Effects of Light Pollution on Habitat Selection in Post-Metamorphic Wood Frogs (*Rana sylvatica*) and Unisexual Blue-spotted Salamanders (*Ambystoma laterale × jeffersonianum*)

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Abstract.—Light pollution is problematic for many nocturnal organisms, but our understanding of its effects on vernal pool-breeding amphibians is limited. Further, we know little about leaf litter preference of these amphibians in their recently metamorphosed stage. Our objectives were to determine if newly metamorphosed Wood Frogs (*Rana sylvatica*) and unisexual Blue-spotted Salamanders (*Ambystoma laterale × jeffersonianum*) prefer either deciduous or coniferous leaf litter when given a choice between these two common cover types and if artificial light affects microhabitat preference of either of these species. We conducted choice experiments in outdoor tanks with recently metamorphosed frogs and salamanders. We divided each tank into two compartments, filling one half with soil and leaf litter from a coniferous stand and the other with soil and leaf litter from a deciduous stand. Animals had one night to choose a substrate in the dark, as a control, and we recorded their positions the next morning. We then conducted illuminated trials in the same tanks, with a flashlight illuminating one substrate one night and the other substrate the following night. Frogs did not have a leaf litter preference in the dark and did not show a preference when either substrate was illuminated. Salamanders preferred deciduous litter in dark trials and when it was illuminated, however, they chose coniferous litter more often when it was illuminated. Additionally, artificial lighting could attract unisexual Blue-spotted salamanders to substrates they would not normally prefer and possibly settle in drier habitat.

Key Words.—choice experiments; disturbance; juvenile amphibians; urbanization; vernal pools

Introduction

Light pollution is a side effect of rapid global development, spreading light into both urban and rural skies across the world, covering two-thirds of the human population (Elvidge et al. 1997; Cinzano et al. 2001). The animals that live near these sources of light are also exposed to artificial nighttime illumination, many of which are accustomed to navigating their environment by the light of the natural night sky. Longcore and Rich (2004) coined the term ecological light pollution to describe light pollution that specifically alters the patterns of illumination in an ecosystem, recognizing that artificial light also affects animals that live in and around artificially illuminated areas. To give context to the amount of light humans are emitting, a streetlight may illuminate the ground to as much as 30 lux or 30,000 times more illumination than under a new moon (Bennie et al. 2016). A vehicle 50 m away from an object can illuminate an area to 50 lux, or 50,000 times brighter than a new moon (Bennie et al. 2016).

Nighttime light can change animal behavior across many taxa. For example, the foraging habits of bats, birds, rodents, and even marine mammals change with the lunar cycle (Brigham and Barclay 1992; Gannon and Willig 1997; Horning and Trillmich 1999; Dawson et al. 2001; Navara and Nelson 2007). The Loggerhead Sea Turtle (*Caretta caretta*) is a highly publicized example because hatchling turtles will move inland toward artificial light instead of orienting toward the sea (Salmon and Wyneken 1987; Witherington and Bjorndal 1991; Witherington 1992). Like the Loggerhead Sea Turtle, many amphibians tend to be nocturnal and may be disproportionately impacted by artificial nighttime illumination.

The known effects of artificial light on amphibians vary among different species, age classes, and different sources of light. For example, Buchanan (1993) found that Gray Treefrogs (*Hyla chrysoscelis*) were less likely to detect and consume prey under red-filtered light and two intensities of white light than under ambient moonlight. Artificial light can, however, enhance the...
ability of the Squirrel Treefrog (*Hyla squirella*) to detect prey (Buchanan 1998). Mazzerolle et al. (2005) found that Green Frogs (*Rana clamitans*), American Toads (*Anaxyrus americanus*), Spring Peepers (*Pseudacris crucifer*), and Wood Frogs (*Rana sylvatica*) were more likely to stop in roadways when approached by vehicle headlights increasing their risk of automobile-related mortality. Collectively, these studies suggest that frog responses to artificial light may depend on the source, intensity, movement, or timing of light.

