

**Movement, Occupancy, and Detectability of  
Green Salamanders (*Aneides aeneus*) in Northern Alabama**

by

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## Abstract

Green salamanders (*Aneides aeneus*) are a species of concern throughout their range due to habitat modification and population declines. With few short-term movement studies on the species, the information is vital to better understanding the natural history of green salamanders. Understanding movement patterns and their distribution better are crucial to effectively managing and conserving the species. We studied nightly movements of the species in northern Alabama during the spring breeding season in 2015-2016. During summer (2015-2016), we conducted presence-absence surveys of green salamanders and explored habitat characteristics important to the species distribution in northern Alabama. Adult green salamanders moved on average 4.98 m (SE=0.56) per night, but ended up on average about 1.62 m (SE=0.42) from their start locations at W. B. Bankhead National Forest in Winston County, Alabama. There was strong philopatry and general circular movement patterns. There were no strong environmental factor relationships influencing overnight movement or tortuosity during spring. Summer occupancy surveys were conducted at 148 sites, surveyed between 5-10 times each, on Redstone Arsenal in Madison County, Alabama. Estimated detection probability for surveys was 0.33 (0.15-0.57, 95% CL) based on the top model. More surveyor experience increased detection probability drastically. Detection probability was not clearly related to temperature and relative humidity. Based on the model with the most support, we demonstrate a strong relationship between rock characteristics (rock

cover percentage and rock height). We estimated the occupancy of green salamanders at 0.29 (0.18-0.43, 95% CL) in one of two general areas surveyed on the base. The species is patchily distributed throughout its range. Our conclusions about habitat characteristics used by the species and the short-term movements between them are the first step to better understanding patterns important to detecting and managing populations.

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## List of Abbreviations

°C	degrees Celsius
AIC	Akaike information criterion
AICc	Akaike information criterion accounting for sample size
ANHI	Alabama Natural Heritage Inventory
ANOVA	analysis of variance
ATF	Bureau of Alcohol, Tobacco, Firearms and Explosives
BNF	William B. Bankhead National Forest
CI	confidence intervals
CL	confidence limits
cm	centimeter
ESA	Endangered Species Act
GPS	Global Positioning System
IUCN	International Union for Conservation of Nature and Natural Resources
km	kilometer
m	meter
MaxEnt	Maximum Entropy
mm	millimeter
n	sample size

NASA	National Aeronautics and Space Administration
NLCD	National Land Cover Database
RA	Redstone Arsenal military post
RH	relative humidity
SD	standard deviation
SE	standard error
sqrt	square-root
SVL	Snout-vent length measuring from the anterior tip of the body to the posterior edge of the cloaca on green salamanders.
USFWS	United States Fish and Wildlife Service
UV	ultra violet

## Chapter 1: Understanding Daily Movement of Green Salamanders (*Aneides aeneus*) in William B. Bankhead National Forest, Alabama

### Abstract

Understanding movement patterns and their biotic and abiotic drivers is crucial to effectively manage and conserve salamander populations of concern. With minimal information on green salamander (*Aneides aeneus*) movement patterns, we tested several environmental and biological hypotheses on 69 fluorescently tracked individuals during the springs of 2015 and 2016 at W. B. Bankhead National Forest in northern Alabama. Green salamanders have high desiccation tolerance and morphological features aiding in climbing ability, we expected to see high overnight mobility. This was the case; adult green salamanders moved an average of 4.98 (SE=0.56) meters per night. We compared 17 models of biologic interest using AICc analysis to explore factors influencing individual salamander maximum distances traveled overnight and their circularity of movements. Maximum distance traveled overnight varied with ambient relative humidity and a body size-by-sex interaction. There was also a weak relationship between body size and the tortuosity or circularity of movements where smaller salamanders showed higher circularity than larger salamanders. Green salamanders were found inside refugia of both tree and rock substrates, with rock crevices dominating use. There was a strong similarity of salamander body temperatures with daily refugia temperatures, indicating that refugia effectively buffered the species from ambient conditions. Philopatry was observed in that on average adults returned to 1.62 (SE=0.42) meters from their nightly starting point. Our short-term movement study provides insight into the scale of movements during the breeding season. Future work is encouraged to better understand movements of different seasons and at larger scales to efficiently manage and conserve this species of concern.

### Introduction

Studies on movements of salamanders are crucial in building an understanding of the natural history of these amphibians (Carlson et al. 2004, Lowe et al. 2008, Pittman et al. 2014). The more we know about movement behavior, the more efficiently we can

maintain populations (Kramer et al. 1993, Semlitsch and Bodie 2003, Smith and Green 2005, Cushman 2006, Scheffers and Paszkowski 2012, Pittman et al. 2014, Bani et al. 2015). This is particularly important in the case of salamanders since they are excellent indicators of ecosystem health (Welsh and Droege 2001). Small changes in the environment may have large impacts on small animals. Understanding individual and population behaviors can help conservation efforts and determine the scale of conservation management (Green 2003, Heiss et al. 2015).

Movement studies of salamanders often focus on large-scale spatial and temporal behaviors such as migration (Rothermel 2004, Todd and Winne 2006, Timm et al. 2007, Graeter et al. 2008, Semlitsch 2008, Todd et al. 2009, Wang et al. 2009, Orloff 2011, Pittman and Semlitsch 2013, Woolbright and Martin 2014). All species of salamanders travel to find mates, though the degree to which they traverse the landscape is highly variable. For example, pond-breeding salamanders may migrate kilometers to breeding ponds (Palis 1997, Regosin et al. 2005, Todd et al. 2009, Coster et al. 2014), while terrestrial Plethodontid (lungless) salamanders have special breeding habits and often forgo large-scale migrations, possibly giving them smaller home ranges (Petranka 1998, Sapp 2002, Marsh et al. 2005). Similarly, home ranges of salamanders are varied and range up to 48 m<sup>2</sup> (Barbour et al. 1969, Merchant 1972, Kleeberger and Werner 1982, Ovaska 1988). Short-term movement patterns have not been studied extensively for any salamander taxa, but are important behaviors that help us build effective conservation studies and management actions (Peterman and Semlitsch 2013).

Short-term movements of salamanders can be influenced by both biotic and abiotic factors. Territoriality and interspecific competition influence movements, especially in plethodontids (Griffis and Jaeger 1998, Anthony et al. 2002, Pough et al. 2004, Hasumi et al. 2014). Plethodontid salamanders require moist microhabitats to survive (Grover 1998), and generally need to travel between microhabitats for important feeding and breeding opportunities. Most notably, temperature, humidity, and precipitation tend to influence movement events between microhabitat patches and are thus important to salamander movement (Heatwole 1962, Spotila 1972, Full et al. 1988, Canterbury 1991).

Humidity is an especially important abiotic factor that may influence movements of plethodontids (Spotila and Berman 1976). These salamanders require moist skin for cutaneous respiration (Feder 1988, Full et al. 1988, Orlofske et al. 2009, Williams et al. 2014). Desiccation is a threat plethodontids face when traveling between refugia (Gordon 1952, Ray 1958, Spotila 1972, Keen 1984). Precipitation has been shown to be important to fecundity and movement patterns of salamanders (Milanovich et al. 2006, Liebgold and Jaeger 2007). Both biotic and abiotic factors impact a variety of species of plethodontids, but the species of interest for this study is the green salamander.

Green salamanders (*Aneides aeneus*) are lungless and terrestrial plethodontids that are habitat specialists (Gordon and Smith 1949, Gordon 1952). These cryptic salamanders live in the Appalachian Mountains from southwest Pennsylvania, western Maryland, and southern Ohio to northern Alabama and northeastern Mississippi, typically in disjunct, patchy populations (Petranka 1998, Behler 1979). Green salamanders require mature hardwood forest cover and exposed rock outcroppings, escarpments, or caves (Gordon and Smith 1949, Gordon 1952). They have unique adaptations for climbing, such as a prehensile tail, long limbs, and squared toe tips that allow them to use microhabitat features such as small crevices along rock and tree surfaces (Canterbury and Pauley 1994, Diefenbacher 2008, Schaaf 2010). They have a high water-loss tolerance allowing the use of moist to dry crevices within their environment (Gordon 1952). Crevices are used during the day as refuge from the ambient environment (Armstrong 2010). The species is nocturnal and thought to move from crevices at night to forage or search for mates. Due to habitat loss and conversion, the International Union for Conservation of Nature and Natural Resources (IUCN) categorizes green salamanders as Near Threatened because populations appear to be declining throughout their range (Hammerson 2004). The species has state Endangered status in Indiana, Ohio, Maryland, North Carolina, and Mississippi. They are listed as Threatened in Pennsylvania and listed as Protected in Georgia and Alabama (Hammerson 2004, Rainer 2012).

Green salamanders have a slow life history, not reaching breeding maturity until 7-8 years of age, with females limited to breeding once every two years (Waldron and Pauley 2007). These factors contribute to slow population growth of the species (Cantrell 2012). Localized population declines of 98% have been recorded in the southern portion

of their distribution (Corser 2001). Recently, the Center for Biological Diversity petitioned the U.S. Fish and Wildlife Service (USFWS) for federal protection of the green salamander under the Endangered Species Act (ESA) (Giese et al. 2012). The USFWS is currently investigating whether listing the green salamander under the ESA is warranted.

Movement studies of green salamanders are rare. Miloski (2010) studied nightly movements using fluorescent powdering. She found that season, site, and habitat did not affect individual movements in West Virginia. On average, females moved 2.45 m (n=16), males 4.87 m (n=6), and juveniles 4.80 m (n=25). Gordon (1952) noted an observation of individual movement of about 30 meters but the time frame was not known. Gordon (1961) displaced green salamanders and recorded movements up to 8.84 meters within a few weeks; however, paths taken and substrates used by individual salamanders were unknown. Waldron and Humphries (2005) recorded a dispersal movement of 42 meters within an active season (March-November). Canterbury (1991) observed movements between 6.4 to 46.3 meters over a period of two weeks to eight months. Johnson (2002) explored long-term movements through genetic variation, suggesting gene flow between populations in the range of 1-3 km. With the high tolerance of desiccation, and climbing ability, it is important to consider the possibility that movements may exceed the ranges of other plethodontid salamanders, implicating a larger conservation scale (Miloski 2010).

Substrate use of trees and rocks were noted by Gordon (1952) and explored in 2005 by Waldron and Humphries, but movements between these substrates have not been explored. Environmental factors, such as roads and streams are barriers for several species of terrestrial salamanders (Gibbs 1998, Marsh et al. 2005, Ousterhout and Liebgold 2010, Semlitsch et al. 2012, Whiteley et al. 2014, Bani et al. 2015). It seems reasonable that different substrates could limit green salamander movements.

Here, we explored short-term movements of green salamanders to build our understanding of its natural history and scale of movement. There are a variety of movement goals this study aims to understand. We analyze how far individual salamanders move from crevices within a nightly time frame, comparing biotic (sex, size, reproductive status) and abiotic (relative humidity, temperature, and precipitation) factors

to determine their influence on movements. We examine path circularity of movements using individual tortuosity ratios, also comparing biotic (sex, size, and distance) and abiotic (relative humidity, temperature, and precipitation) factors to investigate influence, and look at the tortuosity ratio in relation to crevice philopatry. We look at the diurnal temperature relationship between refugia, ambient conditions, and green salamanders, and determine which substrates are used in Alabama by the species. Lastly, we also add natural history observations for the species. Plethodontid movements are expected to be about 10 meters maximum per night, with males moving further than females (Miloski 2010). With green salamanders being territorial (Cupp 1979, 1980, Canterbury 1991, Cupp 2014), crevice philopatry is expected. Cantrell (2012) argues the importance of specific microhabitat features in green salamanders and we expect to see strong buffering capabilities of crevices used as diurnal refugia by the species. We also expect to find green salamanders present in rock as well as tree refugia (Gordon 1952, Waldron and Humphries 2005, Miloski 2010). Given the small number of studies on green salamanders, particularly short-term movements, it is important to build our understanding of movement patterns and behavior.

## Methods

Green salamander movement data were collected between March-June in 2015 and 2016 in the William B. Bankhead National Forest (BNF), Winston County, northern Alabama. The three collecting locations within BNF were Natural Bridge Recreation Area (lat 34.176247°, long -87.277347°), Sipsey River Recreation Area (lat 34.284477°, long -87.399228°), and Brushy Lake Recreation Area (lat 34.294537°, long -87.274192°). At each location, we searched for green salamanders along rock escarpments and surrounding trees up to seven meters. All locations were characterized by having long, vertical sandstone and limestone rock escarpments, surrounded by mixed mature hardwood with eastern hemlock (*Tsuga canadensis*).

Green salamander daily movement data were collected during the spring breeding season, post brumation (March-June, 2015-2016). Green salamanders are nocturnally active and often hide in refugia during daylight hours (Behler 1979). Green salamanders

were found during the day in refugia. For each refuge, a habitat description was recorded (rock, tree, ground, etc.) and body temperatures were collected using a digital infrared thermometer (HDE infrared thermometer ST 380A, accuracy to  $\pm 1.5^{\circ}\text{C}$ ) to 0.01 degree Celsius prior to handling. Microhabitat temperature was collected using a thermocouple (Signstek 3 1/2 6802II Dual Channel Digital Thermometer  $1300^{\circ}\text{C}$   $2372^{\circ}\text{F}$  with 2 K-Type Thermocouple Sensor Probe, accuracy to  $\pm 0.4^{\circ}\text{C}$ ) which is expected to be closely related to salamander body temperature since the species is ectothermic. Ambient temperature and relative humidity were recorded upon capture (Kestrel 3000 Environmental Meter, accuracy to  $\pm 1^{\circ}\text{C}$ ) about 1 meter from the substrate surface. We used ANOVA testing with a Tukey's post hoc test testing whether daytime ambient air temperature differed significantly from daytime refuge temperature or salamander body temperature.

Each salamander was removed from the refuge carefully, to minimize stress. Animals were immediately placed in a plastic sealable sandwich-sized bag where snout-vent length (SVL) and tail length were recorded to the nearest millimeter. Snout-vent length measures the length from the tip of the snout of a salamander to the posterior-most part of its cloaca. Salamander gender was determined using dentition (enlarged premaxillary teeth present in males), mental glands (present in males), swollen cloacae (present in females), and visible eggs (present in gravid females; Gordon 1967, Wake 1963). A GPS location was collected for each salamander (Garmin GPSMAP 62S Handheld GPS Navigator, Universal Transverse Mercator NAD83 UTM). At each capture, photographs were taken of each salamander's dorsal and ventral aspects. Adult salamanders could be individually identified by their unique dorsal patterns that do not change throughout their lifetime, with the exception of possible scarring (Gordon and Smith 1949).

Following measurement recordings, each animal was dusted with a non-toxic fluorescent powder (Eco Powder, Dayglo Color Corp.) and released into its original discovery location. Because salamanders were collected during the daylight, when they were not active, released salamanders stayed in their refugia and acclimated to powdering before moving during nocturnal hours. As powdered salamanders moved they left trails

of foot prints, body drags, and tail drags along the surface of rocks, leaves, and tree bark (Roe and Grayson 2008). To track salamanders within close proximity of each other, three colors were used (magenta, bright orange, and neon yellow). Because fluorescent powder wears off the animals within two days, this method of tracking allowed examination of short-term movement (e.g. nightly). Powdering was assumed not to affect movements of tracked salamanders because this technique is minimally invasive to the subcutaneous oxygen consumption rates of salamanders and does not alter behavior (Orlofske et al. 2009, Williams et al. 2014). No evidence of predator attraction to the fluorescent powder was observed.

We returned to the locations of release 24 hours after capture and used ultra violet (UV) light to follow the trails left by the fluorescent powder. Powder trails were followed and total movement distance was recorded in centimeters. The net start-to-end distance was also recorded in centimeters. Powder trails were sketched for each salamander. The trails allowed us to determine substrates used, pathways traveled, and philopatry of each green salamander for the capture refuge after a full night activity period. Pathway tracking provided more information than exclusively using Euclidean (net start-to-end) distance, however, Euclidean distance was important in examining site philopatry and tortuosity (Almeida et al. 2010).

