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2015

# Effects of Roads and Trails on Peaks of Otter Salamander (Plethodon hubrichti) and Eastern Red-backed Salamander (Plethodon cinereus) Movement Behavior

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# EFFECTS OF ROADS AND TRAILS ON PEAKS OF OTTER SALAMANDER (*PLETHODON HUBRICHTI*) AND EASTERN RED-BACKED SALAMANDER (*PLETHODON CINEREUS*) MOVEMENT BEHAVIOR

A thesis submitted to the Graduate College of Marshall University In partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Organismal, Evolutionary and Ecological Biology by Cory Benjamin Goff Approved by Dr. Jayme L. Waldron, Committee Chairperson Dr. Anne C. Axel Dr. Thomas K. Pauley Dr. Shane M. Welch

> Marshall University August 2015

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## **ABSTRACT**

Habitat fragmentation negatively affects movement and dispersal of wildlife by altering useable space and creating barriers. The objective of this study was to assess impacts of fragmentation by roads and trails on the movement behavior of two terrestrial, woodland salamander species. I evaluated whether proximity to roads and trails affected the movement behavior of two species: the Peaks of Otter Salamander (*Plethodon hubrichti*), a species of special concern, and the Eastern Red-backed Salamander (*Plethodon cinereus*). I examined movement behavior in three treatments: paved roads, gravel roads and hiking trails. I compared treatments to controls located at least 200m from treatment edges to assess movement behavior based on fragmentation type. I detected salamanders by flipping cover objects during the day. I tracked movement patterns using a fluorescent pigment powder applied to each individual. I mapped and measured each powder trail to quantify travel distance and direction. I analyzed treatment and abiotic effects on movement behavior. I failed to detect a significant effect of treatment or distance from the treatment edge on individual foraging movements. Analysis indicated that abiotic factors including moon phase and precipitation were significant predictors of movement. This study provided additional insight into plethodontid movement patterns. Research will require managers to consider road and trail effects when designing and implementing long-term management plans for the survival of species within the Peaks of Otter area and other regions of the Appalachian Mountains.

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#### **CHAPTER 1**

# **EFFECTS OF ROADS AND TRAILS ON PEAKS OF OTTER SALAMANDER (***PLETHODON HUBRICHTI***) AND EASTERN RED-BACKED SALAMANDER (***PLETHODON CINEREUS***) MOVEMENT BEHAVIOR**

# **INTRODUCTION**

Fragmentation, the process of breaking contiguous habitat into smaller sections, is a problem when managing wildlife populations as new roads are built and habitats are altered for forest management (Forman and Alexander 1998). Roads create a barrier effect, fragmenting continuous populations into smaller, partially isolated subpopulations (Forman and Alexander 1998). Such subpopulations may decrease along with patch size as habitat quality declines (Semlitsch et al. 2007). Habitat alterations and perceived risk associated with roads and other barriers affect movement and dispersal of various taxa (Mazerolle 2001; Marsh and Beckman 2004; Marsh et al. 2005; Semlitsch et al. 2007; Connette and Semlitsch 2013). In addition, roads and trails facilitate anthropogenic activity including vehicular and foot traffic, which also acts as barriers to animal movements (Andrews et al. 2008; Balkenhol and Waits 2009).

Habitat patches and decreased habitat suitability created by edges limit dispersal and movement for many fauna (Marsh et al. 2005; Semlitsch et al. 2007; Row et al. 2012). Roads cause direct and indirect mortality of terrestrial animals, affecting population demographics (Andrews et al. 2008; Balkenhol and Waits 2009; Clark et al. 2010; Brehme et al. 2013). Direct mortality, or roadkill, is often associated with distance moved by organisms and their movement frequency and ability (Forman et al. 2003; Andrews et al. 2008). Research on some taxa has demonstrated dispersal limitations and subsequent decreased genetic relatedness due to clearings and roads (Stephens et al. 2013). Roads and habitat fragmentation, in general, create gene flow barriers for mammals, reptiles, and amphibians (Forman et al. 2003; Clark et al. 2010; Row et al. 2012; Stephens et al. 2013). Roads and trails, whether in-use or abandoned, reduce salamander abundance and fragment habitat (Semlitsch et al. 2007; Andrews et al. 2008). Environmental changes associated with roads and clearings may alter terrestrial plethodontid salamander abundance, distribution, genetic diversity, and movement activity (Kramer et al. 1993). Increased desiccation rates occur when the environment is changed and suitable moisture content is not maintained (Andrews et al. 2008). Roads negatively impact surrounding habitat and included organisms anywhere from 20-800m from the road edge, designated the "road-effect zone" (Forman and Alexander 1998; Marsh and Beckman 2004; Andrews et al. 2008; Semlitsch et al. 2012). Barriers and habitat edges can affect terrestrial salamander activity and movements, potentially causing genetic divergence between subpopulations (Gibbs 1998; Marsh and Beckman 2004; Marsh et al. 2008). In addition, competition for resources (e.g. food and habitat) and interactions between species may increase as habitat quality declines (Marsh et al. 2005; Kroschel et al. 2014).

