

12-2017

Green Salamander Distribution, Abundance, and Physiology in the Southern Blue Ridge

Jillian C. Newman
Clemson University

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

Recommended Citation

Newman, Jillian C., "Green Salamander Distribution, Abundance, and Physiology in the Southern Blue Ridge" (2017). *All Theses*. 2779.
https://tigerprints.clemson.edu/all_theses/2779

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.

GREEN SALAMANDER DISTRIBUTION, ABUNDANCE, AND PHYSIOLOGY IN THE
SOUTHERN BLUE RIDGE

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

by
Jillian C. Newman
December 2017

Accepted by:
Dr. Kyle Barrett, Committee Chair
Dr. David Jachowski
Dr. Michael Sears

ABSTRACT

Green salamanders, *Aneides aeneus*, are a priority species throughout their range and have been negatively affected by habitat loss, climate change, disease, and over-collection. Many historical locations for this species in the Blue Ridge Escarpment have not been visited for ~25 years and thus were in need of a status update. I constructed both small-scale and large scale distribution models for green salamanders. For the small-scale distribution model, I conducted visual encounter surveys across three counties in South Carolina using a headlamp to search rock outcrops and binoculars to search trees. I detected green salamanders at 30 of the 61 (49.2%) surveyed sites and collected a variety of habitat variables and compared a suite of N-mixture models using an AIC framework. Time of day emerged as the most important predictors for salamander detection, while aspect, habitat size, and elevation influenced salamander abundance. It appears that there may have been a range contraction as well as local extinctions in South Carolina for this species, although low detection probability and a lack of access to some sites makes conclusions on this issue difficult to state with certainty. For the large-scale distribution models, I compared the predictions generated by a correlative-only model to those from a model with mechanistic data added to the correlative framework focusing on Green Salamanders in their disjunct range (North Carolina, South Carolina, and Georgia). I conducted a laboratory study to measure resistance to water loss (R_i) and metabolism (VO_2) under a range of environmental conditions. The distribution model under current climatic conditions was similar for both the correlative and correlative + mechanistic approaches. Under two different climate change scenarios, models incorporating mechanism predicted less suitable habitat than correlative-only models. Because future climate projections may include non-analog climates (a

lack of appropriate training data), incorporating mechanism may be useful for forecasting climate vulnerability.

DEDICATION

In dedication to my grandfather, Marcel Blanchette, a three-time cancer survivor who gave me the strength and courage to never give up. Thank you for watching over me. Business has been taken care of.

ACKNOWLEDGMENTS

The project was funded by Clemson University, the South Carolina Department of Natural Resources, and the Greenville Zoo. I am indebted to Will Dillman, for his continual support and assistance throughout my two years at Clemson.

I would like to thank my advisor, Dr. Kyle Barrett, for everything he has done for me. He is an exceptional advisor and mentor who has provided me with invaluable tools that I will be able to use in the next stage of my career. His guidance and encouragement not only aided me with several different research projects but helped push me through difficult personal situations. I would also like to thank my committee members Dr. David Jachowski, and Dr. Michael Sears for offering new perspectives and insight to my project. I am very grateful for my lab mates who have supported me from day one. Special thanks to my field technicians Joel Mota, Cameron Sabin, and Ben Bagwell for their hard work in the field. Thank you to the volunteers who donated time to my project. I am appreciative of those who helped my project by providing us access to their land – South Carolina State Parks, Naturaland Trust, and homeowners. I also thank Eric Riddell for field, lab, and writing assistance. Thanks to my fellow graduate students who supported me throughout my time at Clemson. Last but not least, thank you to my family for supporting me and my passion for wildlife.