Powder trails were classified as either complete or incomplete. A complete path was one that could be followed to its terminal point (e.g. diurnal refugia) and the animal relocated. An incomplete path was one for which the animal could not be located to the trail terminus. Some tracks were lost due to not using enough powder, moving too high or deep into the rock where tracks could not be observed, or traveling over running water on the surface of rocks, washing the powder off of the salamander. Incomplete paths potentially underestimate movements of green salamanders overnight and do not contribute to our understanding of philopatry. Site philopatry was examined only for complete paths using Euclidean distance measurements between the start and terminal points of each green salamander. Tortuosity was calculated as the ratio of the Euclidean distance (start-to terminal location) divided by the total distance each salamander moved. A tortuosity ratio approaching 1 suggested straight movement, while a ratio close to zero

suggested more circular movements between the start and terminal points of pathways. Due to philopatric movements we observed and net displacements close to zero in some cases, our interpretation of the tortuosity ratio changes from its traditional use as a measure of path complexity measurement to a measure for interpreting how circular path movements are.

We used linear models to examine how sex, size, temperature, relative humidity, precipitation and chosen interactions influenced the distance and tortuosity of salamander movements overnight. Linear models for each metric were compared using AICc in the statistical program R (R Development Core Team 2008). Interactions of biological interest were tested in addition to different combinations of factors, chosen for biological relevance. The interactions explored the relationship of salamander movement and tortuosity with size\*relative humidity, and temperature\*relative humidity, as well as interactions between sex for each variable. The global model examined the following relationships:

$$\begin{aligned} \text{Distance} = & \text{Sex} + \text{Size} + \text{Temperature} + \text{Humidity} + \text{Precipitation} + \text{Year} + \\ & (\text{Sex} * \text{Size}) + (\text{Sex} * \text{Temperature}) + (\text{Sex} * \text{Humidity}) + (\text{Sex} * \text{Precipitation}) + \\ & (\text{Sex} * \text{Year}) + (\text{Temperature} * \text{Humidity}) + (\text{Size} * \text{Humidity}) \end{aligned}$$

Size of salamander referred to SVL to account for individuals with broken or regenerated tails. Overnight temperature and overnight relative humidity were calculated using average ambient air temperature and average relative humidity between 8pm-6am of each nightly moving period. Precipitation measurements were collected through daily rainfall for each 24-hour period of tracking. Since salamanders moved at night, overlapping two days, precipitation across those two days was summed for each tracking occasion. Nightly temperature, humidity, and precipitation data encompassing all study areas was collected from the Bankhead National Forest weather station (USDA Forest Service weather station, ID:BHFA1, WIMS ID:10702, Name:Bankhead, lat 34.344167°, long -87.3374°, Elevation:804 feet, MNET:Raws).

Movement measurements were square-root transformed to improve normality and homogeneity of variance (Figure 1-1). Validity of the transformation was checked using

Box-Cox plots and quantile-quantile plots in R (Sakia 1992, Osborne 2010). Two sets of analyses were conducted for linear regression tests on the maximum distance green salamanders moved overnight, one with movement paths of all adults tracked (including complete and incomplete tracks; hereafter, the full data set;  $n=60$ ), and one excluding incomplete paths (hereafter, the filtered data set;  $n=39$ ). The full data set was used to increase sample size; however, its incomplete tracks likely underestimate the maximum distances green salamanders moved overnight.

Additionally, females were analyzed separately from males to determine if gravid female movements differed from non-gravid female movement. We used linear a regression model to test movement distances between the sexual status of all females.

## Results

We tracked 69 green salamanders, 36 in 2015 (14 females; 19 males; 2 adults of unknown sex; and 1 juvenile) and 33 in 2016 (13 females; 14 males; 1 adult of unknown sex; and 5 juveniles). All individual salamanders were captured only once during the study. Of the 69 salamanders, seven did not move from their original crevices (12%; Table 1-1). The remaining salamanders moved between 0.00 and 17.15 meters (Table 1-2). On average, male green salamanders moved 5.36 meters ( $SE=0.82$ ,  $n=33$ ) over a 24-hour period (Table 1-2A). On average, female green salamanders moved 4.53 meters ( $SE=0.76$ ,  $n=27$ ) overnight (Table 1-2A). Male and female movements do not differ significantly ( $p=0.38$ ). The average distance moved did not change much for filtered tracks, where males moved on average 5.38 meters ( $SE=1.22$ ,  $n=19$ ) and females 3.82 meters ( $SE=0.83$ ,  $n=20$ ) (Table 1-2B). Filtered male and female movements do not differ significantly ( $p=0.28$ ). In all cases, the standard deviation of the average overnight distances moved for each sex was large. Environmental conditions for green salamanders were similar between the full data set (temperature between 6.21-27.12 ( $^{\circ}C$ ), relative humidity between 49.72-98.36 percent, and precipitation between 0-1.06 (inches);  $n=60$ ) (Table 1-3A) and the filtered data set (temperature between 6.21-27.12 ( $^{\circ}C$ ), relative

humidity between 49.72-96.09 percent, and precipitation between 0-1.06 (inches); n=39) (Table 1-3B).

#### *Movement Observations and Substrate Use*

Green salamanders were found in rock crevices 68 out of 69 (99%) occasions. All green salamanders were tracked across rock substrate, except one gravid female that was found in a scar of an American Beech tree (*Fagus grandifolia*). That female did not move from her original location for the two-day span she was tracked. Four green salamanders were tracked across leaf litter. In two of these cases, such travel appeared to be due to salamanders falling or leaping off of a vertical rock surface, and returning to the rock after traveling across the litter. In the other two cases, travel on leaf litter appeared intentional, as each salamander climbed down a rock surface, crossed leaf litter, and moved to a different location. One green salamander was tracked to a different rock surface, the other (male) to a chestnut oak tree (*Quercus montana*). Tracking across leaf litter showed green salamanders traveled across the surface litter as well as under the litter.

Only three tracked individuals (4%) used tree substrate (one female, two males). One was a gravid female, found in an American Beech tree (47 mm SVL female tracked May 28-30, 2016 at Brushy Lake Recreation cliffs). Another was an adult male (36 mm SVL) that traveled to a chestnut oak tree from a rock surface, crossing leaf litter to reach the tree (May 19, 2015 at Natural Bridge Recreation area). The third salamander was a male (38 mm SVL) that moved along a rock surface, onto a tree growing from the rock, climbed to the tip of a branch of a young hardwood tree (branch length was 1.7 meters long), and returned along the branch to resume climbing along the vertical rock surface.

Of the tracked females (n=27), 11% did not move from their original crevices, and 19% moved from their original location, but returned to the same crevice. Females moved from their original locations and were found in different crevices following the nightly activity period 44% of the time. We lost 26% of tracked females due to challenges of field tracking. For males (n=33), 9% did not move, 9% moved and returned to the same crevice, 42% moved and ended in a different crevice, and 39% had

incomplete tracks (Table 1-1). Overall, we obtained a 61% success rate in tracking complete trails of males and 74% for tracking females. For juvenile green salamanders, 83% of trails were complete.

### *Refugia, Philopatry, and Tortuosity*

Refugia used during green salamander searches consisted of thin crevices in sandstone and limestone rock faces and scars in tree bark. Refugia temperatures were, on average, lower than ambient temperatures by 4.6°C. Mean body temperatures of salamanders closely matched crevice temperatures, where body temperature was on average 1.55 degrees C lower (Figure 1-2) than crevice temperature. ANOVA testing showed that daytime ambient air temperature differed significantly from daytime refuge temperature ( $p < 0.00$ ) and salamander body temperature ( $p < 0.00$ ). Green salamander body temperature did not differ significantly from refuge temperatures during the day ( $p = 0.30$ ).

Refuge philopatry was explored through net movements of each salamander using Euclidean distance from start to terminal location. Of all tracked green salamanders, 13.0% returned to their original trapping locations (Table 1-1), while 46.4% moved to a different location. The percentage of incompletely tracked salamanders was 30.4%. Removing incomplete tracks, the percentage of salamanders returning to their original crevices increased to 14.6%. The percentage of salamanders that moved to different crevices increased to 66.7%. Female green salamanders moving to new crevices ended up, on average, 1.64 meters (SE=0.75, n=11) from their original crevices, while males were found, on average, 1.89 meters (SE=0.68, n=10) from their original location of capture (Figure 1-3). Juveniles were found on average 0.94 meters (SE=0.40, n=4) from their original locations. Green salamander philopatry did not differ significantly between sex ( $p = 0.77$ ).

Tortuosity was calculated for adult filtered green salamanders that moved during spring of 2016 (n=19). Mean tortuosity was 0.41 (SE=0.07) for 2016 adult green salamanders. Tortuosity did not differ significantly between the sexes ( $p = 0.40$ ), with a mean for males of 0.35 (SE=0.08, n=10), and a mean for females of 0.47 (SE=0.12, n=9)

(Figure 1-4). In most cases, green salamanders moved in a more circular fashion, coming back toward the starting location.

Influences of various interspecific and environmental factors on tortuosity were tested using AICc analysis (Table 1-4). Two models, one incorporating size alone and the other incorporating size, relative humidity, and the interaction of these variables, emerged as equivalent top models. The simplest top model estimated, for every 1 mm increase in green salamander body size, there was a 0.04 ( $\pm 0.01$ , 95% CI) increase in tortuosity ratio ( $r^2=0.54$ ), suggesting that larger animals moved less circular than smaller animals.

#### *Full Movement AICc Analyses*

Movement distances were examined using linear regression for the full green salamander data set (n=60). Seventeen models of biological interest were compared through AICc analysis (Table 1-5). The top model suggests that SVL, sex, and a sex-by-SVL interaction influence movements of green salamanders during the breeding season. This top model has five parameters, and an Akaike model weight suggesting that the model has a 34% chance of the 'true' model within the 17 selected models. The next best model was the null model with 2 parameters, a delta AICc of 1.4, but a low Akaike model weight of 0.17.

The top model estimates that male movement is about 4.46 ( $\pm 1.74$ , 95% CI) sqrt-meters less than female movement. For each 1 mm increase in size (SVL) of salamanders, there was a 0.05 ( $\pm 0.03$ , 95% CI) sqrt-meter increase in the distance moved. The sex-by-size interaction indicated that the difference between males and females increased by 0.11 ( $\pm 0.04$ , 95% CI) sqrt-meters for each 1 mm increase in SVL (Figure 1-5A). This interaction suggests that males of larger size increase the distance that they move overnight more than females of increasing size. As females increase in size, they move shorter distances overnight.

#### *Filtered Movement AICc Analyses*

Linear regressions were also conducted for the filtered green salamander data set (n=39) to account for the possibility of underestimating movements. The same 17 models

of biological interest were compared through AICc analysis (Table 1-6). The top model suggests that relative humidity is the most important recorded factor influencing movement distance of green salamanders during the breeding season. This top model has three parameters, and an Akaike model weight suggesting that there is a 22% chance of it being the 'true' model being within the 17 selected models. The next best model was the null model with 2 parameters, a delta AICc of 0.75, and a low Akaike model weight of 0.15. This suggests that the top two models are not necessarily different.

The top model estimates that for each 1 percent increase in relative humidity, there was a 0.02 ( $\pm 0.01$ , 95% CI) sqrt-meter decrease in the distance moved overnight by green salamanders ( $r^2=0.05$ ) (Figure 1-6B). This is weak evidence that as humidity increases, green salamanders move less. Other highly ranked models in the AICc analysis were single variable models and the model pointing toward an interaction between size and sex on the distance green salamanders move at night (Figure 1-5B).

#### *Gravid Female Movements*

Linear regression was used to determine if gravid female movements differed from non-gravid females (n=27) (Figure 1-7). Gravid females did not differ in the distance moved, relative to non-gravid female movements, for either the full (p=0.33, Figure 1-7A) or filtered (p=0.72, Figure 1-7B) data sets. All gravid females were  $\geq 45$  mm in size (SVL), while non-gravid females ranged from 31-52 mm SVL.

Table 1-1: Categories of movement behaviors for all green salamander tracks (n=69), 2015-2016.

	No movement outside of crevice	Moved, returned to same crevice	Moved to different crevice	Moved, lost trail	Total
Female	3	5	12	7	27
Male	3	3	14	13	33
Unknown	1	0	2	0	3
Juvenile	0	1	4	1	6

Table 1-2: Size and overnight movement distance ranges for adult green salamanders with A) full tracking data (n=60), B) filtered tracking data (n=39), and C) completely tracked gravid females (n=8), 2015-2016. SE=standard error and SD=standard deviation.

	Variable	Range	Mean	SE	SD	Median
<b>A: Full Tracking Data</b>						
<b>Males (n=33)</b>	Distance (m)	0-17.15	5.36	0.82	4.70	4.01
	SVL (mm)	29-64	43.40	1.13	6.50	43
<b>Females (n=27)</b>	Distance (m)	0-12.19	4.53	0.76	3.97	3.84
	SVL (mm)	31-55	46.50	1.32	6.84	43
<b>B: Filtered Tracking Data</b>						
<b>Males (n=19)</b>	Distance (m)	0-17.15	5.38	1.22	5.33	4.01
	SVL (mm)	29-53	42.86	1.52	6.64	43
<b>Females (n=20)</b>	Distance (m)	0-12.19	3.82	0.83	3.72	2.96
	SVL (mm)	33-55	44.98	1.50	6.71	45
<b>C: Gravid Female Data</b>						
<b>Gravid Females (n=8)</b>	Distance (m)	0-10.13	3.29	1.16	3.28	2.96
	SVL (mm)	45-55	49.56	1.41	3.98	48.75

Table 1-3: Environmental conditions for green salamanders with A) full tracks (n=60), B) filtered tracks (n=39), and C) gravid female tracks (n=8), 2015-2016. SE=standard error and SD=standard deviation.

Variable	Range	Mean	SE	SD	Median
<b>A: Full Tracking Data</b>					
Temperature (C)	6.21-27.12	21.04	0.55	4.27	19.32
Relative Humidity (%)	49.72-98.36	80.15	2.10	16.30	86.41
Precipitation (inches)	0-1.06	0.14	0.04	0.34	0
<b>B: Filtered Tracking Data</b>					
Temperature (C)	6.21-27.12	21.64	0.71	4.41	23.58
Relative Humidity (%)	49.72-96.09	77.24	2.31	14.40	73.27
Precipitation (inches)	0-1.06	0.12	0.05	0.33	0
<b>C: Gravid Female Data</b>					
Temperature (C)	23.89-27.12	24.90	0.50	1.42	24.44
Relative Humidity (%)	49.73-73.27	66.20	3.61	10.22	70.91
Precipitation (inches)	0	0	NA	0	0

Table 1-4: AICc table of model outputs for filtered salamander tracks testing interspecific and environmental factors on the tortuosity ratio of movements, 2016 (n=19).