Amphibians make excellent models for examining the effects of fragmentation, urbanization, and changing environmental conditions on ecosystems. Amphibians are particularly susceptible to habitat changes because they use cutaneous respiration and have limited movement ability, making them vulnerable to environmental changes and desiccation (Marsh and Beckman 2004; Marsh et al. 2005; Andrews et al. 2008). Such traits make amphibians appropriate habitat quality indicators (Welsh and Droege 2001; Marsh et al. 2005). Though previous studies have examined salamander abundance and condition in response to habitat fragmentation by roads (Marsh et al. 2004; Semlitsch et al. 2007), and others have assessed dispersal through fragmented areas by translocated individuals (Marsh et al. 2004; Marsh et al. 2005; Connette & Semlitsch 2013), no research to-date has examined foraging

movements in response to roads and trails. This project examined natural foraging patterns for individual salamanders near roads and trails. I analyzed movement patterns to examine the effects of roads and trails on salamander surface activity.

In this study, I assessed indirect impacts of roads and trails on movement behavior of two salamander species. Specifically, I examined movement data in four ways: 1) movement probability (move or no move), 2) total distance moved, 3) net distance moved, and 4) movement direction. Salamanders from the genus Plethodon are lungless, terrestrial, woodland salamanders that generally require cool, moist environments to survive (Connette and Semlitsch 2013). In this study, I focused on two plethodontid species: 1) the Peaks of Otter Salamander (*Plethodon hubrichti*, POS), a habitat specialist endemic to high elevations (>450m) along a 19km stretch of ridge bordering two counties in central Virginia (Kramer et al. 1993; Mitchell et al. 1996; Reichenbach and Sattler 2007; Kniowski and Reichenbach 2009); and 2) the Eastern Red-backed Salamander (*Plethodon cinereus*, RBS), a habitat generalist whose range extends from parts of North Carolina, north into Canada, and west as far as Minnesota, inhabiting forests at all elevations (Petranka 1998; Marsh et al. 2004; Marsh et al. 2008; Beane et al. 2010). The Peaks of Otter Salamander is globally imperiled, is listed as "vulnerable" on the IUCN Red List, is on the federal sensitive species list, and is under a US Fish and Wildlife Service candidate conservation agreement with the US Forest Service and Blue Ridge Parkway (Hammerson and Mitchell 2004; ECOS). I examined movement behavior for POS in allopatric sites and where it was sympatric with RBS to determine whether species interactions affected a salamander's response to decreased habitat quality and fragmentation. I assessed movement behavior in response to salamander proximity to roads and trails for both species to determine whether a habitat specialist (POS) differed in movement behavior from a habitat generalist (RBS). I expected that

individuals in close proximity to roads and trails would move less than individuals at control sites. I also expected individuals to avoid treatment edges when foraging, due to decreased habitat quality, increased risk exposure to predation, and decreased canopy cover that would result in lower moisture and higher temperature.I expected individuals to move farther distances under cooler and moist conditions. Finally, I expected POS in sympatry with RBS to move less than POS in allopatric sites and that RBS would move more on average than POS. Studies of movement patterns help ecologists and wildlife managers better understand species life histories and environmental effects on activity. Additional studies on movement behavior will demonstrate long-term implications of habitat fragmentation.

## **METHODS**

To examine the effects of roads and trails as barriers to terrestrial salamander movement, I used a natural experimental approach to quantify effects of habitat alteration and anthropogenic disturbance by gravel roads, paved roads, and hiking trails. I assumed impacts on salamander movements would decrease as distance from the barrier increased, indicating a linear effect (Marsh and Beckman 2004; Andrews et al. 2008). Further, I included candidate models that only contained conditional covariates known or suspected to drive plethodontid surface behavior, which allowed us to assess the validity of tracking methods. I assumed significant effects of conditional covariates (e.g., temperature, precipitation, and moon phase) would indicate that our tracking method did not adversely affect salamander movement behavior.

#### **Study Sites**

I assessed POS and RBS movement near roads and trails within the Jefferson National Forest along the Blue Ridge Parkway in Bedford and Botetourt Counties, Virginia. I sampled 16

sites, all located within sympatric and allopatric zones of POS distribution. Sites consisted of three treatments: 1) paved roads (i.e., Blue Ridge Parkway), 2) gravel roads (i.e. Forest Service roads), and 3) recreational trails (i.e., the Appalachian Trail). The edge effect is thought to influence terrestrial salamanders 80-100m from road edges (Marsh and Beckman 2004; Semlitsch et al. 2007). To ensure independence, control sites were located at least 200m from treatment edges and study sites were located approximately 200m apart.

# **Data collection**

To locate salamanders during diurnal searches, I flipped natural cover objects in treatment (along roads and trails) and control sites between 5:00pm and sunset. I used fluorescent pigment powder (DayGlo ZQ-11 Aurora Pink; Figure 1) to track salamander movements. Fluorescent pigment powder has been used to track an array of organisms, including small mammals, reptiles, insects, and several amphibian species with no negative effects (Rittenhouse et al. 2006; Roe and Grayson 2008; Orlofske et al. 2009; Furman et al. 2011; Popescu and Hunter 2011). Once I captured a salamander, I measured mass (g) with a Ziploc bag clipped to a10g Pesola spring scale, snout-vent length (SVL; mm) using a simplified "mandermasher" (Wise and Buchanan 1992) and ruler, determined sex by examining morphology (males exhibit square nose and swollen cloaca), dipped the posterior third of the salamander body in fluorescent pigment powder, returned them to their original capture location, and measured the salamander's distance (m) from the road or trail using a measuring tape stretched from the salamander's location to the closest point on the treatment edge. I returned to the site approximately six hours later to 1) determine if the salamander left its cover object (binary data; 1= moved; 0= did not move), 2) trace the pigment powder trail, 3) determine salamander trail direction (i.e. mark North and direction to treatment edge), and 4) photograph the fluorescent