Light may also influence salamander behavior and movement. Even the fossorial Red-backed Salamander (*Plethodon cinereus*) forages less in brighter areas than in darker ones (Perry et al. 2008). Perry et al. (2008) also found that these salamanders performed more visual threat displays in illuminated areas, which expend a great deal of energy. Both of these behavior changes could lead to lower energy reserves in artificially illuminated areas. Spotted Salamanders (*Ambystoma maculatum*), Blue-spotted Salamanders (*Ambystoma laterale*), and unisexual Blue-spotted Salamanders (*Ambystoma laterale × jeffersonianum*) migrate on rainy nights and, like frogs, tend to respond to oncoming vehicle lights by stopping in the roadway, increasing the risk of road mortality (Mazzerolle et al. 2005).

Constible et al. (2001) found that adult Wood Frog presence positively correlated with deciduous leaf litter in Alberta, Canada. The specific microhabitat requirements of metamorph Wood Frogs are unknown, though they are believed to be similar to those of adults (Redmer and Trauth 2005). The microhabitat preferences of metamorph unisexual Blue-spotted Salamanders have also not been studied. Post-breeding adult diploid Blue-spotted Salamanders prefer areas with low duff temperature (taken just beneath the decaying leaf litter, above the mineral soil), high soil moisture, and deep leaf litter (Ryan and Calhoun 2014). Belasen et al. (2013) found unisexual Blue-spotted Salamanders prefer Red Maple (*Acer rubrum*) to Black Cherry (*Prunus serotina*) leaf litter, but no one has studied their use of coniferous litter. After metamorphosis, these two species disperse from vernal pools to forests, traveling over the leaf litter until they find suitable habitat in which to settle.

We focused our study on understanding how artificial light affects leaf litter preferences of recently metamorphosed (metamorph) Wood Frogs and the unisexual Blue-spotted Salamanders. We explored if there is a leaf litter preference of these species during this period of emigration from natal ponds in the presence of artificial light. Because coniferous and deciduous litters are the two most common ground surface covers around the ponds our study animals came from, we used both of these in our design. The first goal of this study was to determine if Wood Frog and unisexual Blue-spotted Salamander metamorphs prefer coniferous or deciduous litter when given a choice between the two in dark conditions. Secondly, we wanted to determine if this preference changed in the presence of artificial illumination of each leaf litter type. We predicted that without artificial light, both Wood Frogs and unisexual Blue-spotted Salamanders would prefer deciduous litter over coniferous litter, as it provides more cover and moisture. Secondly, we predicted that they would avoid either substrate when illuminated, as juvenile Wood Frogs and adult unisexual Blue-spotted Salamanders tend to avoid bright, open areas (deMaynadier and Hunter 1998, 1999; Ryan and Calhoun 2014; Lee-Yaw et al. 2015).

**Materials and Methods**

**Experimental design.**—We collected metamorph unisexual Blue-spotted Salamanders and Wood Frogs of both sexes using drift fences surrounding two vernal pools in Orono, Maine, USA, from 13–27 August 2014. In total, we collected 46 Wood Frogs and 42 Blue-spotted Salamanders. We housed all amphibians in 19.1 L (5-gal) buckets with wet leaf litter and ventilated lids between trials (for 3 d total), with four animals per bucket.

We conducted choice experiments to determine whether metamorphs prefer deciduous or coniferous leaf litter in their migration away from their natal ponds. We used seven circular cattle tanks, 208 cm in diameter and 65 cm deep. We placed tanks in a mixed coniferous-deciduous forest adjacent to the University of Maine campus. Skyglow from the city of Bangor is present in the night sky at this location, but there were no point sources of light shining into or around the tanks other than our flashlights. We covered the tanks with 3 × 3 m clear plastic tarps to prevent accumulation of leaf litter from the forest canopy. The tarps were perforated for ventilation. We harvested leaf litter and the soil directly beneath the litter from coniferous and deciduous tree stands no more than 100 m from vernal pools from which we collected amphibians. We placed a mix of Red Maple, Red Oak (*Quercus rubra*), and White Birch (*Betula papyrifera*) leaf litter 3–5 cm deep and 1 cm of its underlying soil into one half of each tank, and Eastern White Pine (*Pinus strobus*) leaf litter and its underlying soil into the other half of each tank (similar to the design used by Belasen et al. 2013). These were representative of the abundant trees around the vernal pools from which we collected the metamorphs. We did not measure either soil temperature or soil moisture during our trials, but we let the soil and leaf litter sit in the tanks for 2 d before our trials began to let it acclimate to the surrounding temperature. A light rain event on the first night of trials allowed some water to trickle through the perforations in the tarps and wet the soil. We hung
an opaque cardboard divider hung between the two leaf litter types, leaving 1 cm of space above the substrate for the animals to travel underneath while keeping the artificial light only on the desired substrate and leaving the other substrate dark (Anderson 1972).