Model	Parameters	AICc	Delta AICc	Likelihood	Weight
Tortuosity~SVL	3	-0.4	0.00	1.00	0.48
Tortuosity~ONRH+SVL+(ONRH*SVL)	5	-0.4	0.07	0.96	0.46
Tortuosity~Sex+SVL+(Sex*SVL)	5	5.4	5.40	0.07	0.03
Tortuosity~Sex+ONTC+ONRH+SVL+sqrtDistM	7	6.3	6.34	0.04	0.02
Tortuosity~Sex+ONTC+ONRH+SVL+sqrtDistM+(ONTC*ONRH)+(SVL*ONRH)	8	12.0	11.99	0.00	0.00
Tortuosity~ONTC	3	12.1	12.14	0.00	0.00
Tortuosity~ONRH	3	12.4	12.35	0.00	0.00
Null	2	12.5	12.45	0.00	0.00
Tortuosity~sqrtDistM	3	13.1	13.13	0.00	0.00
Tortuosity~Sex	3	14.5	14.50	0.00	0.00
Tortuosity~ONTC+ONRH+(ONTC*ONRH)	4	15.3	15.34	0.00	0.00
Tortuosity~Sex+ONRH+(Sex*ONRH)	5	15.4	15.43	0.00	0.00
Tortuosity~Sex+ONTC+(Sex*ONTC)	5	15.6	15.56	0.00	0.00
Tortuosity~Sex+sqrtDistM+(Sex*sqrtDistM)	5	19.4	19.44	0.00	0.00
Tortuosity~Sex+ONTC+ONRH+SVL+sqrtDistM+(Sex*sqrtDistM)+(Sex*ONTC)+(Sex*ONRH)+(Sex*SVL)+(ONTC*ONRH)+(ONRH*SVL)	12	52.5	52.46	0.00	0.00

Table 1-5: AICc table of model outputs for full salamanders testing effects on distance green salamanders moved overnight. Models ordered from least to greatest AICc value (n=60).

Model	Parameters	AICc	DeltaAICc	Likelihood	Weight
Dist~Sex+SVL+(Sex*SVL)	5	177.3	0.00	1.00	0.34
Null	2	178.7	1.40	0.50	0.17
Dist~Precip	3	179.8	2.50	0.29	0.10
Dist~Sex	3	180.1	2.80	0.25	0.08
Dist~Humidity	3	180.4	3.10	0.21	0.07
Dist~Temp	3	180.6	3.30	0.19	0.06
Dist~Year	3	180.9	3.60	0.17	0.06
Dist~SVL	3	180.9	3.60	0.17	0.06
Dist~Sex+Precip+(Sex*Precip)	5	182.8	5.50	0.06	0.02
Dist~Sex+Humidity+(Sex*Humidity)	5	183.9	6.60	0.04	0.01
Dist~Temp+Humidity+(Temp*Humidity)	5	184.2	6.90	0.03	0.01
Dist~Sex+Year+(Sex*Year)	5	184.3	7.00	0.03	0.01
Dist~Sex+Temp+(Sex*Temp)	5	184.4	7.10	0.03	0.01
Dist~SVL+Humidity+(SVL*Humidity)	5	184.6	7.30	0.03	0.01
Dist~Sex+SVL+Temp+Humidity+Precip+Year	8	189.8	12.50	0.00	0.00
Dist~Sex+SVL+Temp+Humidity+Precip+Year+(Temp*Humidity)+(SVL*Humidity)	10	195.1	17.80	0.00	0.00
Dist~Sex+SVL+Temp+Humidity+Precip+Year+(Temp*Humidity)+(SVL*Humidity)+(Sex*SVL)+(Sex*Temp)+(Sex*Humidity)+(Sex*Precip)+(Sex*Year)	15	203.9	26.60	0.00	0.00

Table 1-6: AICc table of model outputs for filtered salamanders testing effects of distance green salamanders moved overnight. Models ordered from least to greatest AICc value (n=39).

Model	Parameters	AICc	DeltaAICc	Likelihood	Weight
Dist~Humidity	3	122.3	0.00	1.00	0.22
Null	2	123.1	0.75	0.69	0.15
Dist~Temp	3	123.8	1.44	0.49	0.11
Dist~Sex+SVL+(Sex*SVL)	5	123.9	1.61	0.45	0.10
Dist~Sex	3	124.2	1.86	0.39	0.09
Dist~Year	3	124.4	2.06	0.36	0.08
Dist~Precip	3	124.7	2.40	0.30	0.07
Dist~SVL	3	125.2	2.89	0.24	0.05
Dist~Sex+Humidity+(Sex*Humidity)	5	125.5	3.16	0.21	0.05
Dist~SVL+Humidity+(SVL*Humidity)	5	126.5	4.17	0.12	0.03
Dist~Sex+Temp+(Sex*Temp)	5	127.2	4.91	0.09	0.02
Dist~Temp+Humidity+(Temp*Humidity)	5	127.4	5.10	0.08	0.02
Dist~Sex+Precip+(Sex*Precip)	5	127.5	5.20	0.07	0.02
Dist~Sex+Year+(Sex*Year)	5	127.9	5.59	0.06	0.01
Dist~Sex+SVL+Temp+Humidity+Precip+Year	8	133.9	11.59	0.00	0.00
Dist~Sex+SVL+Temp+Humidity+Precip+Year+(Temp*Humidity)+(SVL*Humidity)	10	139.7	17.35	0.00	0.00
Dist~Sex+SVL+Temp+Humidity+Precip+Year+(Temp*Humidity)+(SVL*Humidity)+(Sex*SVL)+(Sex*Temp)+(Sex*Humidity)+(Sex*Precip)+(Sex*Year)	15	155.9	33.61	0.00	0.00

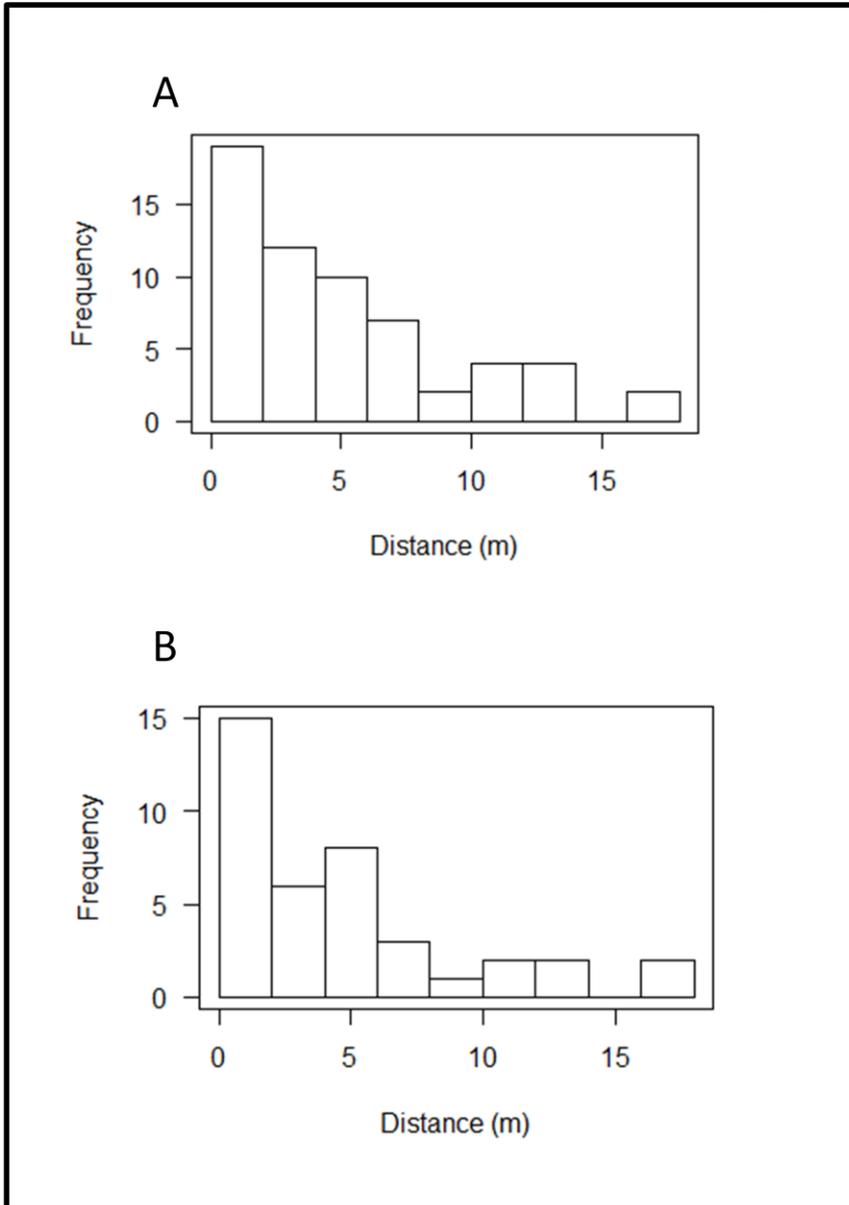


Figure 1-1: Frequency distribution of overnight movement distances by green salamanders with A) full tracks (n=60) and B) filtered tracks (n=39), 2015-2016.

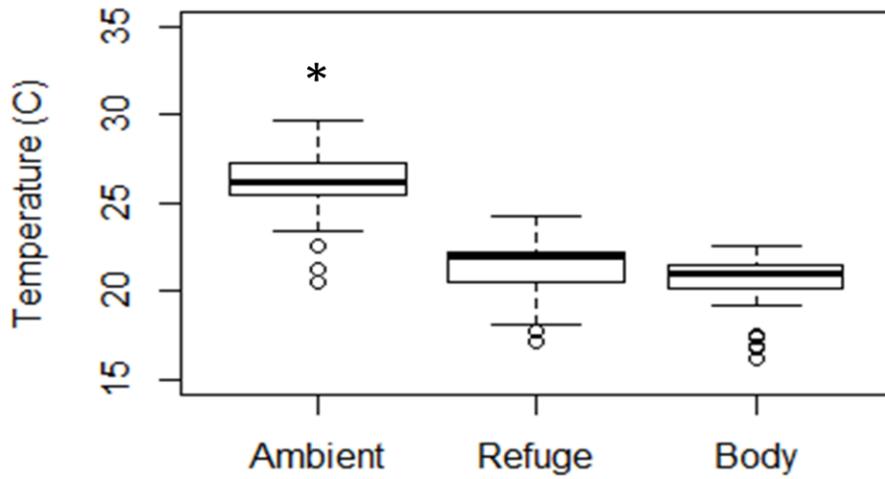


Figure 1-2: Range of ambient air (°C), crevice air (°C), and external body temperatures (°C) (n=45), 2015-2016. \*differs significantly.

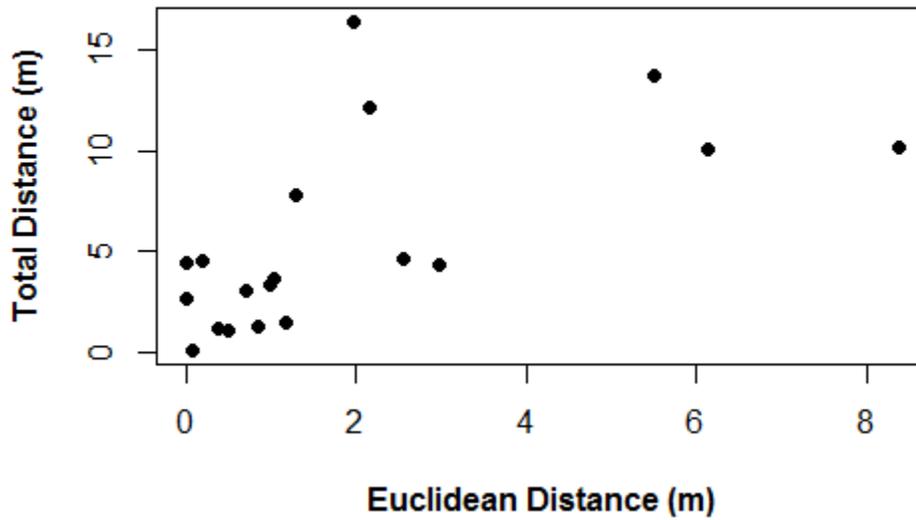


Figure 1-3: Total distance green salamanders moved in relation to philopatry (Euclidean distance from start-to-terminal points of trail).

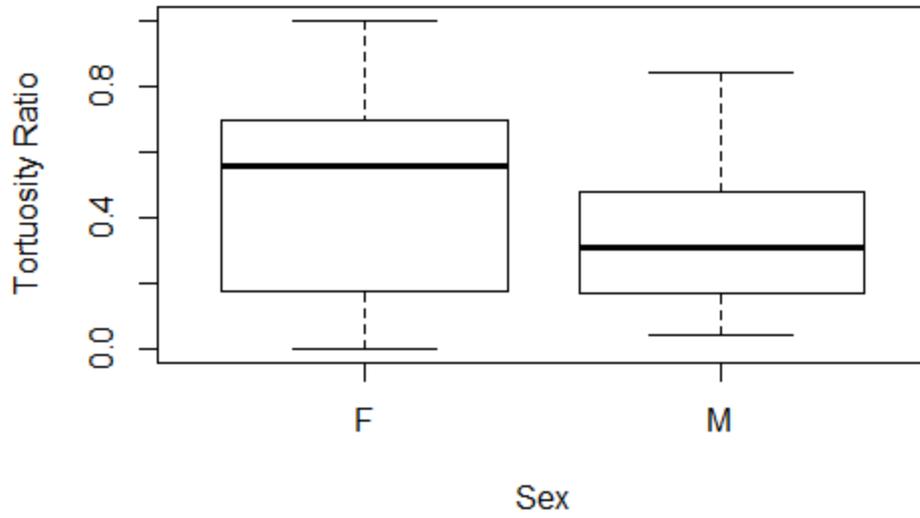


Figure 1-4: Overnight tortuosity ratio distribution of female and male green salamanders in spring 2016 (n=18).

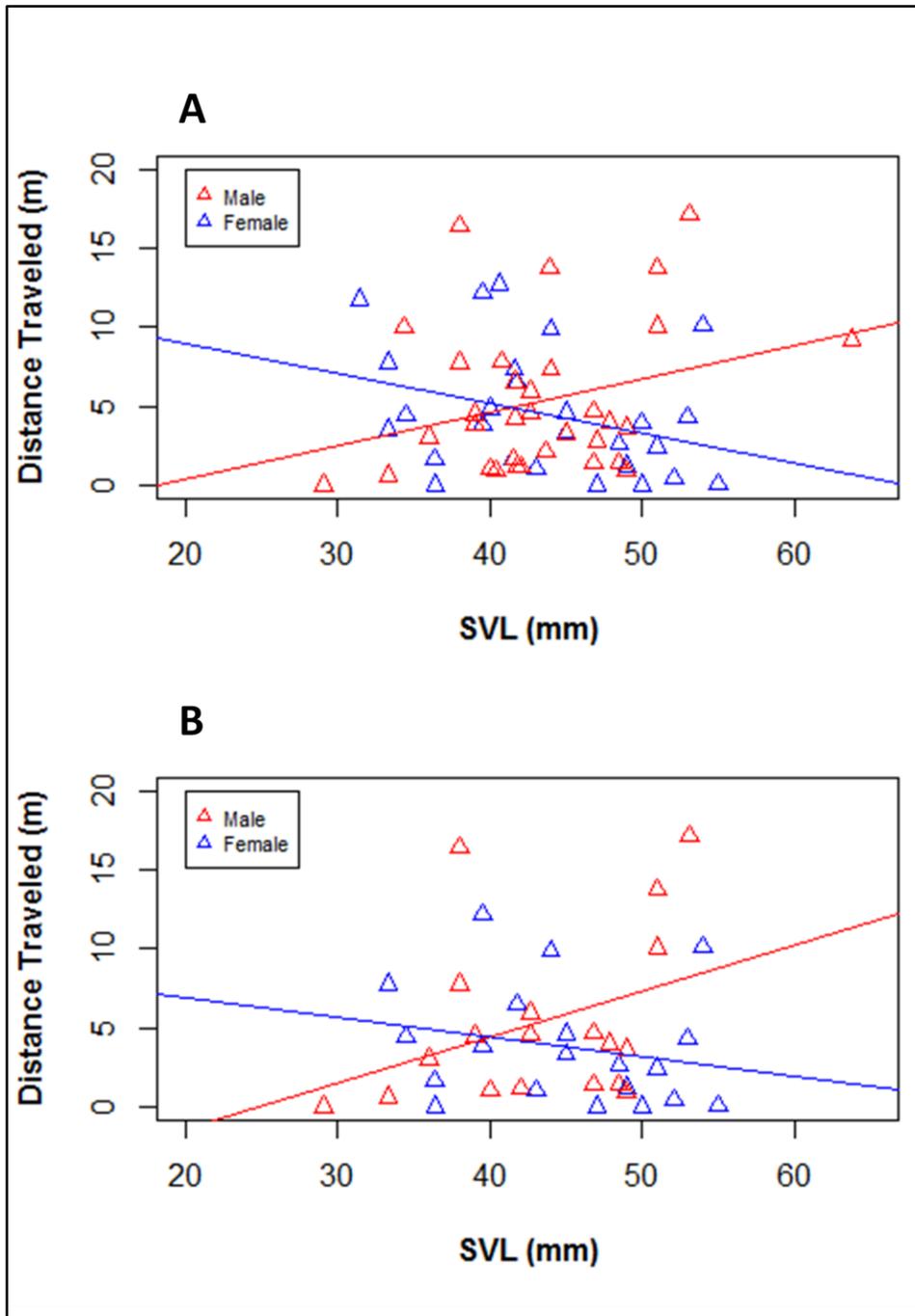


Figure 1-5: Distance (m) traveled overnight vs. snout-vent length (SVL, mm) for green salamanders with A) full tracks (n=60) and B) filtered tracks (n=39), 2015-2016.