powder trail. I used stake flags to mark initial cover objects for easy relocation when returning to trace the path. I recorded the capture location of each salamander with a handheld Global Positioning System (GPS; Trimble Juno SB; 5m accuracy). I recorded sampling covariates, including air temperature (°C) and percent relative humidity (Traceable temperature/humidity pen), and soil temperature (°C; Taylor 9878E thermometer probe) during each survey. Starting in mid-May 2014, I collected and tracked salamanders at two treatments per sampling night with 4- 5 sampling nights per week. I began tracking individual movement trails around midnight, at the end of salamander peak foraging times. I avoided sampling during moderate to heavy rain, as the fluorescent powder would wash away, though I still attempted to sample on nights with forecasted rain. Sampling was conducted through June, or until no salamanders were detected at a site after 30 minutes of searching. In July, I recorded site covariates including canopy cover and elevation.



**Figure 1:** Fluorescent pigment powder applied to the belly and tail of a salamander as it looks in natural light (**A**) and under ultraviolet light (**B**).

I tracked individual salamander movements using a handheld ultraviolet flashlight

(Northwest Marine; 405nm; 82mw) and traced each path using reflective rope. I placed a  $1m<sup>2</sup>$ 

PVC grid over the traced trail. If the trail was not contained within  $1m^2$ , I added additional PVC pieces to create adjacent 1m squares. I used a small dry-erase board to note the individual's identification number and drew an arrow pointing toward the treatment edge (road or trail), where applicable. I used a handheld digital camera (Nikon D60 with 18-55mm DX VR lens or Canon PowerShot A2200) to photograph the  $1-m^2$  plot with all four corners visible (Figure 2). If the trail extended over multiple grids, I individually photographed each grid to minimize distortion. Photographs were oriented to magnetic north using an arrow placed on the ground, aligned with a compass. An additional arrow indicated the direction of the treatment.



Figure 2: Georectified image of a marked salamander movement trail using reflective rope with 1m<sup>2</sup> PVC grid, north arrow (orange), and dry-erase board with identification information and direction of treatment edge (blue arrow).

# **Data Analysis**

I imported the digital photographs into ArcGIS 10.2, georectified each image, and transformed them to ensure edges of the PVC grid measured 1m. I opened the georectified images with ImageJ 1.48v and set the measurement scale to 1m using one of the horizontal edges of the PVC grid from the image. I traced each salamander movement trail using the freehand draw tool and calculated the total distance traveled. I also used the line segment tool to measure net distance traveled from original cover object to the end of the trail.

I assessed movement direction for each salamander to determine if individuals near treatment edges were avoiding edges. Using ArcGIS 10.2, I measured each net movement angle (bearing) and compared the angle to the direction toward each treatment edge (randomized angles for control sites). I then divided the data into three equal directions: toward, away, or parallel (Figure 3).



**Figure 3:** Diagram of movement direction split into three equal bearings for analysis.

**Candidate Models.** I examined the effects of abiotic conditions, moon phase, treatment, and sympatry on salamander movement (Table 1). Prior to analyzing individual models, I used correlation analysis to assess collinearity of predictors, removing correlated variables ( $r \ge 0.70$ ) from the analysis. Natural drivers included soil temperature, relative humidity, percent cloud cover, precipitation totals for the sampling day plus one day prior, as well as precipitation totals for the sampling day plus the three days prior. I measured soil temperature, relative humidity,

and percent cloud cover at the time of sampling, and precipitation totals were gathered from the National Oceanic and Atmospheric Administration's (NOAA) Global Historical Climatology Network (GHCN). The closest station to my study sites, and the station used for precipitation data, was Holcomb Rock (12-19km; GHCND:USC00444039). Soil temperature, humidity, and precipitation totals were z-standardized for analysis. Moon phase models included percent moon luminosity and moon altitude in relation to the horizon at 9:00pm, gathered from www.dailymoonposition.com. Treatment models included a categorical variable for the three treatments (trail, gravel, paved) and controls, as well as the distance from the original capture location to the treatment edge (randomized for control sites). Sympatry models included another categorical variable for whether the site was located in the region of POS allopatry or POS and RBS sympatry.

**Table 1:** Definition of predictor variables used in Peaks of Otter Salamander and Eastern Redbacked Salamander movement behavior models.

Variable	Definition
Cloud	Percent cloud cover at time of initial sampling $(0.00-1.00)$
Moon	Percent moon luminosity at 9:00pm (0.00-1.00)
Altitude	The degrees the moon is located above or below the horizon at 9:00pm (-90-90)
Precip2	Total precipitation for the two days prior to sampling, including the day of sampling, z-transformed
Precip4	Total precipitation for the four days prior to sampling, including the day of sampling, z-transformed
<b>ST</b>	Average soil temperature during initial sampling, z-transformed
<b>RH</b>	Average relative humidity during intitial sampling, z-transformed
Sympatry	Categorical variable to identify site location (Allopatric or Sympatric)
Treatment	Categorical variable to identify site type (Paved, Gravel, Trail, or Control)
Edge	Distance (m) the salamander was found from the treatment edge, randomized for controls

Candidate models assessed natural and anthropogenic variables as sole predictors and in additive models (Table 4). I also included distance to treatment in a nested model to examine linear effects of treatment. I only used the sympatry model when comparing POS movement behavior between allopatric and sympatric sites to examine effects of potential competition.