To begin each trial, we placed four metamorphs in the center of each of seven tanks, oriented along the line between deciduous litter and coniferous litter, between 1900 and 2000. For the duration of the trials, we kept frogs in tanks separate from salamanders. We allowed the amphibians to acclimate to their surroundings for 10 min in an acclimation station. The salamander acclimation station consisted of a one cm tall plastic lid, under which we placed one salamander. The frog station consisted of an inverted 1 cm tall plastic lid with a 10 × 10 cm piece of plastic window screen mesh placed over it to prevent the frog from leaving the station during the acclimation period (Graeter et al. 2008). Each tank we used 14.8 ml of fluorescent powder (DayGlo ECO Pigment™, DayGlo Color Corp., Cleveland, Ohio, USA) and 14.8 ml of mineral oil (Aspen Veterinary Resources®, Ltd., Kansas City, Missouri, USA) to aid in locating the small, camouflaged frogs in the leaf litter. We were able to locate the relatively larger salamanders without placing them in the powder. After we removed the acclimation covers, we placed the perforated plastic tarp over the tanks. We located frogs the following morning (between 0700 and 0900), by shining an ultraviolet light on the leaf litter to reveal their location.

We located the salamanders by gently removing leaves from the tank. We recorded all locations, but removed metamorphs located on the line between coniferous and deciduous leaf litter from the statistical analysis. After each animal was located, we returned it to a holding bucket until the next trial at dusk.

After an initial night of habitat choice experiments, we used the same animals in two more night trials to determine if artificial light affected habitat preference. All seven tanks were set up to imitate conditions of a forest (containing both coniferous and deciduous leaf litter) near a light source, such as a streetlight or garage light. These tests used the same design as the habitat choice experiments, with the addition of a flashlight (Eveready® LED handheld flashlights, Energizer Brands LLC, St. Louis, Missouri, USA: Model 5109L, Series 908D/908A, 1,680 cd intensity, 25 lumens, six-volt battery) attached to the side of the cattle tank approximately 50 cm above the leaf litter, illuminating half of the tank to 14.7 lux (1.7 m²; Anderson 1972). This would be the equivalent to substrate located 5–10 m from an industrial streetlight, illuminating the leaf litter to approximately 14,700 times its brightness on a night with a new moon (Bennie et al. 2016). We chose to use LED lights because they are often used in outdoor light fixtures. We placed the flashlight on either the coniferous or the deciduous side of the tank one night, and switched to the opposite side the next night (see Fig. 1). We started out with two tanks and ran more tanks as we collected more animals. We alternated between lighting deciduous litter and coniferous litter first in all trials. After the three nights of trials, we released the metamorphs back to their respective pools. We repeated this three-night trial sequence for all 42 salamanders and 46 frogs, placing new trial groups of four salamanders in tanks that previously held salamanders, and trial groups of four frogs in tanks that contained frogs.

**Statistical analyses.**—We used a chi-square analysis to determine if amphibian movements were affected by the order in which substrates were illuminated (e.g. if lighting deciduous litter during the second trial made the amphibians chose one side or another in the third trial; Table 1). We used chi-square tests to detect a deviation from an expected 1:1 ratio between metamorphs found in deciduous litter and coniferous litter in dark trials (Anderson 1972). After we found an observed ratio between deciduous and coniferous preference in dark trials, we used a chi-square test to determine if there was a difference between observed preference ratios in illuminated trial and these ratios from dark trials. We deemed \( P \) values ≤ 0.05 statistically significant.