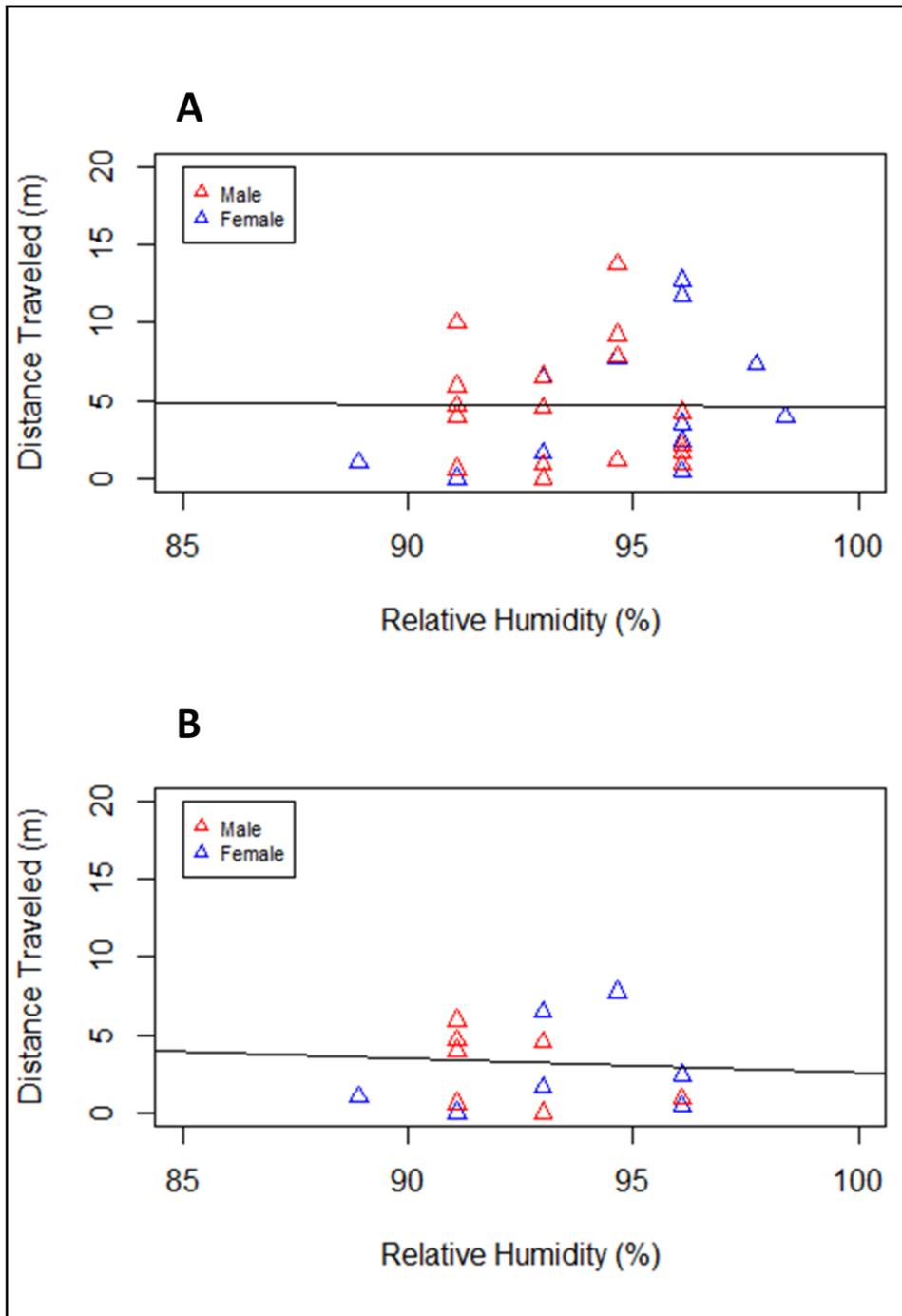


Figure 1-6: Distance traveled (m) vs. average overnight relative humidity (%) for green salamanders with A) full tracks (n=60), and B) filtered tracks (n=39), 2015-2016.

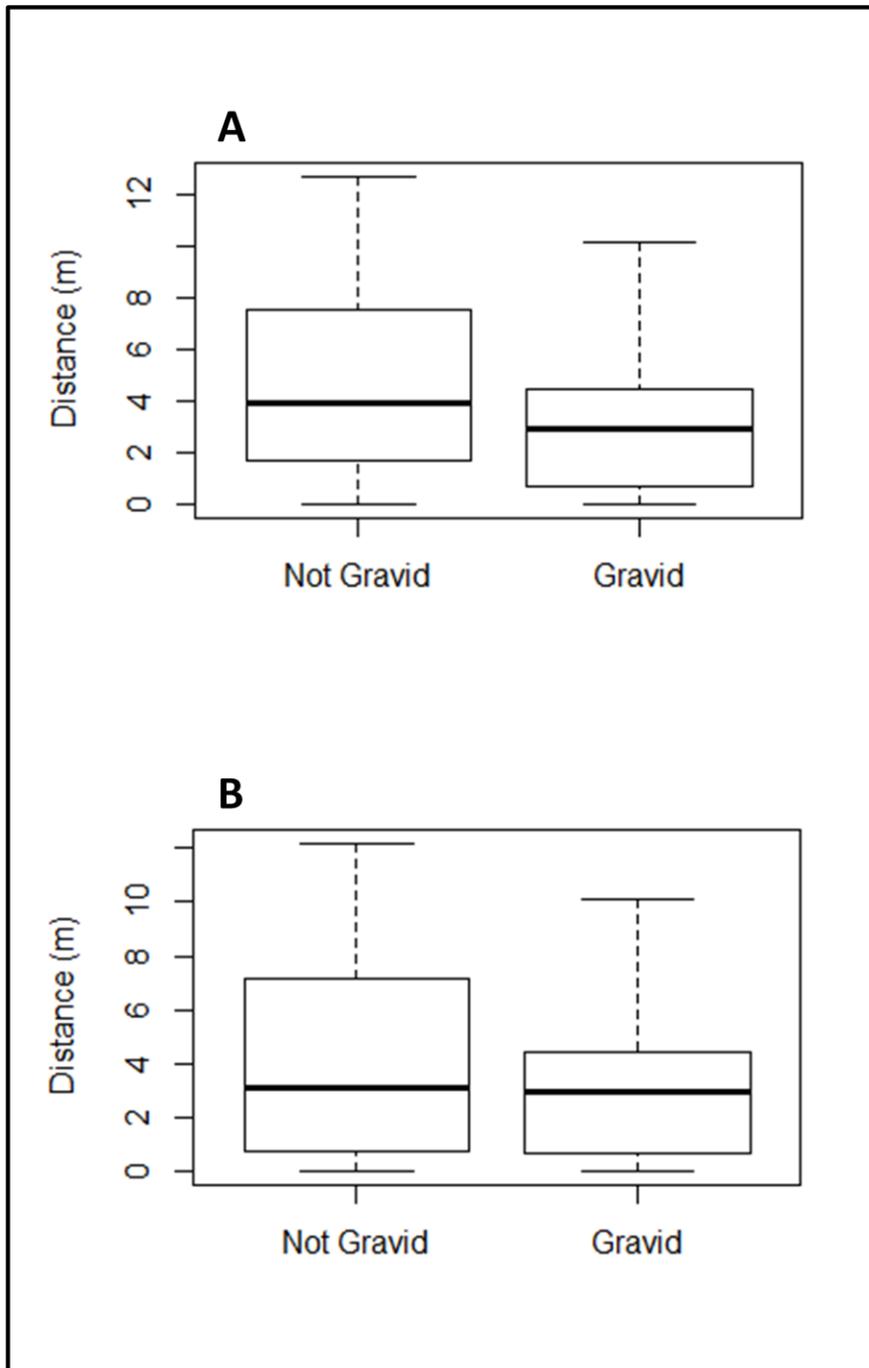


Figure1-7: Reproductive status (not gravid vs. gravid) of female green salamanders in relation to distance traveled (m) for A) full tracks (n=27), and B) filtered tracks (n=20), 2015-2016.

## Discussion

### *Movement Observations and Substrate Use*

A variety of natural history gaps exist in our knowledge of green salamanders, particularly relative to their movements. Use of crevice refugia by green salamanders indicates these crevices have moisture retention capabilities important to the species (Cantrell 2012). Rock crevices are where green salamanders traditionally have been described to occur; but the species has also been found on and under the bark or scars of trees (Gordon 1952, Waldron and Humphries 2005). In our study, both rocks and trees were used as refugia, with use of rocks dominating over use of trees. These observations are consistent with Waldron and Humphries (2005). Leaf litter was used as a transition substrate which did not provide the physical protection from predation or abiotic changes like tree and rock crevices.

Green salamanders moved overnight 89.9% of the time after being powdered. The effect of powder on enticing an individual to move overnight is unknown. The powder did not force a green salamander to immediately move from refugia, providing some evidence that the powder had little effect on salamander behavior. Williams et al. (2014) tested the effect on behavior following powdering in the lab, showing no behavioral impact. Handling of salamanders is also a reasonable explanation for movement rates, however, green salamanders did not immediately move from refugia upon release for several hours until their nocturnal active period.

Despite difficulties of tracking all salamander paths to completion, we did observe important biological information. One such observation included a possible mating. Reproduction by plethodontid salamanders involves a male enticing a female to follow him and depositing a spermatophore, and the female picking up the spermatophore with her cloacal lips (Gordon 1952, Cupp Jr. 1971, Canterbury and Pauley 1994, Petranka 1998). One possible mating was tracked, involving a male and female with overlapping paths for a distance of 2.8 meters on the same night (tracked May 28, 2016 at Brushy Lake Recreation cliffs where the female moved a total of 4.31 m and the male moved a total of 16.44 m). However, we cannot confirm that the salamanders used the same space

at the same time. Different green salamanders often moved along the same rock crevices and ledges over the breeding season, often taking similar paths along refugia.

Only three tracked individuals (4%) used tree substrate while 99% of green salamanders used rock substrate. Waldron and Humphries (2005) saw similar patterns with used trees often within 2 meters of suitable rock. Rock use is important for green salamanders, but tree use should be explored further. Different phenology of movements into and out of trees and rocks for each sex is suggested, but still yet to be explored in depth (Miloski 2010).

### *Refugia, Philopatry, and Tortuosity*

The buffering capability of crevice microhabitat was confirmed by data collected during salamander captures. Green salamanders were on average 8.6 degrees Celsius cooler than daytime ambient air temperature (Figure 1-2). Ectothermic animals are often closely tied to environmental conditions, not being able to regulate their core temperatures internally. They instead modify their core temperature through behavior, such as basking in sun, limiting their activity to specific times of day, or selecting habitat that buffers them from extreme temperature fluctuations (Pough et al. 2004). External body temperatures of green salamanders were similar to microhabitat temperatures upon capture and did not overlap with ambient temperatures (Figure 1-2) (Rosell et al. 2009). Average green salamander body temperatures were 0.9 degree Celsius below the refugia air temperatures. Thigmothermy theory can help explain this difference. Ectotherms often behaviorally thermoregulate through contact with hot or cold surfaces transferring heat energy (Pough et al. 2004). The same trend is possibly true for humidity in crevices vs ambient humidity. Besides regulating body conditions through temperature and humidity, avoiding predation is also a likely factor driving crevice use.

A return rate of 13.0% to original crevices demonstrates a low return rate at the scale of individual crevices for green salamanders. I observed 46.4% moving from their original crevices to new locations. However, 30.4% of all the tracks were incomplete. Removing the incompletely tracked salamanders, the percentage of salamanders returning to the original crevices used increased only slightly (14.6%), suggesting that the

observation of low crevice philopatry was not an artifact of incomplete tracking. The percentage of salamanders that moved and were completely tracked to a different crevice increased to 66.7%. Philopatry is expected to the scale of crevice as it has been observed in the species during the summer (Gordon 1967), but we observed a slightly larger scale for philopatry than crevice during the breeding season (Figure 1-3). Completely tracked female green salamanders moved an average Euclidean (net) distance of 1.64 meters from their original crevices, indicating philopatry to a general area. The same pattern can be seen in completely tracked male green salamanders, which were found on average 1.89 meters from their original location of capture. Juveniles show a smaller scale of philopatry than adults, but still not to the scale of specific crevices. However, the small sample size prevents strong inference about juvenile movement. Philopatry to this scale during overnight activity is noteworthy. Nightly movements can easily be underestimated when looking at mark-recapture events that only take into account Euclidean distance measurements, particularly because studies have suggested limited movement for the species (Gordon 1961).

Tortuosity was investigated in relation to philopatry of green salamanders by including the total distance green salamanders moved overnight. Philopatric movements should have lower tortuosity ratios, or more circular movements; again, this is a different interpretation of tortuosity than historically used. Our measure of circularity using tortuosity ratio may be influenced by various environmental or biotic factors. We used AICc analysis to narrow which factors were likely to influence the tortuosity of the salamander movements (Table 1-4). The top six models all suggest that size is important to how much a salamander's path loops back to itself. The observed decrease in tortuosity ratio with increasing body size suggests smaller green salamanders move along less straight paths. Because size is directly related to the age of salamanders (Staub et al. 1995, Marvin 2001, Liebgold and Jaeger 2007, Waldron and Pauley 2007), we expect to see different behaviors of salamanders at various ages. Younger, smaller salamanders could travel paths that are more circular with possible exploratory excursions, helping them learn their habitat (Crane and Mathis 2011). Another possibility is that younger green salamanders may have smaller home ranges than larger adults. Food abundance of different prey sizes may also play a role. While this size relationship is not strongly

supported by the data, it serves as a launching point for future studies interested in testing factors driving movement patterns.

The top models show that relative humidity may also play a small role in the tortuosity paths of green salamanders. Although the observed relationship between tortuosity and ambient relative humidity was weakly supported, a stronger relationship may be present for the relative humidity within refugia for overnight movements. Green salamanders traveled along crevices and ledges in the rocks, rarely venturing into the unprotected open, even at night. The buffering capabilities of refugia were shown for air temperatures and likely include relative humidity as well. As of now, there are no tools that will allow us to measure the relative humidity within a small crevice, but this information may be helpful in understanding green salamander movement.

#### *Full Movement*

The top model of complete and incomplete tracks (full data set) had the most weight, with a 34% chance of being the top model (Table 1-5). The top model weakly suggests size, sex, and the interaction of sex and size influenced the maximum distance traveled nightly during the spring breeding season (Figure 1-5A). This interaction suggests as body size increases, movement distance of males increases, but decreases for females. As males increase in size, they may travel longer distances, a pattern consistent with mate searching. This fits the pattern of Plethodontid salamander reproduction (Petranka 1998), but larger samples sizes are suggested for greater support of this relationship.