**Statistical Analysis.** I conducted statistical analyses in SAS 9.4 to examine predictors of

movement. I examined fourteen models using anthropogenic and abiotic variables and ranked them using AICc model selection. I used models with ΔAICc less than 2.00 for inference. I assessed model fit by examining model deviance (Pearson chi-square/DF) for the most parameterized model. I used a generalized linear mixed model (GLIMMIX Procedure) in SAS 9.4, fit with a Laplace approximation (Waldron et al. 2013) to examine models using binary movement data (movement probability), continuous trail length data (total distance moved), net movement (straight-line distance from start to end) and movement direction (in relation to the treatment edge) as response variables in separate analyses. In all analyses, I specified site as a random effect to account for a lack of independence among observations from the same site. I used the response variables as the fixed effects for each model (Table 4). I also examined the effect of site location (allopatry or sympatry) on movement to determine if competition affected distance moved.

I used t-tests to examine interspecific differences in mass, SVL, and movement. I used logistic regression to examine binary movement data, modelling the probability a salamander moved from its initial cover object. I used Poisson regression to examine models with total distance or net distance as the response variable. For bearing analysis, which included a categorical response variable, I used a multinomial distribution and multinomial logistic regression. All candidate models were run to assess movement probability, total distance, net distance, and movement direction in two separate analyses: for the Peaks of Otter Salamander (N  $= 141$ ), and for the Eastern Red-backed Salamander (N  $= 46$ ).

# **RESULTS**

I captured and marked 210 adult salamanders (>30mm; Kniowski and Reichenbach 2009)

in May and June, 2014. Heavy rain prevented tracking 23 individuals; therefore data collected from these individuals were not used in analyses. Of the 187 remaining individuals, 46 were RBS in sympatry with POS, 59 were sympatric POS, and 82 were allopatric POS. I recorded 118 individuals that moved from their original cover object (rock or log); the remaining 69 salamanders did not move from cover objects. The average mass and SVL of POS was 1.32g and 46.2mm, respectively. The average mass and SVL of RBS was 1.07g and 43.2mm, respectively (Table 2). Peaks of Otter salamanders were significantly larger than RBS in both mass ( $t = 3.81$ ; *P* = 0.0002) and SVL (*t* = 3.49; *P* = 0.0006).

The average total distance moved by POS was 1.41 meters and the average distance moved by RBS was 1.04 meters (Figure 4; Table 3). Neither total distance moved (*t* =1.76; *P* = 0.081) nor net distance moved  $(t = 1.10; P = .2741)$  were significantly different between species.

**Table 2:** Average mass (g) and snout-vent length (SVL; mm) for Peaks of Otter salamander (POS) and Eastern Red-backed salamander (RBS) with standard deviation and range of values.

	Mass(g)		$SVL$ (mm)		
<b>Species</b>	$Mean + SD$	Range	$Mean + SD$	Range	
<b>POS</b>	$1.32 \pm 0.41$	$0.40 - 2.50$	$46.2 \pm 5.2$	$35.0 - 58.0$	
<b>RBS</b>	$1.07 \pm 0.31$ 0.60 - 1.80		$43.2 \pm 4.5$	$35.0 - 52.0$	



**Table 3:** Average total distance moved (m) and net distance moved (m) for Peaks of Otter salamanders (POS) and Eastern Red-backed salamanders (RBS), including range and standard deviation.



# **Correlation Analysis**

Soil temperature was correlated with daily high air temperature ( $r = 0.7386$ ;  $P =$ 

 $\leq$ 0.0001), daily low air temperature ( $r = 0.7877$ ;  $P = \leq$ 0.0001), average daily air temperature ( $r = 0.7877$ )

0.8577;  $P = 0.0001$ , average three-day air temperature ( $r = 0.7332$ ;  $P = 0.0001$ ), and air

temperature at sampling time  $(r = 0.7419; P = 0.0001)$ . I retained soil temperature for analysis.

Daily precipitation totals were correlated with two day precipitation totals ( $r = 0.9727$ ;  $P =$ 

<0.0001), thus I retained the two day precipitation totals for analysis. Finally, moon luminosity was correlated with moon altitude at midnight ( $r = 0.9340$ ;  $P = 0.0001$ ), but not moon altitude at 9:00pm, so I retained moon luminosity and moon altitude at 9:00pm for analysis. Removing correlated variables resulted in 11 variables retained for analyses, combined into 14 total candidate models (Table 4).

<b>Model</b>
Cloud
Moon
$Moon + Altitude$
$Moon + Cloud$
$Moon + Cloud + Altitude$
Precip2
Precip4
SТ
$ST + RH$
$ST + RH + Precip2$
$ST + RH + Precip4$
Sympatry
Treatment
$Treatment + Edge (Treatment)$

**Table 4:** Predictor variables used in candidate models to analyze Peaks of Otter Salamander and Eastern Red-backed Salamander movement behavior.

I used the most parameterized model to examine model fit for each analysis. Movement analysis had great fit (Pearson  $\chi^2$ /DF = 1.00), whereas analysis of net distance moved was underdispersed (Pearson  $\chi^2$ /DF = 0.69) and analysis of total distance moved was slightly overdispersed (Pearson  $\chi^2$ /DF = 1.12).