TABLE OF CONTENTS

	Page
TITLE PAGE	i
ABSTRACT	ii
DEDICATION	iv
ACKNOWLEDGMENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER	
I. CHAPTER TITLE	1
Introduction	1
Methods	3
Results	7
Discussion	9
Management Recommendations	12
References	15
Figure Legends.....	24
II. CHAPTER TITLE	27
Introduction.....	27
Methods	30
Results	41
Discussion	42
References	48
Figure Legends.....	60
APPENDICES	66
A. Estimated green salamander dispersal for the year 2050	67

LIST OF TABLES

Table	Page
1.1 List of abundance covariates for green salamander N-mixture models	20
1.2 Top candidate green salamander abundance models	22
2.1 List of bioclimatic variable descriptions	55
2.2 Analysis of covariance examining the effect of temperature and body mass on skin resistance to water loss	56
2.3 Analysis of deviance table for metabolism.....	57
2.4 Amount of suitable habitat predicted to be gained/lost when mechanism was added to the correlative model.....	58
2.5 Comparison of average current and future temperature and precipitation variables in the modeled extent.....	59

LIST OF FIGURES

Figure	Page
1.1 Map of study area in South Carolina	25
1.2 Habitat size and elevation as the top predictors of abundance	26
2.1 Flowchart illustrating the inputs, process, and outputs of mechanistic layers	62
2.2 Effect of temperature on skin resistance to water loss	63
2.3 Effect of temperature on metabolism	64
2.4 Species distribution models for green salamanders	65

CHAPTER ONE

ENVIRONMENTAL PREDICTORS OF GREEN SALAMANDER DISTRIBUTION AND ABUNDANCE IN THE BLUE RIDGE ESCARPMENT

INTRODUCTION

Amphibian habitat suitability can be constrained by a wide array of factors attributable to natural habitat heterogeneity (Tockner et al. 1996; Vallan 2002) and anthropogenic changes such as forest fragmentation and climate change (Petranka et al. 1993; Gibbs 1998; Araújo et al. 2006; Barrett et al. 2014). Habitat specialists are particularly susceptible to factors altering distributions at a wide range of spatial scales. Specialists suffer greater population declines when faced with habitat loss and tend to be less resilient to the effects of climate change when compared to generalists (Travis 2003; Munday 2004). Small-bodied specialists that live at higher elevations and have limited ability to evade diseases, are at particularly high risk of extinction (Owens and Bennett 2000; Pounds et al. 2006).

The Green Salamander, *Aneides aeneus* (Cope and Packard 1881), is considered a habitat specialist and is the only member of the “climbing salamander” genus found on the east coast of the United States. This species is typically associated with narrow granitic or sandstone rock crevices (Bruce 1968; Mount 1975). Green Salamanders have specialized toe-tips which allow them to climb up vertical surfaces and a unique lichen-like pattern on their dorsum that allows them to blend in with their surroundings (Mount 1975; Petranka 1998). Green Salamanders occur from southwestern Pennsylvania to northern Alabama and into eastern Mississippi. There is a disjunct population in the Blue Ridge Escarpment (Petranka 1998). Green Salamanders are considered “near threatened” by the International Union for Conservation of Nature (IUCN).

Within the disjunct Blue Ridge Escarpment (BRE) population, Green Salamanders are state listed as “imperiled” in Georgia and North Carolina, and “critically imperiled” in South Carolina (Natureserve 2017).

Snyder (1983) noted that Green Salamanders in the Carolinas are close to extinction. Corser (2001) acknowledges four major threats facing Green Salamanders: habitat loss, climate change, over-collection of the species, and disease. Little is known about Green Salamander dispersal but it has been documented that they can disperse 42 m from the nearest rock outcrop (Waldron and Humphries 2005). Thus researchers believe it is important to have forested buffers around outcrops during clear-cutting (Petranka 1998; Wilson 2001; Waldron and Humphries 2005). The BRE has experienced warmer summer temperatures and colder winter temperatures since the 1960's, and like many other amphibians of high conservation priority, the Green Salamander is expected to lose a significant amount of its climatically suitable habitat in the next half-century (Snyder 1991; Corser 2001; Barrett et al. 2014). Nevertheless, the Carolinas have been identified as an area of resilience to climatic change relative to many other parts of the range (Barrett et al. 2014). Over-collection of Green Salamanders (which are highly coveted for their attractiveness) could potentially lead to population declines (Corser 2001; Wilson 2001). For example, continual collection of egg-brooding Green Salamanders from the same site over consecutive years can result in population decline (Wilson 2001). Green Salamanders are likely vulnerable to disease such as chytrid fungus because they occur in moist conditions at high elevations (Daszak et al. 1999; Young et al. 2001). Recently, cases of chytrid fungus have been detected in both Virginia and North Carolina and Ranavirus was reported in Virginia (Blackburn et al. 2015; Moffitt et al. 2015).