The pattern of decreasing movement distance with increasing body size for females during the breeding season makes biological sense when we look at female reproduction patterns (Gordon 1952, Cupp 2011). Females find brooding crevices in June to start laying eggs late June to mid-July (Canturbury and Pauley 1994, Cupp 2011). Once females find brooding crevices, they will lay eggs and stay within the same crevice for the entire summer while the eggs develop as well as after hatching (Gordon 1952, Petranka 1998, Cupp 2011). Support for this relationship is relatively weak, but better than the other tested models.

### *Filtered Movement*

Of the filtered track, the fact that relative humidity was not an important factor is surprising because it is expected to be important to all lungless salamanders as they require moist skin to exchange oxygen through the skin and respire (Full et al. 1988). Due to the high tolerance of desiccation in green salamanders, relative humidity is likely to have a smaller impact on movements and activity than in other plethodontid salamanders (Gordon 1952).

Ambient temperature was not a significant factor influencing maximum nightly movement distances. Refugia temperature data (Figure 1-2) show that green salamander body temperatures do not match ambient temperatures during the day, but instead match crevice temperatures, and therefore, ambient temp is not likely an important factor pressuring overnight movements. Green salamanders moved along crevices and rock features, effectively buffering them from ambient temperatures (Pauley and Watson 2009). Temperature is more likely important to occupancy of green salamanders on a larger scale (Hardman 2014).

### *Full and Filtered Comparison*

The incomplete tracks may underestimate the maximum distance green salamanders move overnight within the spring breeding season. However, the similar spread of the biotic, abiotic, and movement data collected (Figures 1-2 and 1-3) suggest only a slight underestimation. Use of both complete and incomplete tracks allowed us to explore relationships with a larger sample size.

Movement distances may have been influenced by biotic (SVL and sex) or abiotic (temperature, relative humidity, precipitation, year) factors and, therefore, relationships among these factors were explored above with a subset of linear models for both full and filtered track data sets (Tables 1-5 and 1-6). Although the group of highest ranked models varied for the two analyses, two models were in the highest ranked group in both analyses. These models were the null model and the size, sex, and interaction of size and sex model. Support for the null model suggests that none of the biotic or abiotic factors had an effect on green salamander movement. This may be due to the fact that

movements are so highly variable among individuals, patterns are hard to tease out with the current sample size. The size, sex, and interaction of size and sex suggests a pattern biologically important to green salamanders, but further study with green salamanders is needed.

#### *Gravid Female Movements*

Gravid females did not move significantly different distances overnight than non-gravid females. All gravid females were  $\geq 45$  mm SVL in size, however, not all females  $\geq 45$  mm SVL were gravid. With female reproduction of green salamanders occurring biennially, this is an expected pattern (Gordon 1952, Waldron and Pauley 1997). Waldron and Pauley (1997) noted a 46 mm SVL reproductively mature size estimate, but this was not sex specific. Our data suggest that females are not reproductively active until they reach a minimum of 45 mm SVL.

#### *Summary and Conservation Implications*

With little known specifically about green salamander movements, we examined patterns of overnight movements of green salamanders during the spring breeding season. With green salamanders being territorial (Cupp 1980, Canterbury 1991, Cupp 2014), crevice philopatry was expected, however, philopatry instead was relevant at a scale of within 2 meters of the original location was found to be more accurate. Tortuosity ratios of individual salamander paths are expected to be close to zero for philopatric species, however, they were closer to 0.5, showing a high variability of movement patterns in the species. Linear regression of tortuosity ratios weakly suggested smaller adult salamanders moved in more circular patterns than large adults. We showed crevice buffering capabilities of temperature for the species, but also suggest the same pattern for relative humidity. Green salamanders were present in rock as well as tree refugia (Gordon 1952, Waldron and Humphries 2005, Miloski 2010). Given the small number of studies on green salamanders, particularly short term movements, our study brings us closer to understanding the complex relationship of movements, breeding, and beyond.

With population declines, specific habitat requirements, and slow population growth rates, conservation of the species is a concern (Waldron and Pauley 2007). This

small scale study helps to shed some light on individual salamander movements, substrate use, and other patterns in relation to environmental and biotic conditions. With conservation scale usually focused on long-term population monitoring at the landscape level, our study shows large movements within a shorter temporal scale for individuals. This information can help us formulate small scale conservation goals focused around critical microhabitat refugia availability and connectivity, particularly in the southern most extent of the species range.

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## Chapter 2: Occupancy and Detection Probability of Green Salamanders (*Aneides aeneus*) on Redstone Arsenal in Huntsville, Alabama

### Abstract

Green salamanders (*Aneides aeneus*) are a species of concern throughout their range due to habitat modification and population declines. Occupancy surveys allow us to estimate the probability a species is present in an area, while taking into account imperfect detection. Estimates of occupancy probability throughout Redstone Arsenal are critical for developing effective management plans for habitat and surrounding areas. We conducted visual surveys during summers 2015-2016 at 148 sites within two strata of suitable habitat on Redstone Arsenal, adjacent to Huntsville, Alabama. We detected no green salamanders within the northern stratum. Surveys in the southern strata detected the species at 23 of 85 sites. Model selection using Akaike's Information Criterion (AICc) indicated that detection probability varied most in response to observer experience. Estimated detection probability for surveys was 0.33 (0.15-0.57, 95% CL) based on the top model for the most experienced observer. As observer experience decreased, detection probability also decreased. Detection probability was not clearly related to temperature or relative humidity. Based on the model with the most support, the estimated probability of occupancy in the southern stratum was 0.29 (0.18-0.43, 95% CL). A strong relationship between rock characteristics (rock height and rock cover percentage) and occupancy was detected at Redstone Arsenal. The survey protocol designed can be used outside of Redstone Arsenal, throughout the species' range, but is especially important in areas of short, scattered exposed limestone and sandstone rock, in hardwood forests.

### Introduction

An understanding of species distributions and their relationships with habitat factors is crucial for conservation and management (Guerry and Hunter 2002). Occupancy modeling is one way to model these relationships accounting for the fact that it is difficult to determine with 100% certainty that a species is not present just because it

was not found during sampling (MacKenzie et al. 2003, 2004, 2005, Bailey et al. 2007, Nichols et al. 2008, Maerz et al. 2015). Variability in detection probability (detectability) can come from multiple sources (O'Donnell and Semlitsch 2015, Edwards et al. 2016). For example, species characteristics, such as being small, cryptic, and rare, make it harder to detect that species at sites where it is present. Another example is the level of observer experience because some surveyors have stronger search images and higher effectiveness than others. These multiple factors calculated together with presence-absence observations and habitat parameters can be used to estimate the probability of an area being occupied accounting for imperfect detection (MacKenzie et al. 2005, 2006). Occupancy modeling has been applied to a variety of salamander species, allowing for a better understanding of their habitat requirements, management needs, and conservation strategies (Bailey et al. 2004, Kroschel et al. 2014, Joseph et al. 2016, Lamb et al. 2017).

Green salamanders (*Aneides aeneus*) are a species of concern throughout their range due to habitat loss and conversion (Corser 2001, Wyatt 2010). These salamanders live in the Appalachian Mountains from southwest Pennsylvania, western Maryland, and southern Ohio to northern Alabama and northeastern Mississippi in disjunct, patchy populations (Behler 1979, Petranka 1998). The International Union for Conservation of Nature and Natural Resources (IUCN) categorizes green salamanders as Near Threatened with the population trend of decreasing throughout their range (Hammerson 2004). They have state Endangered status in Indiana, Ohio, Maryland, North Carolina, and Mississippi, are categorized as Threatened in Pennsylvania, and are categorized as Protected in Georgia and Alabama (Hammerson 2004, Rainer 2012).

Green salamanders have a slow life history, not reaching breeding maturity until 7-8 years of age, with females limited to breeding biennially (Waldron and Pauley 2007). These factors contribute to a low rate of potential population growth for the species (Cantrell 2012). Localized population declines of 98% have been recorded in the southern portion of their geographic distribution (Corser 2001). Recently, the Center for Biological Diversity petitioned the U.S. Fish and Wildlife Service (USFWS) for federal protection of the green salamander under the Endangered Species Act (ESA) (Giese et al. 2012). The USFWS is currently investigating whether green salamander protection is warranted.

Green salamanders (*Aneides aeneus*) are lungless and terrestrial salamanders dependent on refugia (Gordon and Smith 1949, Gordon 1952). Green salamanders require mature hardwood forest cover and exposed rock outcroppings, escarpments, or caves (Gordon and Smith 1949, Gordon 1952). They have unique adaptations for climbing, such as a prehensile tail, long limbs, and squared toe tips that allow them to use surface imperfections for moving to and using small crevices along rock and tree surfaces (Canterbury and Pauley 1994, Diefenbacher 2008, Schaaf 2010). These salamanders have a high water loss tolerance, allowing them to use moist or dry crevices within their environment (Gordon 1952). Crevices are used during the day as refuge from the ambient environment (Armstrong 2010). The species is nocturnal and moves from crevices at night to forage or search for mates (Gordon and Smith 1949, Gordon 1952).

Hardman (2014) used logistic regression and Maximum Entropy (MaxEnt) models to predict green salamander occurrence in western North Carolina. These models showed soil type, depth-to-bedrock, elevation, and canopy cover as important factors contributing to occupancy at the 30-m scale across the western North Carolina landscape. Her models showed forested areas with intact canopy, at intermediate elevations, and shallow soils of particular types were the best predictors of green salamander presence. Hardman (2014) also suggested the site covariate depth-to-bedrock may be more important in some regions but not others. Specifically, areas with smaller, more dispersed rock outcroppings are less likely to be linked to bedrock layer depth. Lipps Jr (2005) showed bedrock type, elevation, slope, percent canopy cover, and distance to water were predictive of green salamander occupancy. Dolomite, limestone, and sandstone were observed to be important bedrock types. Additionally, Lipps Jr (2005) predicted green salamander occupancy across a 900 m<sup>2</sup> area in Ohio using an inductive model. He highlighted sites occupied by green salamanders as having the following characteristics: elevation 183-244 m, slope >37%, percent canopy cover >82%, and distance to water < 325m. However, his models did not account for false absences because he used presence only data (versus presence-absence). Hardman's (2014) predictive models were built from presence-absence data, modeling false-negatives of occupancy data.

A limitation of these previous studies is that both Lipps Jr (2005) and Hardman (2014) found modeling of rock outcroppings were not possible with digital data. They were also unable to model cliff faces accurately at the 30 m resolution scale; instead, slopes  $>37\%$  were used to try and capture this cliff habitat. This is problematic because sandstone and limestone cliffs are often noted as ideal habitat for the species (Gordon 1952, Petranka 1998). Given the natural history of green salamanders, information on rock outcroppings is important to represent microhabitat availability for green salamanders. The amount of refugia available for the species may impact occupancy and densities for the species, particularly since they heavily rely on crevices (Gordon and Smith 1949, Gordon 1952). During summer months, female attend their eggs, cleaning them and protecting them from predators (Gordon 1952, Cupp 2011). During this time, females do not leave the crevice to forage, eating only opportunistically for the duration of the incubation period (Gordon and Smith 1949).

Our goals for this study center on short-term (2-year) occupancy and detection of green salamanders at a 30 x 30 meter scale, at Redstone Arsenal, Alabama, in the southern end of the species range. Our objectives were to 1) develop and assess an occupancy survey technique that incorporates both rock and tree habitat, 2) estimate impacts of survey covariates on detection probability of green salamanders to better understand their impacts on survey efforts, and 3) estimate impacts of site covariates on the probability of occupancy on Redstone Arsenal, specifically including rock characteristics that may be strongly related to occupancy probability for green salamanders.

Our survey design incorporated trees as well as rock habitat to avoid underestimations of green salamander presence (Waldron and Humphries 2005). We investigated impacts of temperature, relative humidity, and observer on detection probability. We investigated impacts of aspect, canopy cover, rock cover, and rock height of each site on the probability of occupancy on Redstone Arsenal. We predicted that, as temperature increased, our ability to detect salamanders would decrease because salamanders would retreat far into refugia (Spotila and Berman 1976). We expected that, as humidity increased, our ability to detect salamanders would increase due to slower

desiccation rates (Gordon 1952, Ray 1958, Spotila 1972, Keen 1984). We expected observers would have a strong effect on detection ability. Canopy cover has been shown to influence green salamander occupancy (Hardman 2014), so we expected higher occupancy of sites with more canopy cover. We expected that a northern aspect (direction of the slope) of the site would increase green salamander occupancy due to more moist physical conditions (Lipps Jr 2005). We predicted that rock characteristics would have a strong positive impact on green salamander occupancy where higher rock cover and taller rock height would increase the probability of occupancy sites on Redstone Arsenal because of microhabitat availability (Gordon 1952, Lipps Jr 2005, Hardman 2014). Rock characteristics have not been modeled for the species at a 30 x 30 meter scale previously. This information helps us to better understand green salamander distributions on Redstone Arsenal, but can also be used outside of the area with similar habitat characteristics.

## Methods

### *Study Area*

Redstone Arsenal is a US Military installation in Madison county, adjacent to Huntsville, Alabama (lat 34.684166°, long -86.654166°) (Figure 2-1). The US Army Garrison manages the tenants of multiple US Army departments and NASA Marshall Space Flight Center on the 15,429 hectares of land ([www.redstone.army.mil/](http://www.redstone.army.mil/)). The installation is made up of 4 zones: residential, city center, professional, and industrial (Figure 2-2). The city center and professional zones are developed relatively densely. Residential is also developed, but contains large recreation areas with little development. The industrial zone tests projectiles, contains some development, and tracts of protected land including the Wheeler Wildlife National Refuge.

Within Redstone Arsenal, two main strata were surveyed for green salamander presence. The first area contained Madkin and Weeden Mountains in the northern section of the military installation (3.19 miles<sup>2</sup> area; or 826 hectares), within the residential zone.

The other area was concentrated along the southern border of the post (1.82 miles<sup>2</sup> area; or 471 hectares), in the industrial zone (Figure 2-3).

### *Strata and Site Selection*

Areas to survey were selected at Redstone Arsenal based on presence of suitable forested cover types and rock outcroppings. Suitable forest types included South-Central Interior Highlands Dry Oak Forest or South-Central Interior Mesophytic Forest (NLCD 2011). Sites with hardwood deciduous forest are areas dominated by trees generally greater than 5 meters tall, making up at least 20% of the total vegetation. More than 75% of the tree species shed foliage simultaneously in response to seasonal change during fall months. Redstone Arsenal US Army Garrison, Environmental Division, Cultural Resource Manager/Archaeologist Ben Hoksbergen was consulted on rock outcropping distribution on base because no GIS information was available for emergent rock availability. This consultation restricted the survey areas to the north and south strata, where the north stratum contained Madkin and Weeden Mountains and the south stratum encompassed Testing Area-5 and the ATF Capano Range (Figure 2-3). Using ARCMAP (version 10.3.1), a 30 x 30 m grid (fishnet tool) was overlaid on top of the suitable forest habitat within the two strata. Possible sampling points were selected at random within each stratum and ground surveyed for the presence of rock outcroppings. Sites containing rock outcroppings with microhabitat crevices for green salamanders were retained for field sampling because of the dependence on the proximity of emergent rock (Waldron and Humphries 2005). The northern stratum contained 63 sites in 2015, but was restricted to 10 sites re-surveyed in 2016 so that more effort could be placed in the southern stratum. The southern stratum contained 36 sites in 2015, to which 49 sites were added in 2016.

Site size was determined to be 30 x 30 m through logistical practicality and known daily movements of the species. Green salamanders have been tracked over 15 meters in one night during the highly active spring breeding season, often returning within 2 meters of their original locations, showing philopatry to an area (see Chapter 1). Thus, a 30 x 30 m site was judged as an appropriate scale for assessing occupancy and for meeting the assumption that occupancy status of each site is closed to changes within a

single season (summer). If sites are too small to capture this movement, occupancy would not be constant for the model between surveys within a single season.