### **Movement Probability**

**Peaks of Otter Salamander.** I retained three models for inference out of 14 candidate models  $(N = 141$ ; Table 5). The most important predictors of POS movement probability were moon luminosity ( $\omega_i$  = 0.46), moon altitude ( $\omega_i$  = 0.36), and Precip2 ( $\omega_i$  = 0.23; Table 4). The top ranking model of movement probability for POS included moon luminosity and moon altitude. I failed to detect an effect of moon luminosity on POS movement probability ( $\beta$  = -0.0663  $\pm$ 0.0495;  $P = 0.1827$ ). Moon altitude was positively associated with movement probability ( $\beta =$  $0.0129 \pm 0.0058$ ;  $P = 0.0293$ ). The model containing precipitation totals for the two days prior to sampling (Precip2) as the sole predictor was the second highest ranking model, but I failed to detect a significant effect of Precip2 on POS movement probability ( $\beta$  = 0.2862  $\pm$  0.1839; *P* = 0.1221). The third supported model included moon luminosity, altitude, and cloud cover. I failed to detect significant effects of moon luminosity ( $\beta$  = -0.0624  $\pm$  0.05286; *P* = 0.2200), cloud cover (*β* = 0.1904 ± 0.5286; *P* = 0.7194) or altitude (*β* = 0.0121 ± 0.0062), though altitude approached significance  $(P = 0.0530)$ .

**Table 5:** Candidate models examining Peaks of Otter Salamander (N = 141) movement probability (binary logistic regression), ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling; Precip4 = precipitation totals for four days prior to sampling;  $ST = soil$  temperature;  $RH =$  relative humidity; sympatry = categorical variable for allopatric or sympatric site location; treatment = categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc  $=$  the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i =$ model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.



**Red-backed Salamander.** Analyses examining RBS movement probability resulted in four supported models out of 13 candidate models ( $N = 46$ ; Table 6). Cloud cover was the top predictor of movement for RBS, accounting for 57% of model weights, followed by moon luminosity with 49% and soil temperature with 29% (Table 5). In the top ranking model, cloud cover and moon luminosity were negatively associated with RBS movement probability, though I failed to detect a significant effect of moon luminosity ( $\beta$  = -2.0773  $\pm$  0.9887; *P* = 0.0427;  $\beta$  = - $0.3128 \pm 0.408$ ;  $P = 0.4484$ , respectively). The model including cloud cover as the sole predictor of movement probability also indicated that movement probability increased as cloud cover decreased ( $\beta$  = -2.0295  $\pm$  0.9573; *P* = 0.0408). The third supported model included soil temperature and relative humidity as predictors of RBS movement probability. Relative humidity was positively associated with movement probability ( $\beta$  = 10.1309  $\pm$  4.8078; *P* = 0.0421), but I failed to detect a significant effect of soil temperature on RBS movement probability (*β* = -  $0.1375 \pm 0.4520$ ;  $P = 0.7626$ ). The fourth supported model included moon luminosity, moon altitude, and cloud cover as predictors of RBS movement probability. I failed to detect

significant effects of moon luminosity ( $\beta$  = -0.3375  $\pm$  0.4219; *P* = 0.4290) or moon altitude ( $\beta$  =

 $0.0082 \pm 0.0119$ ;  $P = 0.4943$ ) on RBS movement probability. Cloud cover was negatively

associated with RBS movement probability, although the relationship approached significance (*β* 

 $= -1.9301 \pm 1.0101$ ;  $P = 0.0643$ ).

**Table 6:** Candidate models examining Eastern Red-backed Salamander ( $N = 46$ ) movement probability (binary logistic regression), ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling; Precip4 = precipitation totals for four days prior to sampling;  $ST = soil$  temperature;  $RH =$  relative humidity; treatment = categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc = the difference between the AICc value for the current model and the lowest AICc score. *ω<sup>i</sup>* = model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.



# **Total Distance Moved**

**Peaks of Otter Salamander.** I retained four out of 14 candidate models examining total distance moved by POS ( $N = 141$ ; Table 7). Cloud cover was the most important predictor of total distance moved by POS, accounting for 64% of model weights, followed by moon luminosity ( $\omega_i$  = 0.58) and moon altitude ( $\omega_i$  = 0.38; Table 7). The top-ranked model indicated cloud cover was positively associated with total distance moved ( $\beta$  = 0.6281  $\pm$  0.2597; *P* =

0.0171). The second supported model included cloud cover, moon altitude and moon luminosity as predictors of POS distance moved. I failed to detect significant effects of cloud cover  $(\beta =$ 0.4414  $\pm$  0.2747; *P* = 0.1107), moon altitude ( $\beta$  = 0.0053  $\pm$  0.0032; *P* = 0.1039), or moon luminosity ( $\beta$  = -0.0546  $\pm$  0.0419; *P* = 0.1953). The third supported model included moon luminosity and moon altitude as predictors of POS movement distance. I failed to detect a significant effect of moon luminosity on total distance moved ( $\beta$  = -0.0646  $\pm$  0.0408; *P* = 0.1158), but moon altitude was positively associated with total distance moved ( $\beta$  = 0.0069  $\pm$ 0.0030;  $P = 0.0243$ ). In the fourth supported model, total distance moved by POS was positively associated with cloud cover ( $\beta$  = 0.5888  $\pm$  0.2604; *P* = 0.0255), but I failed to detect a significant effect of moon luminosity ( $\beta$  = -0.0404  $\pm$  0.0408; *P* = 0.3248) on total distance moved.

**Table 7:** Candidate models examining Peaks of Otter Salamander ( $N = 141$ ) total distance moved (Poisson regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling;  $Precip4 = precipitation$  totals for four days prior to sampling;  $ST$  $=$  soil temperature; RH  $=$  relative humidity; sympatry  $=$  categorical variable for allopatric or sympatric site location; treatment = categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc = the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i$  = model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.