**Results**

We removed from our analysis 16 frogs found on the centerline of the tank in habitat trials, 16 in deciduous-illuminated trials, and 13 in coniferous-illuminated trials. We also removed two salamanders on the centerline in each of the habitat, deciduous-illuminated, and coniferous-illuminated trials. We saw no significant difference in leaf litter choice by metamorphs when either substrate was illuminated first (Table 1). Metamorph Wood Frogs did not exhibit a clear leaf litter preference (Table 2). We compared results from illuminated trials to the 1:2 deciduous:coniferous ratio found in dark trials, and frogs did not exhibit a leaf litter preference when deciduous litter was illuminated (Table 2) or when coniferous litter was illuminated (Table 2).

Metamorph unisexual Blue-spotted Salamanders strongly preferred deciduous litter over coniferous litter (Table 2), with 88% of salamanders found in deciduous litter in dark trials. We compared the illuminated trials to the observed 6:1 deciduous:coniferous ratio in dark trials. The preference for deciduous litter remained when we illuminated it, with 70% of salamanders choosing deciduous litter in these trials (Table 2). The choice ratio shifted when we illuminated coniferous litter, with 55% of salamanders choosing coniferous litter in these trials (Table 2).
We did not observe a clear leaf litter preference in metamorph Wood Frogs, supporting the finding by Patrick et al. (2008) that juvenile Wood Frogs do not exhibit small-scale habitat or leaf litter selection (on the order of 1 m$^2$) during their emigration phase. Wood Frogs also did not exhibit substrate preference when either leaf litter type was illuminated. Metamorph Wood Frogs may only respond to ultraviolet (UV) light, as observed in Connolly et al. (2011), and not to the LED light used in our trials. Avoidance of UV light may be beneficial in avoiding UV-B radiation that can be harmful to some species of the genus *Rana* (Belden et al. 2003; Weyrauch and Grubb 2006).

Metamorph Blue-spotted Salamanders strongly preferred deciduous litter in the dark trials, possibly due to its higher levels of moisture retention and cover, as documented for other salamanders (Renaldo et al. 2011; Belasen et al. 2013; Lee-Yaw 2015). We did not measure leaf litter humidity in this study, and thus do not know the exact mechanism behind our results. However, Lee-Yaw et al. (2015) found metamorph Long-toed Salamanders to prefer deciduous litter over coniferous litter, citing its maximization of moisture and cover. Ousterhout et al. (2014) found that juvenile Spotted Salamanders and Small-mouth Salamanders (*Ambystoma texanum*) chose grass substrate over leaf litter when both were in pulverized form. They hypothesized that attraction to the high moisture content of grass substrate drove this preference, and that these juvenile salamanders may be selecting microhabitats using moisture cues rather than visual cues, because they are entering a novel habitat when they leave their natal pools with no prior knowledge of differences in leaf litter type. More research is needed to determine whether moisture is the driving factor in deciduous preference.

The chemical composition of deciduous and needle-leaved coniferous litter varies due to nutrient content, pH, and degree of refractory materials (and hence decomposition rates and pathways). These differences can affect invertebrate communities available to salamanders (Kuiters and Sarink 1986; Hendriksen 1990; Friberg 1997; Raich and Tufekcioglu 2000; Reich et al. 2005). In Michigan, Belasen et al. (2013) hypothesized Blue-spotted Salamanders to avoid Black

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**Table 1.** Total numbers of frogs and salamanders found in either deciduous or coniferous litter in the second illumination trial (SIT) compared against which leaf litter was illuminated on the first illumination trial (FIT), and the results from chi-square tests including sample sizes (n), chi-square values ($\chi^2$), and $P$ values.