### *Survey Methods*

Multiple surveys were conducted per site allowing us to estimate the probability of detecting an organism, given it is present in an area, and account for the effect of false absences in assessing probability of occupancy of an area (MacKenzie et al. 2003, 2005). Bailey (2002) sampled for various species of terrestrial salamanders in the Appalachian Mountains and calculated optimal survey effort in comparison with detection probability. Five surveys per site is optimal for the level of detection probability expected with some cryptic terrestrial salamander species (Bailey 2002, Bailey et al. 2004). In our case, we expected low detection probability because green salamanders are small, cryptic, and hide. Increasing surveys conducted per site lowers uncertainty due to possible false absences, but there is a tradeoff between survey effort and efficiency of information gained (Mackenzie and Royle 2005, Bailey et al. 2007). Large quantities of additional surveys do not lower uncertainty significantly beyond a threshold and would therefore waste resources proportionally.

Surveys were conducted in June through mid-September, summer, because we assumed this would be the period when green salamanders would both active enough to be detectable and for populations to be closed to immigration and emigration (Otto et al. 2013). We believe the assumption of closure is a reasonable assumption due to female brooding activity of limited movement (Gordon 1952, Petranka 1998, Cupp 2011). Surveys were conducted during the day, when salamanders retreat into crevice refugia. Green salamanders emerge from crevices to forage at night (Gordon 1952). Surveys at night have been shown to provide higher detection probabilities than day surveys (Miloski 2010); however, access and logistics restricted our surveys to daytime hours. Sites were surveyed by one person each survey, but a minimum of two observers surveyed sites within the five repeated surveys, to account for observer impacts on the data collected.

During each survey, observers used flashlights to look into rock and tree crevices and along leaf litter. Crevices varied in size and shape and surveyors searched to the best of their abilities, looking as far back as the depth and shape of the crevices allowed. Sites were surveyed in a non-destructive manner so as not to disturb microhabitat; rocks were not moved or destroyed during the survey process. Surveyors could only look about 2 meters up the base of trees and thus it is possible green salamanders higher were not detected. Waldron and Humphries (2005) surveyed 10 meters up trees and suggested higher surveys, however, use of a ladder or climbing equipment was not feasible or practical in this study. Tall rocks were surveyed by climbing as high as safely possible, making sure to survey as many crevices as possible. Surveys took on average about one hour to complete at each site.

In addition to presence-absence data collection, site and survey covariates were collected. Site covariates were constant for a site, helping us understand what factors may be influencing green salamander occupancy. Site covariates included in our models were aspect, percentage rock cover, rock height, and percentage canopy cover (Gordon 1952, Hardman 2014). Aspect was calculated for each 30 x 30 meter site using LiDAR data provided by Redstone Arsenal. For analyses, aspect measurements were divided into quadrants representing compass direction of north east (NE), south east (SE), south west (SW), and north west (NW) (Table 2-2). Rock height data collection involved measuring the height of the tallest rock at each site to the nearest 0.1 meters. To quantify percentage rock cover and percentage canopy cover, each site was sampled at 36 points with 6 meter spacing and averaged (Figure 2-4). We collected percentage rock data in present/absent format at each of the 36 points, and calculated the average percentage ground area covered with rock. Canopy cover was defined as the proportion of the ground area occupied by the above ground parts of plants (Jennings et al. 1999) collected at each sample point using a densitometer about 1.5 meters off ground (Korhonen et al. 2006). Two observers independently collected non-spherical densitometer measurements estimating the amount of vertical leaf cover blocking light at each of the 36 points. This technique reduces observer, tree length, and tree height bias in the densitometer readings (Jennings et al. 1999, Korhonen et al. 2006). Percentage vertical leaf coverage was

recorded to the closest 5% (Terry and Chilingar 1955) and averaged across points for each site (Table 2-2).

Survey covariates change between surveys, affecting detection probability for green salamanders. Survey covariates collected were ambient temperature, relative humidity, and observer experience. Ambient temperature (degrees Fahrenheit) and relative humidity (percent) were recorded (Kestrel 3000 Environmental Meter, accuracy to  $\pm 1^\circ\text{C}$ ) at the center of each site at the beginning of each survey. A GPS location was collected for each salamander detected (Garmin GPSMAP 62S Handheld GPS Navigator, Universal Transverse Mercator NAD83 UTM). Surveyor names were recorded for each survey and multiple observer parameters were used in the model to test experience on detection probability. This allowed us to estimate whether detection probability of green salamanders varied between three categories of observers (A = an observer with the most survey experience for cryptic animals; B = a second observer with moderate experience; C = a third category consisting of all other observers with little experience).

### *Analyses*

Because no detections were recorded in the northern stratum, we limited occupancy analyses to the southern stratum. Site and survey covariates were evaluated for a significant correlation with presence of green salamanders using the program Presence (Version 8.4) with a set of candidate models thought biologically important. We choose a best model by Akaike's Information Criterion (AIC) (Anderson and Burnham 2002, Arnold 2010). The most explanatory model of the collected data suggests which factors are most significant in estimating presence of green salamanders and their detection probability. We scaled all continuous site and survey covariates by subtracting either the mean of the covariate for the stratum (rock height, rock cover) or a reference value (75 degrees F for temperature, 75% for relative humidity and canopy cover). Scaling was done to make model interpretation easier and improve model convergence.

Models tested estimated  $\Psi$  (psi) for the occupancy probability for the area and  $p$  for detection probability of each site surveyed (Table 2-3). We used both multi-season models that assumed occupancy status could change between years for the 36 sites

measured both summers (psi, gamma, p parameterization; MacKenzie et al. 2006), and single-season models that assumed occupancy was constant across both years. The null model was  $\psi(\cdot)p(\cdot)$ , where no factors influenced occupancy or detection probability. In other models, the detection probability component of each model was kept fixed as  $p(\text{temperature, relative humidity, observer1, observer2})$ . The modeled observer effects were contrasts between the moderate experienced observer vs. most experienced surveyor, and between the group of less experienced surveyors vs. the most experienced surveyor. Models for psi, in addition to the intercept-only model, included the following forms: a)  $\psi(\text{aspect})$ ; b)  $\psi(\text{canopy cover})$ ; c)  $\psi(\text{rock height} + \text{rock cover})$ ; d)  $\psi(\text{canopy cover} + \text{rock height} + \text{rock cover})$ . Because of the limited number of sites at which green salamanders were detected (23 sites), in forming this set of candidate models, we avoided fitting overly complex models and included only models that added 3 or fewer parameters to the intercept only psi model (e.g. we did not include models with interactions of other variables \* aspect, which had 4 levels). Since rock height and rock cover both were measures of essential rock microhabitat, we kept both together in fitting models. Because both forest cover and rock variables are believed to determine site suitability for green salamanders, we included one model for psi incorporating both rock and canopy cover variables.

To use the fitted model to estimate detection probability or occupancy at specific values of the survey or site covariates, we used the inverse-logit back-transformation. To calculate 95% confidence limits (CL) for these estimates, we used a normal approximation to form 95% CL for these estimates on the logit scale, then back-transformed them with an inverse-logit transformation. We used model parameters from the top model to calculate odds ratios to interpret the estimated effects of covariates on detection and occupancy probability.

We assessed whether site covariates important in the top occupancy model were similar by comparing southern stratum site values to the site covariate ranges of 28 randomly selected sites in the northern stratum. We did this by using a binomial logistic regression with stratum as a response variable. This analysis examines whether the

probability that site is in one stratum vs. the other is related to the main and interacting effects of the top site covariates.

Extrapolating from the sample of sites visited in the field to the total southern stratum, the total area occupied in the southern stratum was estimated as the product of the total area of the stratum, the estimated (null-model) occupancy probability of sites retained for sampling, and the estimated proportion of the southern stratum that contained potentially suitable habitat (number of random sites retained for sampling, 85, divided by the total number of sites visited, 412).

$$\hat{A}_{occ} = A_{total} * \psi_{suitable} * \frac{n_{suitable}}{n_{total}}$$

A 95% confidence interval (CI) for this estimate was calculated as the estimate  $\pm 1.96 * \text{standard error of the estimate}$ . The variance ( $SE^2$ ) of the estimate was calculated based on the Delta Method, with covariances of zero between terms, using the standard error of the occupancy estimate and using the binomial variance as the variance of the estimated proportion of the stratum that contained potentially suitable habitat:

$$\text{var}(\hat{A}_{occ}) = (A_{total})^2 * \text{var}\left(\psi_{suitable} * \frac{n_{suitable}}{n_{total}}\right)$$

$$\text{var}(\hat{A}_{occ}) = (A_{total})^2 * \left[ (SE(\psi_{suitable}))^2 * \left(\frac{n_{suitable}}{n_{total}}\right)^2 + (\psi_{suitable})^2 * \left(\frac{\left(\frac{n_{suitable}}{n_{total}}\right) * \left(1 - \frac{n_{suitable}}{n_{total}}\right)}{n_{total}}\right) \right]$$

## Results

In 2015, we surveyed 63 sites at Madkin or Weeden mountains in the northern stratum and had no detections of green salamanders in 315 surveys. In 2016, we resurveyed 10 sites in the northern stratum and did not detect any green salamanders in the 50 surveys (Figure 2-5). These mountains do not have any previous recordings of green salamanders between 1992 and 1994 based on an Alabama Natural Heritage Inventory (ANHI) report (Godwin and Hilton 1995). The range of covariates values between the two strata were similar (Tables 2-1 and 2-2).

In 2015, we surveyed 36 sites at the ATF Capano Range in the southern stratum and we detected green salamanders at 10 sites during 180 surveys. In 2016, we resurveyed the same 36 sites and detected green salamanders at 14 sites during 180 surveys. We detected green salamanders both years at 8 of the same sites. At 3 of the resurveyed sites, we detected green salamanders the first year, but not the second. In 2016, we also visited 49 additional sites (selected at random with the same procedure as the first year) and detected our target species at 6 sites during these 245 surveys. Green salamanders were detected at a total of 23 sites of 148 sites on Redstone Arsenal, all in the southern stratum (Figure 2-6). All green salamanders were found on rock habitat.

We found green salamanders during 47 surveys of the 605 surveys in the south stratum (16 of 180 in 2015 and 31 of 425 in 2016). On most occasions, only 1 green salamander was found during a survey on a site. On a few occasions, 2 green salamanders were found during a survey. On 1 occasion, 3 green salamanders were found during a survey in 2016. There was also one brooding crevice with eggs detected twice during 2016 where over five green salamanders were found.

Because the apparent absence of green salamanders in the north stratum may be at least partly driven by factors other than local site conditions (e.g. landscape isolation), we ran occupancy analyses only for the southern stratum. We first ran a simple multi-season model where  $\psi(\text{year}), \gamma(\cdot), p(\cdot)$  showed occupancy for the southern stratum to be relatively constant between year, where 2015 estimated occupancy probability was 0.294 (SD=0.091, 0.150-0.479 95% CI) and the 2016 probability of occupancy was 0.297

(SD=0.067, 0.184-0.441 95% CI). Estimated colonization probability between years was low ( $\gamma = 0.120$ ; SD=0.078, 0.031-0.366 95% CI), and simpler single-season models fit the data as well or better. Therefore, we proceeded with single-season modeling, treating occupancy status of a site as closed to changes within 2015 to 2016, with 5 surveys per site for those sampled in just one year and 10 surveys per site for those surveyed both years. The highest ranked single-season model incorporated rock characteristics (rock height and rock cover) as the most important factor influencing occupancy. Factors important to detection probability were not separated into various models, therefore, observer, temperature, and humidity survey covariates were in the top model (Table 2-3). The top model was chosen because it had the highest model weight of 45%. This means that there was a 45% chance of this model being the top model of the selected models. The next closest top model had the same components influencing occupancy probability, but also included canopy cover as an important site covariate. With a delta AIC value of 0.63 and a single parameter increase, these models are comparable. We chose to interpret the simpler model as the top model.

Observers had strong impacts on detection probability in the top model. Our estimate of expected per-survey detection probability at an occupied site when the most experienced observer was the surveyor, relative humidity was 75%, and temperature was 75°F was 0.33 (0.15-0.57, 95% CL). The estimated odds of detection under the same environmental conditions when observer B was the surveyor indicated she was 0.50 (0.22-1.14, 95% CL) times as likely to find green salamanders at occupied sites compared to observer A. The estimated odds of detection under the same temperature and humidity conditions for observer C showed this group of observers were 0.15 (0.05-0.45, 95% CL) times as likely as observer A to find green salamanders at occupied sites.

There was no strong effect of temperature or humidity on detection probability in the top model. The estimated odds of temperature on detection probability was 0.98 (0.89-1.08, 95% CL). This means that for each 1 degree F increase in temperature, the odds of detection decreased by approximately 2%. With an odds ratio CL range both above and below 1, we conclude that there is no clear effect of temperature on detection probability. The estimated odds of relative humidity on detection probability was 1.02

(0.98-1.05, 95% CL), meaning that for each 1% increase in relative humidity, the odds of detection increased by approximately 2%. The CL range of the humidity odds ratio crosses 1, indicating no clear effect of humidity on detection probability.

Based on the top model, the estimated probability of occupancy for surveyed sites was 0.29 (0.18-0.43, 95% CL) for the south stratum on Redstone Arsenal when rock height was 1.5 meters and rock cover was at 33%. Estimates indicated that for every 1 meter increase in rock height, there was a 2.11 (1.10-4.07, 95% CL) percent increase in the probability of occupancy. The estimated effect of rock cover on the probability of occupancy showed for every 10 percent increase in rock cover, there was a 1.26 (0.87-1.83, 95% CL) percent increase in the probability of occupancy. Rock cover had more uncertainty in the estimated effect than rock height. We can assess the estimated effects of both rock variables on the probability of occupancy for the southern stratum on Redstone Arsenal (Figure 2-7). Rocks over 3.5 meters tall with 2% rock cover correspond to an estimated 0.50 probability of green salamanders being present at Redstone Arsenal within the southern stratum. Rocks at 2 meters tall with at 50% rock cover also correspond to an estimated 0.50 probability of green salamanders being present at Redstone Arsenal within the southern stratum. There were no significant differences between rock characteristics in the north stratum compared to the south stratum (rock height,  $p=0.23$ ; rock cover,  $p=0.94$ ; interaction of rock height and rock cover  $p=0.18$ ) (Figure 2-8). The estimated total area occupied in the southern stratum was 33.42 ha (18.97-47.86, 95% CI).

Table 2-1: Table showing mean, standard deviation, and range of site covariates collected for north and south strata, 2015-2016. SE=standard error. \*n=28 random subset of sites in north stratum.

Variable	North Stratum (n=63)			South Stratum (n=85)		
	Range	Mean	SE	Range	Mean	SE
Slope (degrees)	9-78	31	2.31	8-69	35	1.25
Rock Height (m)	0.1-8*	1.7*	0.33*	0.2-5.1	1.5	0.10
Rock Cover (%)	0.14-0.75*	0.42*	0.04*	0.03-0.69	0.34	0.02
Canopy Cover (%)	0.62-0.85*	0.72*	0.01*	0.45-0.91	0.73	0.01
Elevation (m)	215-349	279	4.00	174-229	196	1.22

Table 2-2: Number and percentage of plots within each quadrant of aspect for north and south strata.