Model	AICc	$\triangle$ AICc	$\omega_i$
<b>Cloud</b>	358.16	0.00	0.27
$Moon + Cloud + Altitude$	358.59	0.43	0.21
Moon + Altitude	359.05	0.89	0.17
Moon + Cloud	359.14	0.98	0.16
$ST + RH$	361.50	3.34	0.05
Moon	362.21	4.05	0.04
$ST + RH + Precip4$	363.33	5.17	0.02
$ST + RH + Precip2$	363.57	5.41	0.02
<b>ST</b>	363.62	5.46	0.02
Sympatry	364.02	5.86	0.01
Precip2	364.05	5.89	0.01
Precip4	364.05	5.89	0.01
Treatment	366.32	8.16	0.00
$Treatment + Edge(Treatment)$	368.48	10.32	0.00

**Red-backed Salamander.** Two models out of 13 candidate models were retained for inference in the analysis examining total distance moved for RBS ( $N = 46$ ; Table 8). Top predictors of total distance moved by all RBS included moon luminosity ( $\omega_i = 0.50$ ), moon altitude ( $\omega_i$  = 0.37), and soil temperature ( $\omega_i$  =0.31; Table 8). The top predictive model indicated moon altitude had a significant positive effect on total distance moved by RBS ( $\beta$  = 0.0105  $\pm$ 0.0049;  $P = 0.0391$ ). I failed to detect a significant effect of moon luminosity ( $\beta = -0.1390 \pm 0.0391$ ). 0.1743;  $P = 0.4304$ ) or soil temperature ( $\beta = 0.2523 \pm 0.1946$ ;  $P = 0.2028$ ) on total distance moved by RBS.

**Table 8:** Candidate models examining Eastern Red-backed Salamander ( $N = 46$ ) total distance moved (Poisson regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling; Precip4 = precipitation totals for four days prior to sampling; ST  $=$  soil temperature; RH  $=$  relative humidity; treatment  $=$  categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge.  $\triangle AICc$  = the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i =$ model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.

Model	<b>AICc</b>	$\triangle$ AICc	$\omega_i$
Moon + Altitude	103.36	0.00	0.29
<b>ST</b>	105.29	1.93	0.11
$ST + RH$	105.45	2.09	0.10
Moon	105.70	2.34	0.09
$Moon + Cloud + Altitude$	105.74	2.38	0.09
Cloud	106.19	2.83	0.07
Precip2	106.19	2.83	0.07
$ST + RH + Precip4$	106.30	2.94	0.07
Precip4	106.93	3.57	0.05
$Moon + Cloud$	107.38	4.02	0.04
$ST + RH + Precip2$	107.79	4.43	0.03
Treatment	110.77	7.41	0.01
$Treatment + Edge(Treatment)$	119.21	15.85	0.00

# **Net Distance Moved**

**Peaks of Otter Salamander.** I retained four models for inference out of 14 models examining net distance moved for POS ( $N = 141$ ; Table 9). Top predictors of net distance moved by POS included moon luminosity, cloud cover, and whether the individual was found in allopatry or sympatry, accounting for 54%, 22% and 16% of model weights, respectively (Table 8). I failed to detect a significant effect of any predictor on net distance moved among the supported models. I failed to detect a significant effect of moon luminosity on POS movement distance in any of the supported models (model 1:  $\beta$  = 0.0459  $\pm$  0.0460; *P* = 0.3201; model 3:  $\beta$  = 0.0524 ± 0.0470; *P* = 0.2669; model 4: *β* = 0.0434 ± 0.0464; *P* = 0.3519). I failed to detect an effect of sympatry on POS movement  $(\beta = 0.1275 \pm 0.2468; P = 0.6063)$ . I failed to detect a significant effect of moon altitude or cloud cover, though both were positively associated with total distance moved by POS ( $\beta$  = 0.0029  $\pm$  0.0037; *P* = 0.4379;  $\beta$  = 0.2322  $\pm$  0.3216; *P* =

0.4717, respectively).

**Table 9:** Candidate models examining Peaks of Otter Salamander (N = 141) net distance moved (Poisson regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip $2$  = precipitation totals for two days prior to sampling; Precip $4 =$  precipitation totals for four days prior to sampling;  $ST =$ soil temperature;  $RH =$  relative humidity; sympatry  $=$  categorical variable for allopatric or sympatric site location; treatment = categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc = the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i$  = model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.



**Red-backed Salamander.** I retained three supported models out of 13 candidate models in the analysis of net distance moved by RBS ( $N = 46$ ; Table 10). Moon luminosity was the top predictor of net distance moved by RBS, accounting for 49% of model weights, followed by moon altitude with 37% (Table 9). The top supported model for net distance moved by all RBS indicated a significant positive association with moon altitude ( $\beta = 0.0120 \pm 0.0059$ ;  $P = 0.0496$ ). I failed to detect a significant effect of moon luminosity on RBS movement ( $\beta$  = -0.1475  $\pm$ 0.2179;  $P = 0.5028$ ). I failed to detect a significant effect of soil temperature on net distance moved ( $\beta$  = 0.2680  $\pm$  0.2318; *P* = 0.2551). The third supported model included moon luminosity as a predictor of RBS movement. I failed to detect a significant effect of moon luminosity on RBS movement  $(\beta = -0.1926 \pm 0.3459; P = 0.5811)$ .