With the growing threat of habitat loss and global climate change, I sought to determine the current status of Green Salamanders within South Carolina. The last extensive inventories for the species in the area were done in 1968 and 1990 (Bruce 1968; Hafer and Sweeney 1993). These surveys identified different habitat affiliations; specifically, salamanders appeared more frequently on south-facing slopes in the 1960s survey and a wider range of elevations (Bruce 1968), but more commonly on north-facing slopes and higher elevations in the Hafer and Sweeney (1993) survey. It is an open question whether this is a real shift driven by temperature or some other factor, or if it resulted from sampling error. To identify the current distribution and status of Green Salamanders in the southern portion of the range, I sampled prospective Green Salamander habitat in the Blue Ridge Mountains of South Carolina. I did so by reassessing known historical Green Salamander localities and some newly located prospective sites in South Carolina (*sensu* Corser 2001). I assessed a wide range of habitat features within and around sites to evaluate potential predictors of site-level abundance. I also documented several new occupied sites in South Carolina.

METHODS

Data Collection

I collected a comprehensive list of historical Green Salamander records in South Carolina from the South Carolina Department of Natural Resources and three publically-accessible online databases (Price and Dorcas 2007; Cicero et al. 2010; USGS 2013). I also identified potential localities through conversations with South Carolina state park officials and through searching rock outcrops while traveling to historical locations. A total of 96 distinct sites were identified within three counties containing the Blue Ridge Region of South Carolina (Fig. 1.1, inset map).

Thirty-five of these sites were not surveyed because sites had no rocky outcrops or large trees with flaky bark that could be identified at the locale ($n = 24$), sites were inaccessible from roads or trails ($n=10$), or sites were on private land that I did not have permission to access ($n = 1$).

For the remaining 61 accessible sites with appropriate habitat (an emergent rock outcrop), I surveyed them three times each (with the exception of two sites which were only surveyed once due to time constraints) between May and August 2016 (Hafer and Sweeney 1993; Corser 2001; Waldron and Humphries 2005). Surveys were spread across the entire survey period with two rounds of surveys conducted mid-morning to mid-day, and one round of surveys conducted at dawn (no surveys were conducted at night due to logistical and safety concerns). Surveys were done in a standardized fashion using a similar method outlined by Miloski (2010) by 1–2 observers depending on the rock outcrop size. I established circular plots around a rock outcrop within historical Green Salamander sites and I created four 25–m transects representing the four cardinal directions (N, E, S, and W). Each visit consisted of a two-part visual encounter survey by the observer(s): (1) a thorough search of the entire rock outcrop using a headlamp, and (2) a line-transect survey in which the observer(s) walked all four transects searching trees (2 m on each side of the transect line) using binoculars and flipping cover objects checking for salamanders. All herpetofauna encountered throughout surveys were recorded. I also collected habitat variables during every survey (except for habitat size which was measured once), assuming measurement error and thus taking an average measurement (Table 1.1). I measured habitat size (outcrop size) by assuming the sites were roughly rectangular in shape, so I multiplied the north-south and the east-west distance of the rock outcrop using a reel measuring tape (Keson 300-ft Tape, Keson Industries, Inc.). I collected elevation using a Garmin GPS (GPSmap 62s, Garmin, Ltd.), slope using a clinometer (PM5/1520, Suunto), and aspect using a

compass (MCB CM/IN/NH, Suunto). I assessed drainage presence/absence within 400 m of the site based on a visual assessment and Google Earth (v7.1.8.3036, Google, Inc.), and land cover within a 25-m radius of the outcrop was categorized as mixed forest, hardwood, softwood, or shrub based on our observations during site visits. I measured basal area using a 10-factor prism (Jim-Gem Square-shaped, Forestry Suppliers) and canopy cover (to the nearest 0.01) using a concave densitometer (Spherical Crown, Forestry Suppliers) at the beginning of each of the four line transects. I downloaded four bioclimatic variables (BIO1, BIO5, BIO12, BIO17) from World Clim (Hijmans et al. 2005) and extracted the raster values to the Green Salamander presence points in ArcMap (ArcGIS 10.3.1, ESRI). These data correspond to mean annual temperature, maximum temperature of the warmest month, annual precipitation, and precipitation of the driest quarter for the period 1960 – 1990.