<table>
<thead>
<tr>
<th></th>
<th>FIT</th>
<th>Deciduous</th>
<th>Coniferous</th>
<th>$\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frogs</td>
<td>Deciduous</td>
<td>10</td>
<td>2</td>
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<td></td>
<td>Coniferous</td>
<td>21</td>
<td>10</td>
<td>11</td>
<td>2.30</td>
</tr>
<tr>
<td>Salamanders</td>
<td>Deciduous</td>
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<td>8</td>
<td>12</td>
<td>0.80</td>
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<td></td>
<td>Coniferous</td>
<td>20</td>
<td>14</td>
<td>6</td>
<td>3.20</td>
</tr>
</tbody>
</table>

**Table 2.** Total numbers of frogs and salamanders found in either deciduous or coniferous litter (Substrate Chosen) compared against which leaf litter was illuminated at the time of that choice (Substrate Illuminated; Sub. Illum.), and the results from chi-square tests including sample sizes (n), chi-square values ($\chi^2$), and $P$ values.

<table>
<thead>
<tr>
<th>Sub. Illum.</th>
<th>Substrate Chosen</th>
<th>n</th>
<th>Deciduous</th>
<th>Coniferous</th>
<th>$\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frogs</td>
<td>Neither (Dark)</td>
<td>30</td>
<td>10</td>
<td>20</td>
<td>3.33</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>Deciduous</td>
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<td>13</td>
<td>17</td>
<td>1.35</td>
<td>0.245</td>
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<tr>
<td></td>
<td>Coniferous</td>
<td>33</td>
<td>13</td>
<td>20</td>
<td>0.55</td>
<td>0.460</td>
</tr>
<tr>
<td>Salamanders</td>
<td>Neither (Dark)</td>
<td>40</td>
<td>35</td>
<td>5</td>
<td>22</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Deciduous</td>
<td>40</td>
<td>28</td>
<td>12</td>
<td>12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Coniferous</td>
<td>40</td>
<td>18</td>
<td>22</td>
<td>66</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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**Figure 1.** The three-night trial sequence in one tank of frogs. Night one was a dark habitat preference trial and nights two and three were illuminated preference trials with either the coniferous or deciduous side illuminated.
Cherry leaf litter due to its high densities of invertebrates that feed on and sequester its cyanide compounds. In general, Mueller et al. (2016) found that deciduous litter supports more species of beetles and spiders, common prey items for unisexual Blue-spotted Salamanders. Preferring deciduous litter may be beneficial as these metamorph salamanders grow into adulthood and begin to feed. Possibly, the salamanders were drawn to the invertebrate communities attracted to deciduous litter or the artificial illumination, rather than the illumination itself.

Salamanders showed strong preference for deciduous substrate when it was illuminated and weaker preference for coniferous substrate when it was illuminated. These results contradict results from previous light pollution studies on adults and larval of other ambystomatid salamanders. Anderson (1972) found two subspecies of Long-toed Salamander (Ambystoma macrodactylumcroceum and A. m. sigillatum) to be strongly photonegative as adults and found A. m. croceum to be weakly photonegative as small larvae (< 50 mm snout-vent length, SVL). Larvae became photopositive when they reached 50 mm SVL, and then became photonegative again after they started to metamorphose. Schneider et al. (1991) found A. tigrinum, A. punctatum, and A. mexicanum to be photonegative as larvae, but they illuminated their trials to 286 lux, whereas Anderson (1972) illuminated his trials to only about 17 lux. We assume that these studies used incandescent bulbs in their studies, as the use of LED lighting did not become common until the early 2000s.

In contrast to previous research, our study focused on how these animals respond to anthropogenic LED light pollution in their natural surroundings, using outdoor tanks (Anderson 1972; Schneider et al. 1991; Ousterhout et al. 2014). In this setting, metamorph Wood Frogs and unisexual Blue-spotted Salamanders did not actively avoid LED light. Further, LED light may attract metamorph unisexual Blue-spotted Salamanders away from deciduous leaf litter, which they normally prefer in dark conditions. This could draw salamanders traveling from their natal ponds to substrates they would not normally prefer, which may be drier and have different invertebrate communities present. There is a similar potential risk for metamorph Wood Frogs that do not actively avoid or move toward illuminated substrate and could be traveling through light-polluted areas that make them more visible at night. Further research is needed to determine the effects of light pollution on juvenile Wood Frogs and unisexual Blue-spotted Salamanders in natural settings, and whether moisture requirements, soil chemistry, or cover from light drive leaf litter preference in these species in light polluted areas.

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Herpetological Conservation and Biology


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