**Table 10:** Candidate models examining Eastern Red-backed Salamander (N = 46) net distance moved (Poisson regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling;  $Precip4 = precipitation$  totals for four days prior to sampling; ST  $=$  soil temperature; RH  $=$  relative humidity; treatment  $=$  categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc = the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i =$ model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were



retained for inference.

# **Movement Direction**

**Peaks of Otter Salamander.** One out of 14 candidate models was retained for inference in the analysis examining movement direction by POS ( $N = 85$ ; Table 11). Precip4 was the top predictor of movement direction, accounting for 57% of model weights (Table 10). I failed to detect a significant effect of Precip4 on POS movement direction (toward versus away, *β* = -  $0.4327 \pm 0.3645$ ;  $P = 0.2408$ ; toward versus parallel,  $\beta = 0.2811 \pm 0.2611$ ;  $P = 0.2868$ ).

**Table 11:** Candidate models examining Peaks of Otter Salamander ( $N = 141$ ) movement direction (multinomial logistic regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling; Precip4 = precipitation totals for four days prior to sampling;  $ST = soil$  temperature;  $RH =$  relative humidity; sympatry = categorical variable for allopatric or sympatric site location; treatment = categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc  $=$  the difference between the AICc value for the current model and the lowest AICc score.  $\omega_i =$ model weight. Models are listed in order of support. Models with ∆AICc < 2.00 (in bold) were retained for inference.



**Red-backed Salamander.** I retained three models out of 13 candidate models for inference in the analysis examining RBS movement direction  $(N = 33;$  Table 12). Soil temperature was the most important predictor of RBS movement direction, accounting for 32% of model weights, followed by Precip2 ( $\omega_i$  = 0.30) and moon luminosity ( $\omega_i$  = 0.21; Table 11). I failed to detect a significant effect of Precip2 on RBS movement direction (toward versus away, *β* = -3.5983 ± 80.115; *P* = 0.9648; toward versus parallel, *β* = 13.156 ± 37.309; *P* = 0.7299). I failed to detect significant effects of other predictors, though supported models included soil temperature (toward versus away,  $\beta$  = -0.3522  $\pm$  1.0082; *P* = 0.7317; toward versus parallel,  $\beta$  = 1.0235 ± 0.6958; *P* = 0.1620) and moon luminosity (toward versus away, *β* = -0.3428 ± 2.0767; *P* = 0.811; toward versus parallel,  $\beta$  = 2.3691  $\pm$  1.6416; *P* = 0.1695).

**Table 12:** Candidate models examining Eastern Red-backed Salamander ( $N = 46$ ) movement direction (multinomial logistic regression) ranked according to AICc model selection. Cloud = percent cloud cover; Moon = percent moon luminosity; Altitude = moon altitude; Precip2 = precipitation totals for two days prior to sampling; Precip4 = precipitation totals for four days prior to sampling;  $ST = soil$  temperature;  $RH =$  relative humidity; treatment  $=$  categorical variable for treatment type: paved, gravel, trail, or control; Edge = salamander distance from the treatment edge. ∆AICc = the difference between the AICc value for the current model and the lowest AICc score. *ω<sup>i</sup>* = model weight. Models are listed in order of support. Models with ∆AICc



< 2.00 (in bold) were retained for inference.

# **DISCUSSION**

Many factors influence *Plethodon* salamander movement activity during peak foraging times. I failed to detect an effect of treatment or distance from treatment edge on movement behavior, indicating that the barriers I examined may not have an effect at the scale at which I examined them. Behavior was strongly influenced by natural predictors (e.g. cloud cover, relative humidity, and moon altitude), indicating decreased habitat quality near barriers may not drive salamander foraging behavior. Salamanders living in close proximity to barrier edges may have adapted to living in such conditions and therefore did not show changes in foraging behavior as compared to salamanders farther from a treatment edge. In addition, because salamander abundance is generally lower near road edges (Marsh and Beckman 2004; Semlitsch et al. 2007), individuals that live in these areas would most likely have lower competition with other individuals and potentially higher food and cover availability. Salamanders near road edges can be larger (Semlitsch et al. 2007), which may indicate individuals with higher body condition are able to live in these barrier edges. Future research should examine the time a salamander

spends on the surface and compare results at varying distances from barriers to determine habitat quality differences and road effects.

Though I failed to detect effects of treatment or distance from edge on salamander movements, my analyses indicated significant effects of abiotic predictors known to drive salamander surface behavior. Precipitation, soil temperature, cloud cover, and relative humidity were all top predictors of salamander movement, following conclusions from other studies (e.g., Taub 1961; Welsh and Droege 2001; Kniowski and Reichenbach 2009; MacNeil and Williams 2014). These results also indicate my use of fluorescent powder did not significantly affect movement behavior. Cloud cover and relative humidity were significant predictors of RBS movement probability. Salamanders were more likely to leave cover objects as relative humidity increased. My results also indicated RBS were more likely to move from cover objects when cloud cover decreased, which may suggest individuals moved underground or to more suitable cover objects. As cloud cover increased, however, total distance moved by POS increased, indicating salamanders might make longer foraging treks with increasing cloud cover.