Abundance Analysis

Using data from visual encounter surveys, I developed an N-mixture model for Green Salamanders in South Carolina to investigate the relationships between species counts and environmental site covariates. I analyzed count data using the unmarked package (Fiske and Chandler 2011) in Program R 3.3.1 (R Core Team 2017). I used the “p-count” function to fit N-mixture models to the count data. Abundance models assume that the population is closed and counts between sites (rock outcrops) are independent of other sites. I assessed the weight of evidence for a model using the Akaike’s Information Criterion (AIC). I standardized all continuous covariates before putting them into the models and removed highly correlated variables *a priori*. I transformed the aspect variable on a north/south gradient by taking the absolute value of the difference of the aspect value and 180. The land cover variable was

removed from the analysis because there was only a small proportion of sites with softwood and shrub-dominated habitats. The drainage variables were removed because all sites had a drainage present within 400-m of the site. Bioclimatic variables were removed because each of the measures had high pairwise correlation values with elevation ($\geq \pm 0.96$). I began by exploring three possible model structures on the null model: negative binomial, zero-inflated Poisson, and Poisson. A comparison of these structures via AIC revealed the most support for the negative binomial, so all subsequent models were created with this structure.

I first identified survey-specific covariates that may have influenced detection probability (observer experience, total search time, time of day, cloud cover, temperature, and Julian calendar day number). Observers were given a ranking between 0–2 (“0” referred to a low level of experience and “2” referred to a high level of experience). Observers new to the field or naïve to field equipment were designated as having less experience than those observers who have had 3+ years in the field and have worked with a variety of field equipment. With time, less experienced observers became more experienced and earned a ranking of “2” as the field season progressed. If multiple observers were conducting the survey, then their experience score was averaged. Total search time was measured as the amount of time it took the observer(s) to complete a survey effort, and I divided this measure by total habitat size to generate the search effort variable (hereafter, “duration”). Time of day was included because searches ranged from dawn to mid-day. Cloud cover was broken up into two categories: overcast and sunny. Rain events were considered overcast. I took air temperature using a thermometer (6-1/4” Pocket Case Enviro-Safe, Forestry Suppliers). I recorded the Julian day number based on the 2016 leap year calendar.

I began identifying possible covariates of detection by comparing a null model to all possible univariate models of detection covariates, while keeping abundance covariates constant across sites. Detection covariates with strong support ($\Delta AIC < 2$) were evaluated in all possible combinations to explore support for additive models. Once I determined which detection model had the most support ($\Delta AIC = 0$), I incorporated this detection covariate model in all subsequent models exploring covariates of abundance. Similar to our process for identifying detection covariates, I first generated all possible univariate models with abundance covariates, identified those variables with the most support ($\Delta AIC < 4$; which also represented weights > 0.1), and then examined all possible combinations of those covariates. Our final model comparison (via AIC) involved the null model, strongly supported univariate models, and all possible multivariate models involving the top abundance covariates.

RESULTS

Distribution and Arboreal Use

Out of the 61 sites that I surveyed, 30 had Green Salamander detections (49.1%). Ten of those sites were new potential Green Salamander locales in the South Carolina Blue Ridge region. These new locales were located in Pickens County, SC (n=7) and Oconee County, SC (n=3). The majority of these sites were south-facing (n=8), ranged in elevation from 399–641 m, and in size from 136–6649 m². Out of the ten newly discovered potential sites that I surveyed, seven had detections (70%). I found six Green Salamanders using arboreal habitats during surveys. In addition, I found six salamanders (three on one occasion) on a Red Oak, *Quercus falcata*, at Table Rock State Park that was not in a survey plot. The farthest distance I documented a Green Salamander from a rock outcrop to an arboreal habitat was 35.2 m. The

highest observation of a Green Salamander on a tree was approximately 9 m from the ground on a mossy patch of a Red Oak. Green Salamanders were documented on hardwoods including Red Oaks, Red Maples (*Acer rubrum*), Black Cherries (*Prunus serotina*) as well as other arboreal/woody habitats such as rotten logs and tree snags.