	NE Aspect	SE Aspect	SW Aspect	NW Aspect
<b>North Stratum (n=63)</b>	9 (0.14)	23 (0.37)	10 (0.16)	21 (0.33)
<b>South Stratum (n=85)</b>	11 (0.13)	24 (0.28)	21 (0.25)	29 (0.34)

Table 2-3: AIC results table of single season occupancy model of southern strata, 2015-2016.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.
psi(RockH,RC),p(b0,Temp,RH,Obs1,Obs2)	284.41	0	0.4468	1	8
psi(RockH,RC,CC),p(b0,Temp,RH,Obs1,Obs2)	285.04	0.63	0.326	0.7298	9
psi(RockH,RC,CC,SE,SW,NW),p(b0,Temp,RH,Obs1,Obs2)	287.43	3.02	0.0987	0.2209	12
psi(CC),p(b0,Temp,RH,Obs1,Obs2)	288.73	4.32	0.0515	0.1153	7
psi(·),p(b0,Temp,RH,Obs1,Obs2)	289.02	4.61	0.0446	0.0998	6
psi(SE,SW,NW),p(b0,Temp,RH,Obs1,Obs2)	289.66	5.25	0.0324	0.0724	9
psi(·),p(·)	303.34	18.93	0	0.0001	2

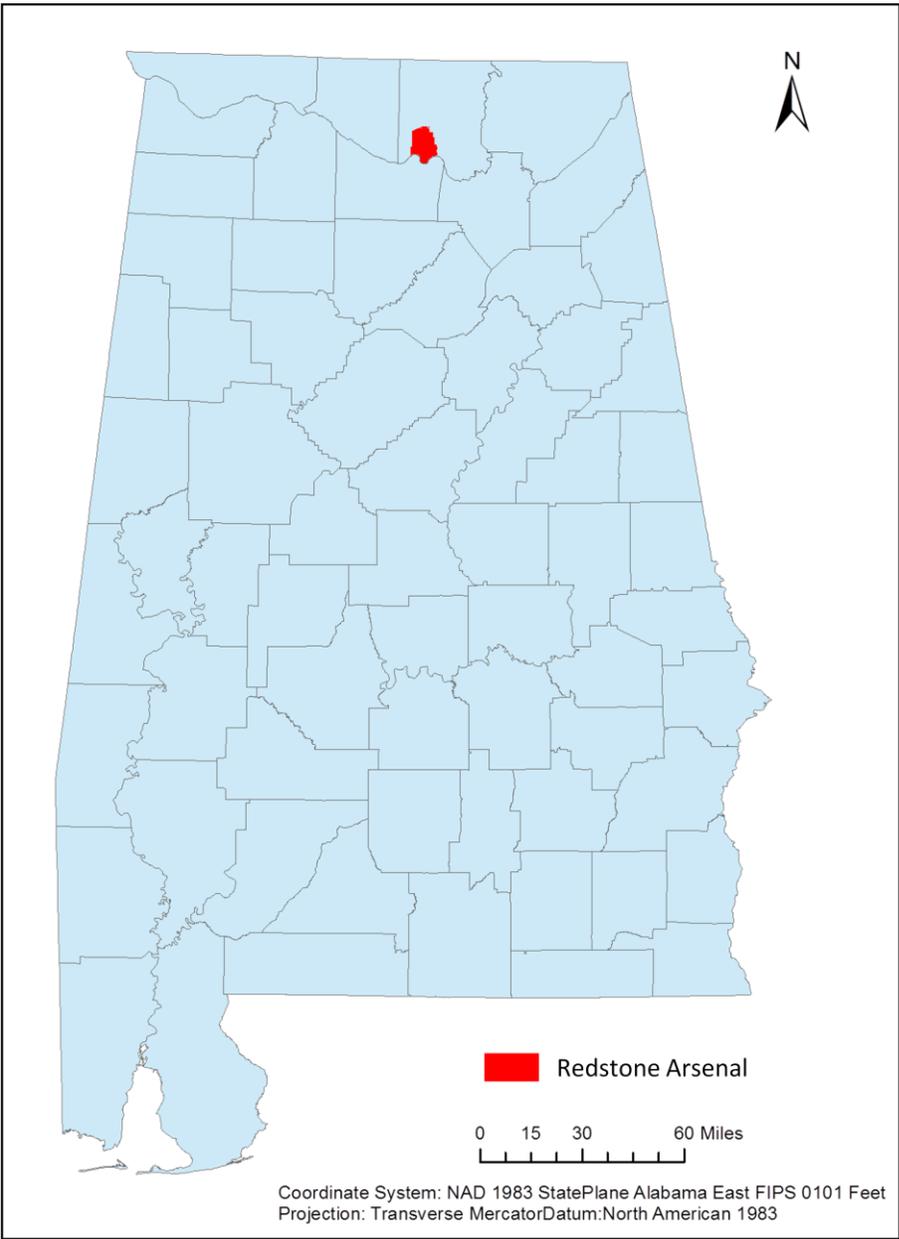
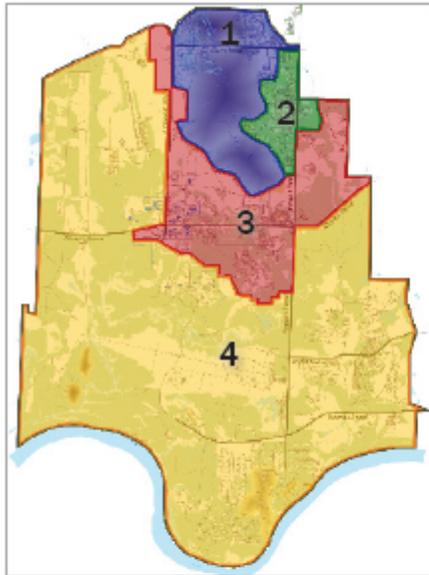


Figure 2-1: Redstone Arsenal location in the State of Alabama.



## Redstone Arsenal is comprised of four major zones:

- 1. Residential Zone:** includes our residential housing areas, recreational opportunities, shopping and many other quality of life services
- 2. "City Center":** mixed-use zone akin to a city downtown, consisting of a centralized services corridor, recreation destinations, professional office/lab space, and future temp lodging
- 3. Professional Zone:** houses our organizational headquarters, major office complexes/research laboratories and is the highest concentration of our workforce
- 4. Industrial Zone:** contains our industrial and explosive operations, test areas, warehousing and ammunition storage, Wheeler Wildlife Refuge, and our river recreation areas

Figure 2-2: Redstone Arsenal zones of development ([www.redstone.army.mil/](http://www.redstone.army.mil/)).

## Redstone Arsenal Strata

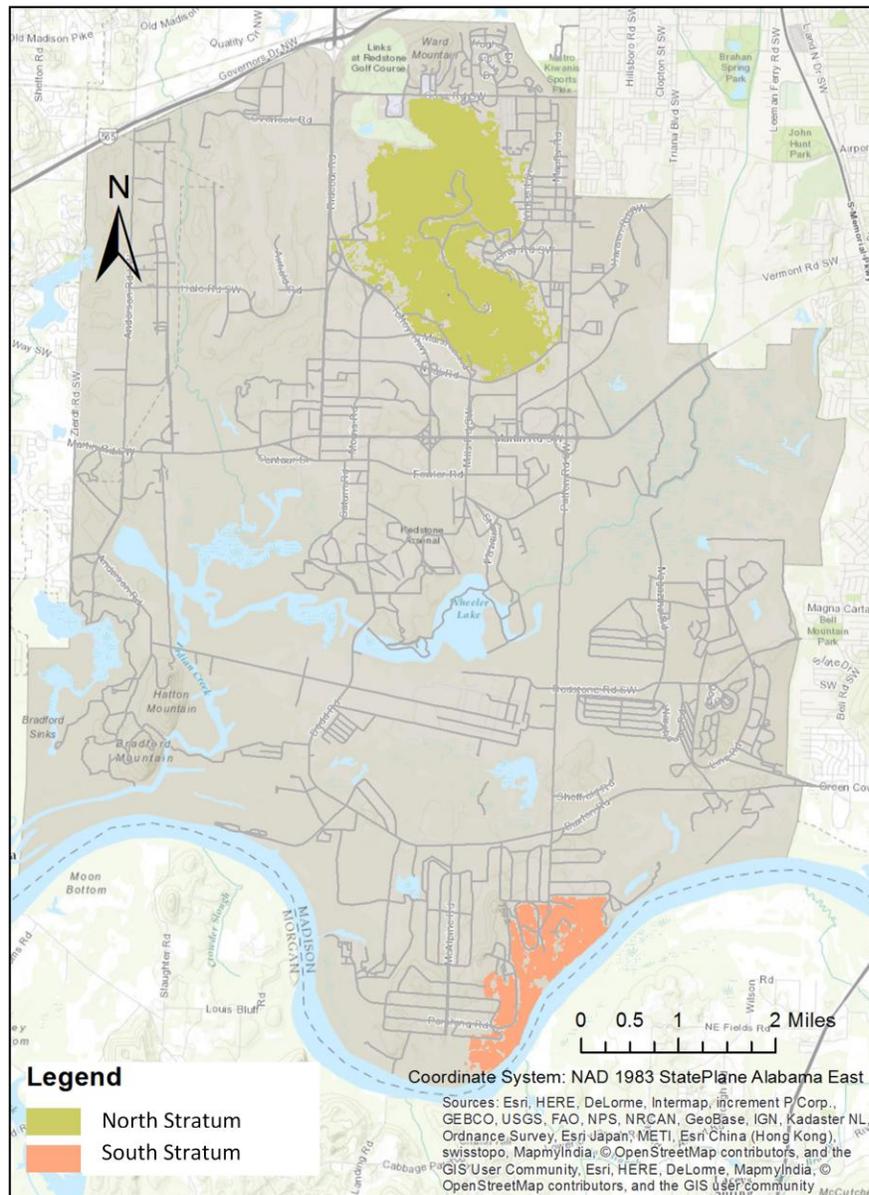


Figure 2-3: Strata within Redstone Arsenal, Huntsville, Alabama.

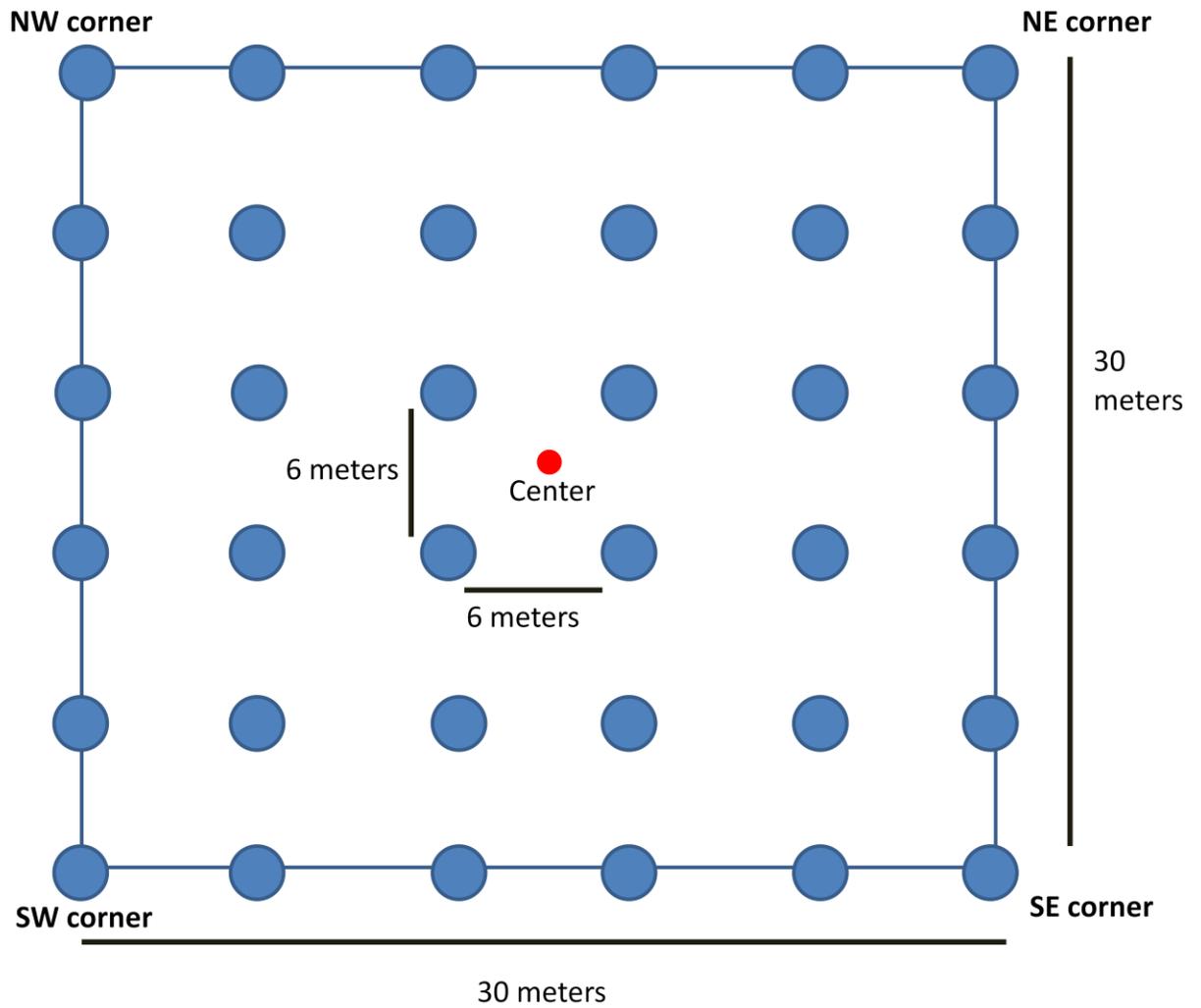


Figure 2-4: Sample grid for each site showing the spatial layout of rock outcrop and canopy cover measurements. The data was averaged for each site and used as a site covariate for occupancy estimation.

## Redstone Arsenal North Stratum

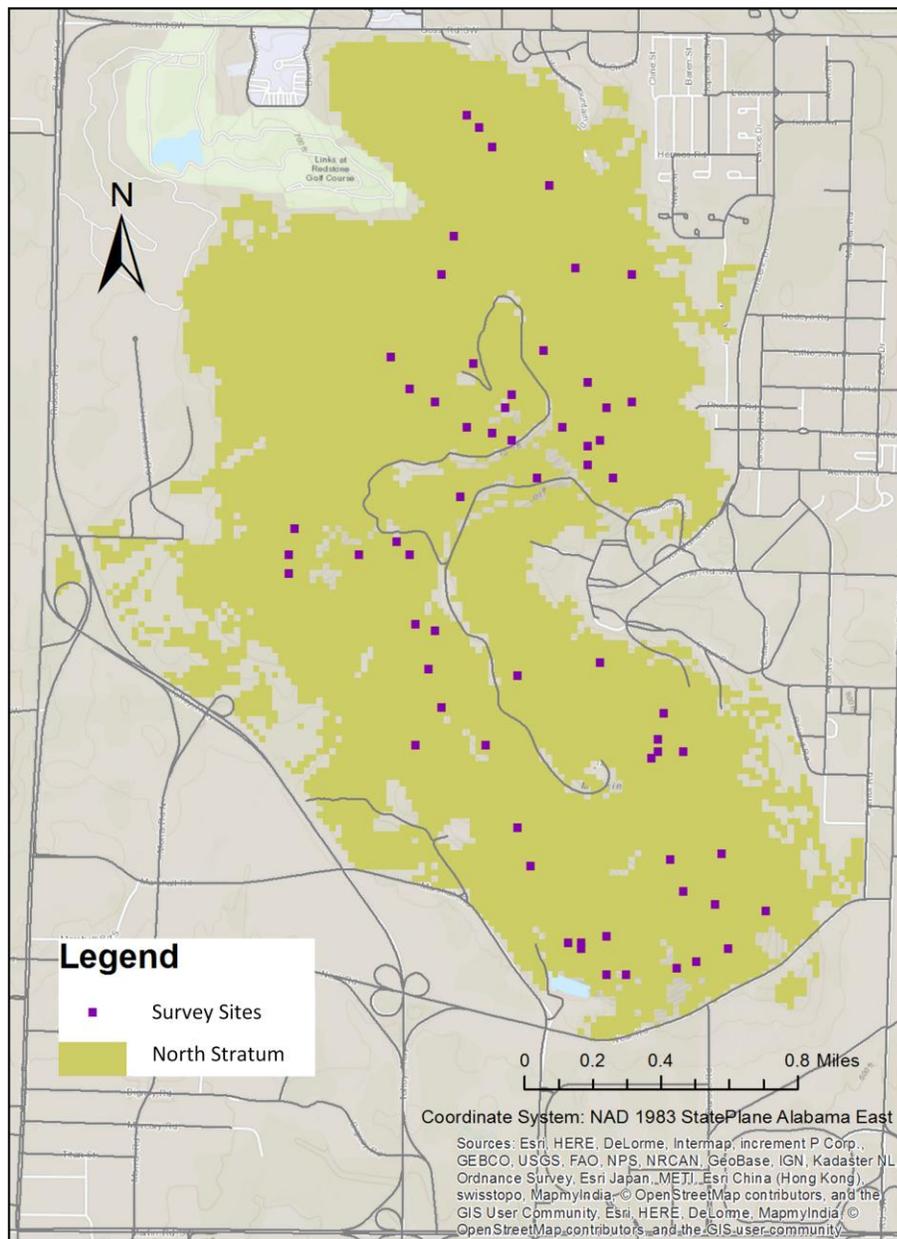


Figure 2-5: Location of randomly selected sites in forested areas containing rock cover in the north stratum (Weeden and Madkin mountains).

