests have suggested an avoidance of black cherry when salamanders are presented a choice between a neutral substrate soaked with a solution of black cherry "tea" (leaf litter soaked in rainwater, then strained) versus a plain rainwater soaked substrate (Belasen, unpubl. data). However, this test was performed later in the season when it was clear that the salamanders had begun to respond to the onset of winter, and therefore these data were omitted from this study.

**Preference, avoidance, or both?**—There were no differences found in leaf litter biomass, depth, or percent moisture content across the three patch types. However, we did detect a difference in leaf litter macroinvertebrate biomass. If macroinvertebrates are the major driver in salamander site selection, we may expect to see a hierarchy following the observed spatial distribution of Blue-spotted Salamanders, where red maple patches host the highest biomass, followed by witch-hazel and then black cherry. Since this was not the trend shown in our analysis, red maple may be a higher quality or "preferred" habitat for reasons other than food abundance. The clustered distribution of the salamanders around red maple could be related to the relatively high

decomposition rate of red maple leaf litter (Day, 1982; Aber et al., 1990), which may contribute some other beneficial quality to the salamander's habitat in these patches that was not measured in this study.

Alternatively, salamanders could be utilizing other cues in habitat selection. Since we did not detect a difference in physical quality of leaf litter between patches, we posit that Blue-spotted Salamanders may be selecting refugia based on chemical cues in the substrate, a phenomenon that has been observed in other salamander species (Mushinsky, 1975; Wyman and Jancola, 1992). We hypothesize that the possible avoidance of black cherry patches may be related to detection of cyanogenic chemicals in the surrounding habitat (Swain et al., 1992). Like many other stone fruit trees of the Rosaceae family, black cherry is known to contain strongly cyanogenic compounds (Swain et al., 1992). Poisonous glycosides and catabolic enzymes are compartmentalized within plant matter while it is whole, but these chemicals mix upon laceration producing hydrogen cyanide (Kingsbury, 1964; Poulton, 1990). Amphibian skin allows for cutaneous respiration and osmoregulation and lacks protective structures, which renders these animals particularly vulnerable to environmental irritants (Whitford and Hutchinson, 1963; Pessier, 2002). The effects on amphibians of exposure to low levels of cyanide are not well understood, although toxicity at high concentrations has been well established (Eisler, 1991). The potential link between the intolerable chemicals contained within black cherry leaf litter and the environmental sensitivity of amphibians could help explain the spatial distribution of Blue-spotted Salamanders found in our analysis; however, further research and chemical analysis of the leaf litter within our study site would be necessary in order to support this conclusion.

Qualities of the leaf litter and arthropod populations in monospecific tree patches may also have longer-term implications for survival. Based on our experimental results, in which black cherry substrate was avoided in the absence of other influencing factors, we suggest that this tree species could have either a direct negative effect on Blue-spotted Salamanders or an indirect effect mediated through invertebrate food sources. Many insects are known to preferentially feed on cyanogenic plants and sequester cyanide compounds for chemical defense or metabolic functions (Zagrobelny et al., 2008). The effects of cyanide entering the detritus pool via insect frass (Fitzgerald, 2007) on habitat quality are not well-understood, nor are the impacts of consuming the insects. These may be unpalatable or toxic to salamanders, thus exacerbating the already lower macroinvertebrate density found in these patches.

Because lower arthropod biomass exists in black cherry patches, these areas may be less suited to the Blue-spotted Salamander's nutritional needs. This low nutritional quality could serve as a reliable signal for habitat type or quality and may have contributed to an evolved behavioral selection mechanism. Salamanders have been shown to prefer olfactory cues of home ponds over unfamiliar ponds (McGregor and Teska, 1989). For terrestrial juveniles and adults, home habitats are not ponds but vegetation patches. Olfactory cues used to locate home or preferred patches may be associated with the leaf litter substrate, and therefore with higher quality habitat or more abundant food resources.

**Metapopulations and the terrestrial habitat matrix.**—The distribution of terrestrial salamanders is governed in part by their need for moisture and protective cover (Degraaf and

Yamasaki, 2002). These habitat requirements have been examined at varying scales: forest vs. field (Rothermel, 2004), stand vs. sapling (Degraaf and Yamasaki, 2002), and in this study, preferred vs. non-preferred sub-canopy patches. Although prior studies have shown that moisture and humidity (Heatwole, 1962) and temperature (Belden et al., 2000) may influence salamander movement and distribution, our species-specific study adds to a body of evidence that finer-scale microhabitat cues could play an important role (Rittenhouse et al., 2004). At this scale, Blue-spotted Salamanders appear to preferentially occupy red maple habitat over potentially unsuitable black cherry habitat.

Determining the density at which patches of red maple or black cherry become a preferred or unfavorable environment for Blue-spotted Salamanders is important in understanding the distribution patterns and habitat use of this species. These trees occur in different monospecific patch densities within the study plot (Fig. 1) and across their overlapping distributions (Allen and Vandermeer, unpubl.). Our study suggests that 15 m and 20 m encompasses the approximate range of influence that red maple and black cherry respectively have on a Blue-spotted Salamander's reception of environmental cues. These distances likely represent the area around the tree for which the effects on salamander habitat choice demonstrated in our experiments hold true. This may reflect the size of the leaf-litter shadow or the radius to which these trees influence soil and leaf-litter biota, which, in turn, may affect Blue-spotted Salamander habitat suitability and dispersal.

The limited dispersal ability observed in the focal species is a defining characteristic of a metapopulation (Hanski et al., 1995). Traditional amphibian metapopulation models have defined ponds as patches and distance from breeding ponds as the dispersal limiter (Smith and Green, 2005). However, our results indicate that distance from ponds, at this scale, does not play a role in the spatial distribution of the Blue-spotted Salamander, at least during the autumn. In contrast, our results indicate that when a matrix is heterogeneous, some patches may be of lower quality or lower "preference" than others. In theory, if these patches are avoided because they are less suitable as semi-permanent sites, these may actually limit dispersal further than metapopulation models suggest. Disparity in habitat quality could potentially result in barriers (in this case, black cherry patches) to recolonization of suitable habitat (red maple patches) or migration to breeding ponds. Further testing is necessary to determine whether black cherry is actually unsuitable habitat and presents the risk of limiting dispersal. Nevertheless, potential matrix effects provide an interesting and potentially important addition to the amphibian metapopulation concept.

## ACKNOWLEDGMENTS

We would like to thank E. Werner for use of the ES George Reserve and valuable comments on our manuscript, and the Werner lab for background data; J. Vandermeer for advice, comments, and guidance; J. Foufopoulos and the Foufopoulos Lab for review; A. Moore, B. Otero, M. Baiz, A. Taylor, M. Valencia, M. Beaudoin, and R. Hom for help with data collection; and all of the Field Ecology 2011 students.

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