Detection and Abundance Analyses

Time of day emerged as the most important variable for detecting Green Salamanders. Detection probability of Green Salamanders across all sites and across sites known to be occupied ranged from ~0.03 – 0.13 as a function of time of day. Salamanders had a higher probability of being detected later in the day. Aspect, size, and elevation were the only three variables that were supported among our candidate set of abundance covariates (Table 1.2). The top candidate model contained all three abundance covariates – aspect, habitat size, and elevation (in its linear form; Table 1.2). There were also two other models with a $\Delta AIC < 2$ and therefore, had some support in my analysis (Table 1.2). In the second top model (Aspect+Size), parameter estimates for both aspect and size were similar for both models and so this second model is not adding any new additional information to my analysis. In the third top model (Aspect+Size+Elev² model), the quadratic term crosses zero and thus is not an informative term. Aspect had a negative influence on Green Salamander abundance and size had a positive influence on abundance (Table 1.2; Fig. 1.2). I found support for models where elevation had a direct negative on abundance (Table 1.2; Fig. 1.2), however, the quadratic effect was not informative (Table 1.2). Out of the 51 historical locations that I surveyed, 23 of these sites had detections. I adjusted for detection probability by using what I know about aspect, habitat size, and elevation at a site to predict abundance at a particular site and determined that 45/51

(88.24%) historical sites that I surveyed were predicted to have at least two individual green salamanders. I used two individuals as the threshold because that was my estimate of abundance at the least-abundant site with confirmed occupancy. For a survey of average habitat size and elevation, abundance increased by ~4.7-fold (from 1.72 to 8.08) as aspect shifted from more northerly- to southerly-facing sites. For a survey of average aspect and elevation, abundance increased by ~5-fold (from 5.93 to 29.24) as habitat size ranged from approximately 1–6650 m². For a survey of average aspect and habitat size, abundance increased by ~15-fold (from 1.21 to 18.06) as elevation ranged from approximately 280-1040 m.

DISCUSSION

Green Salamander abundance was influenced by aspect, habitat size, and elevation (Table 1.2; Fig 1.2). Interestingly, sites with south-facing slopes (which tend to be xeric) had higher estimated abundances of Green Salamanders than those with north-facing slopes. This is consistent with Bruce (1968) who suggests that rock outcrops sites on south-facing slopes may be buffered from sunlight penetration because of the narrowness and irregularity of the crevices in which Green Salamanders are found in. Our findings, however, are inconsistent with more recent literature suggesting a preference for northerly-facing slopes (Hafer and Sweeney 1993). Hafer and Sweeney (1993) based their criteria for “high probability of containing suitable Green Salamander habitat” off of 14 known Green Salamander locales (with ten of those sites having a northerly-facing aspect), thus it is likely this small sample size may have biased their conclusions. As expected, larger sites had higher estimated abundances of salamanders than smaller sites. Larger sites represent opportunities for higher Green Salamander numbers and thus contribute to genetic diversity (Petranka et al. 1993; Noël et al. 2007). The model with the most

support indicated a negative relationship between estimated abundance and elevation, however the quadratic elevation covariate was uninformative (Table 1.2). A study in Ohio suggested that Green Salamanders preferred low elevations between 183–244 m (Lipps 2005). The Bruce (1968) Green Salamander surveys characterized rock outcrop sites in the BRE to have a wide elevational range, including low elevations (305-m and above). He suggests that although higher elevations may be available to salamanders in the BRE, they may not be able to disperse to them because of the topography. Further, salamanders may prefer the stable microclimates provided by lower elevation gorges of the BRE (Bruce 1968). Hafer and Sweeney (1993) characterized habitat suitability of Green Salamanders in South Carolina to increase with elevation, which is contrary to our findings. Knowledge of site-specific population growth rates and genetic diversity would be valuable contributions toward further contextualizing the environmental associations I describe here.