## Redstone Arsenal South Stratum

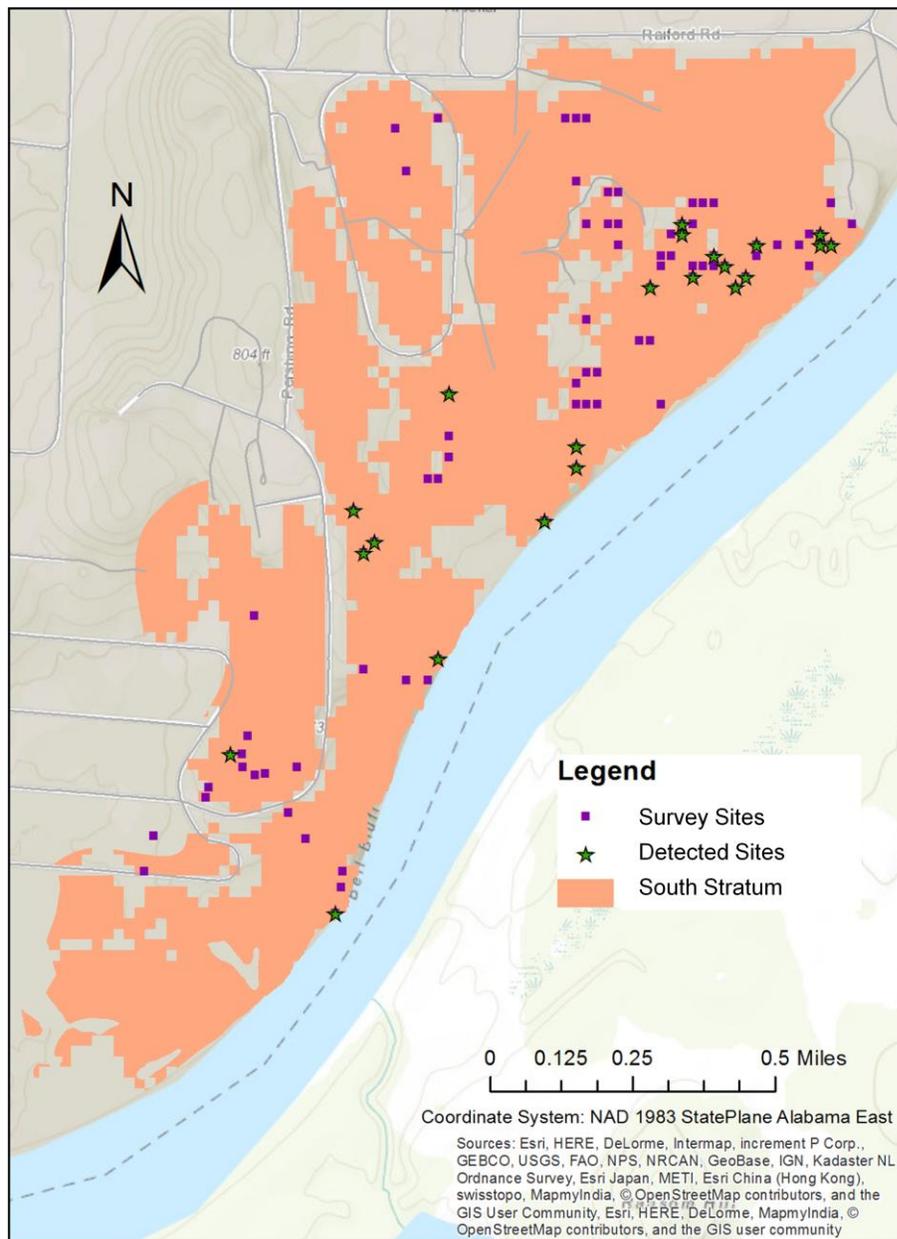


Figure 2-6: Location of randomly selected sites in forested areas containing rock cover in the south stratum. Site detections are indicated.

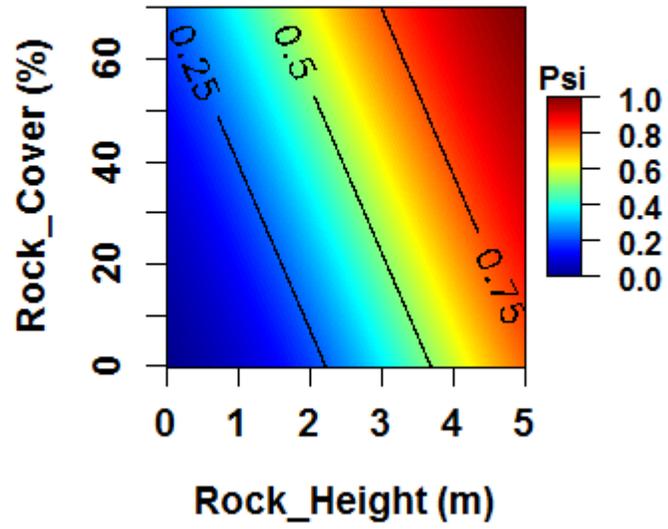


Figure 2-7: Rock height and rock cover predictions of the probability of occupancy in the southern stratum of Redstone Arsenal for surveyed sites.

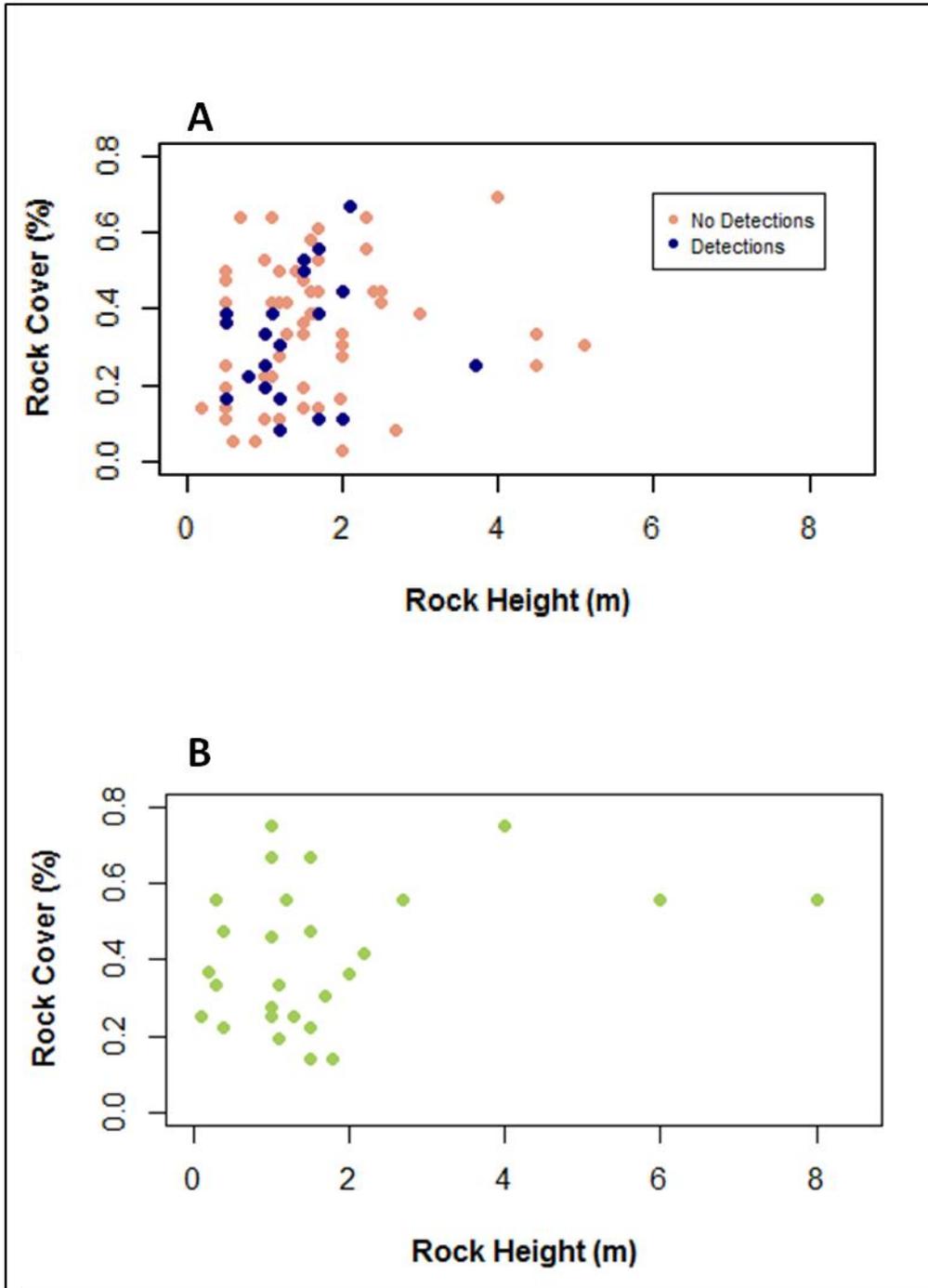


Figure 2-8: Rock characteristics in A) all south stratum plots (n=85), with detections noted, and B) random subset of north stratum plots (n=28).

## Discussion

Our survey method of including searches in trees as well as rock habitat is important in not underestimating the detection of the species. While no green salamanders were found in tree habitat during these surveys, the species has been shown to use tree substrate (Waldron and Humphries 2005, Miloski 2010). Future surveys should continue to incorporate tree habitat so biases of microhabitat use do not make inaccurate conclusions about the species distribution.

Of the survey covariates included in the top model, temperature and humidity had weak effects due to a wide range of confidence intervals incorporating possibly both positive and negative relationships for each variable on detection probability. Observer effects had a drastic influence on the detection of green salamanders. Observer A's 33% detection probability indicates that 5-7 surveys must be conducted to accurately account for false absences at an occupancy probability of 30% (MacKenzie and Royle 2005). With observer B's detection probability of about 17%, 16-18 surveys would be needed. With observer C's detection probability at about 5%, even more surveys would be expected. Hafer (1992) suggests up to 24 surveys may be needed to accurately detect green salamanders in some areas, calculating detection probabilities close to 0.20. This indicates that more surveys at Redstone Arsenal would be needed to better account for low detection probability. Strong effects from observers have conservation implications. In lieu of an exuberant number of surveys in areas with green salamanders we suggest surveys be conducted by biologists with more experience at detecting small, cryptic species to better account for false-absence data estimates needed for occupancy modeling.

At Redstone Arsenal, low salamander density may contribute to a low detection probability (MacKenzie et al. 2005). Areas outside of Redstone Arsenal, such as W. B. Bankhead National Forest (about 115 km west) contain larger numbers of green salamanders along rock escarpments. Large densities of green salamanders can be found within a 30 x 30 m rock cliff face, raising detection rates significantly.

Few differences in habitat characteristics are documented between the two surveyed strata (Tables 2-1 and 2-2), however, we only detected green salamanders in the south stratum. Of the site covariates, rock height and rock cover contributed to the top model, but did not differ significantly between the strata (Figure 2-8). Rock cover appeared to have a weaker effect on occupancy than rock height, but was included with rock height because these factors combined provide a better representation of fine scale microhabitat availability. The more exposed limestone rock on a site, the greater the number of rock crevices in which green salamanders are found. Similar occupancy probabilities can be obtained when a site has tall rocks but low overall rock cover, vs. a site with high rock cover of short rocks. This information can be used by Redstone Arsenal for management for the species of concern, highlighting areas highly likely to be occupied as possible areas of conservation priority.

Extrapolating the top site covariates in the southern stratum to test the occupancy of the northern stratum, we saw no significant difference between the strata rock characteristics (Figure 2-8). This would lead us to believe that green salamanders would be present in the northern stratum. However, with previous surveys in the northern stratum by ANHI between 1992-1994 (Godwin and Hilton 1995) and our surveys between 2015-2016, no detections have been made in that area of Redstone Arsenal. We are unsure if green salamanders have been extirpated from the area, or if they were never present in the first place. Therefore, we cannot make conclusions about the relative effects of habitat characteristics, isolation, development, or management between the areas.

Increasing accuracy of green salamander occupancy at this 30 x 30 m scale or smaller would require mapping all crevices available in rocks and trees. This would be highly time consuming and we chose not to collect this information due to the large number of sites and surveys we conducted. Connectivity of microhabitat is also likely a strong factor in green salamander occupancy (Bendik et al. 2016). We chose sites at random within the suitable forest habitat both years. However, there were cases where sites were adjacent to each other. With high connectivity between these sites, we would

expect similar occupancy rates; however, rock characteristics often could change greatly between adjacent sites.

### *Summary and Conservation Implications*

Our short-term (2-year) study at Redstone Arsenal allows us to optimize detection probability and better understand 30 x 30 m scale occupancy in relation to rock characteristics for areas with smaller, dispersed rock outcroppings. Our survey design incorporated both tree and rock substrate that are important aspects of green salamanders habitat (Waldron and Humphries 2005). We did not find any green salamanders on tree substrate in Redstone Arsenal, but our survey method is especially important for the species to prevent underestimations of occupancy and population sizes (Waldron and Humphries 2005, Hardman 2014). We determined observer affect to be exceptionally strong on detection probability and recommend investing in training surveyors intensely to reduce the number of site visits to account for false absences. Hourly temperature and relative humidity had highly variable impacts on detection probability and are less important in landscape models than mean annual temperatures (Lipps Jr 2005). Aspect and canopy cover were not found in the top predictive model for green salamander occupancy in the southern stratum on Redstone Arsenal. We modeled rock characteristics with both rock height and percent rock cover, showing they were very important to occupancy probability. Using the percentage of scouted sites suitable with rock in target forest habitat, the south stratum at Redstone Arsenal could have about 33 hectares of occupied green salamander habitat.

Modeling green salamander occupancy with rock characteristic details is very important to understanding the species distribution and relationships with habitat. This information is critical for better conservation and management strategies for the species, particularly with Redstone Arsenal representative of a shorter, more scattered rock outcrop habitat than where green salamanders are traditionally found (in large rock escarpments and cliffs). This is the first occupancy model to explore rock characteristics in the model and they were highly ranked. Future modeling should incorporate rock characteristics to better model species distributions across their range.

It is likely that the addition of other site covariates in our models would provide more insight into habitat characteristics, however, that was not feasible in our case for model convergence due to a low number of detections. Surveying more sites in more areas outside of Redstone Arsenal in the future can make the model more robust and more able to handle a larger number of survey covariates, particularly in the southern range of the species.

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