Moon luminosity and moon altitude were important predictors of movement probability and movement distances for both salamander species. The model containing moon luminosity and moon altitude was consistently a top supported model. These relationships likely reflect risk exposure to predation during times of high luminosity. Plethodontid salamanders are not generally surface active during daytime hours and therefore moon luminosity may play an important role in nocturnal foraging behavior. Ralph (1957) found that not only are RBS more active at night, but they exhibit a reduction in activity when the moon's zenith (highest angle for a given day) occurs during peak times of activity. My results indicated that both species were more likely to leave cover objects and move longer distances when the moon was higher in the

sky, contradicting Ralph's results. Although not significant, moon luminosity (how much light the moon reflected) was consistently supported in top models, reinforcing my supposition that the moon is an important contributor of nocturnal salamander behavior. Salamander activity increased as the moon rose, as long as the moon reflected little light. Amphibian responses to lunar cycles are highly species-specific, with some exhibiting a negative response to moonlight and others exhibiting a positive response (Grant et al. 2013). Salamanders generally show a negative response to light. Wise and Buchanan (2006) found terrestrial salamanders might be photonegative as a response to desiccation (from increased temperature and decreased moisture levels) and predation risk. However, more light helps RBS find and orient toward prey, assisting their foraging ability (Wise and Buchanan 2006; Perry et al. 2008). In addition, salamanders in normal night light can exhibit photopositive responses and increased light can aid foraging (Wise and Buchanan 2006). One explanation for increased movement response to lunar position involves salamander diets. Both POS and RBS feed primarily on insects (Grant et al. 2013), which are more active at night with increased moonlight, supporting my observations of increased movement activity as the moon rose above the horizon. However, predation risk may reduce salamander movement with intense moonlight. Grant et al. (2013) found evidence that not all amphibians avoid activity at full moon despite higher predation risk. Amphibian predator avoidance may depend on visual acuity (Grant et al. 2013), resulting in a combination of factors that influence salamander risk assessment. Such factors might include visual acuity, prey availability, predator presence, and light intensity. Roberts and Liebgold (2008) found that terrestrial animals respond less to predators than aquatic animals. One means RBS and POS may reduce predation risk, even on moonlit nights, is by climbing vegetation (Roberts and Liebgold 2008). Plant-climbing activity is assumed to be a predator avoidance strategy, rather than a

foraging strategy, because the forest floor actually contains more prey for terrestrial salamanders (Roberts and Liebgold 2008). During nocturnal tracking, I observed POS and RBS perched on vegetation and climbing tree trunks up to waist high, usually on rainy or very wet nights.

I failed to detect a significant effect of treatment on individual movement direction, though out of 187 observations used for analyses, only one individual crossed a treatment edge (see Salamander Crossing note in Appendix A). I failed to detect an effect of proximity to treatment on the distance or direction an individual will move when foraging. However, on average, I observed RBS and POS move 1.31m over a 6-8 hour period, and I found individuals an average of 7.10m from treatment edges. Thus, observing foraging behavior over this short time frame might not be accurately demonstrating barrier edge effects. Although previous research has indicated salamander abundance declines near barrier edges (Marsh and Beckman 2004), I postulate that individuals in close proximity to roads and trails have habituated to environmental conditions and my not exhibit differences in foraging behavior as compared to individuals in more suitable, less fragmented, habitat.

Future studies should analyze salamander body condition and movement over longer time periods and through multiple seasons to better examine barrier avoidance. In addition, lunar effects on amphibians and specifically terrestrial salamanders has been poorly studied. Future research should continue to examine lunar position and luminosity as factors influencing terrestrial salamander movement behavior. Because my results indicated moonlight affects terrestrial salamander foraging behavior, light pollution from nearby homes and cities may alter salamander movement behavior, acting as an anthropogenic moon (Grant et al. 2013). In addition to these factors, future studies should analyze total time spent on the surface at varying distances from barriers to determine effects on behavior. The results of my study further support the use of

fluorescent pigment powder to quantify terrestrial plethodontid movement behavior. In addition, research examining the effects of anthropogenic features should include natural covariates to assess method validity and contribute insight into movement ecology.

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### **APPENDIX A**

### **Salamander Crossing Note**

Out of 187 salamanders tracked during my study, 49 individuals were located along hiking trails. On June 9, 2014, I tracked a female Peaks of Otter Salamander (42mm SVL; 1.2g), in an allopatric zone, from its original cover rock, across the hiking trail, to the other side and underneath a downed tree. I originally found the individual 0.40m from the edge of the trail and it moved a total distance of 3.73m, for a net distance of 2.41m. At time of original capture, the air temperature was 19.6°C, soil temperature was 16.1°C, and the relative humidity was 72%. When I returned and tracked the salamander movement trail, the air temperature had dropped to 17.7°C, soil temperature was still at 16.0°C, and humidity had increased to 81%. I documented cloud cover at 70%. This was the only salamander during my study that crossed a barrier.

#### **Salamander Microhabitat Use**

During my research, I observed and documented microhabitat use for the Peaks of Otter Salamander and the Eastern Red-backed Salamander. I noted both species perched on ferns or small shrubs, approximately one foot above the ground (Figure 5). These observations generally occurred on rainy nights. In addition, I observed both species climbing tree trunks (Figure 6), sometimes pressed into the deep folds of oak tree bark, up to one meter above the ground. Again, these observations generally occurred on rainy nights. Finally, though some salamander movement trails ended under other cover objects (rocks and logs), most trails ended when the individual followed small holes in the ground, usually near the base of plants and trees. These notes may provide insight into microhabitat use by both species.



**Figure 5:** A Peaks of Otter Salamander perched on a small plant, covered in fluorescent pigment powder used for tracking.



Figure 6: A Peaks of Otter Salamander climbing the trunk of a tree.



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Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D. Chairperson, IACUC

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Monica A. Valentovic, Ph.D. Chairperson, IACUC

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