Detection of Green Salamanders was influenced by time of day in an unexpected manner. Surprisingly, time of day had a positive influence on detection of salamanders suggesting that salamanders were more surface active (and therefore easier to detect) later in the day. In other words, Green Salamanders were more detectable during the hotter parts of the days. Rock outcrop microclimate is likely buffered from the surrounding warm and dry air associated with the hottest times of the day (Locosselli et al. 2016). Several findings within this study and others suggest Green Salamanders in the BRE may be somewhat resilient to warm and dry conditions (Gordon 1952; Bruce 1968; Barrett et al. 2014). For example, one preliminary laboratory study documented Green Salamanders to have a higher tolerance to drying compared to another plethodontid salamander, *Plethodon metcalfi* (= *Plethodon jordani melavantris*) (Gordon 1952).

This suggests that Green Salamanders may be able to take advantage of sites that are less suitable for other species using rock outcrops (e.g., *Plethodon metcalfi*).

Many of the historical localities in South Carolina that I surveyed fell short of the suggested 100-m forested buffer (Petranka 1998; Wilson 2001; Waldron and Humphries, 2005). For example, fourteen rock outcrop sites had < 20 m of forest between the site and a paved road or powerline cut (eight of which were occupied). Throughout surveys, I only saw six salamanders within arboreal habitats. Occupied trees were predominately hardwoods, similar to those found in the Waldron and Humphries (2005), however two detections were found on rotten logs/tree snags. The majority of detections outside of rocky outcrops occurred on moss, lichen, or flaky bark which likely provide moist refugia. Our farthest documented movement during the survey season was 35 m from the nearest rock outcrop and therefore it is likely that some salamanders at these sites are exposed to a lack of shade due to open canopy. Detections away from rock outcrops may have been influenced by the extreme drought (in part from the 2016 El Niño event), which could have decreased movements away from moist rock crevices. Furthermore, many sites had a thick *Rhododendron* understory so it is possible that I missed detections in this thick shrub. *Rhodoendron* detections were high in North Carolina Green Salamander surveys (pers. communication, M. Hall). Open canopies have been found to limit migration opportunities and lead to patchy distributions (Gordon 1952; Snyder 1991; Corser 2001), but I do not have data on movement among the habitats studied here.

Green Salamanders were detected in less than half of the sites that I surveyed and when they were detected, they were not typically abundant (Fig 1.1, main map). When I adjusted for detection probability, six sites were predicted to have less than two individual Green Salamanders. Three of these sites were located in Greenville County, two of these sites were in

Pickens County, and one of these sites was in Oconee County. Because the species has low detection probability it is possible that some sites were occupied even though I never detected individuals. Nevertheless, our survey methods represent a more intensive survey effort than either of the two previous surveys in South Carolina (Bruce 1968; Hafer and Sweeney 1993). Future status assessments should explore ways to increase detection of individuals by incorporating fall (September and October) and nighttime salamander surveys. Knowledge of distributional shifts relative to historical trends will allow for a better understanding of how Green Salamanders will respond to threats such as land use and climate change, as well as disease and collection.

MANAGEMENT RECOMMENDATIONS

Current Habitat Protection

My results suggest that aspect, habitat size, and elevation have the most influence on Green Salamander abundance. In order to protect current Green Salamander habitat, it would be beneficial to focus on protecting bigger rocky outcrop sites at lower elevations on south-facing slopes (as these were the habitat associations that predicted highest abundances). Although south-facing slopes are known to be xeric, literature suggests that the rock crevices in which Green Salamanders are found in are structured in such a way that they deflect sunlight (Bruce 1968). Additionally, larger habitats (rock outcrops) are likely important for this habitat specialist because they provide additional refugia for salamanders to be active (i.e. foraging and reproduction) and this can lead to increased genetic diversity (Petranka et al. 1993; Noël et al. 2007). Further, the results of my study along with others suggest that Green Salamanders prefer lower elevations (Bruce 1968; Lipps 2005). Literature suggests that the microclimate at lower

elevations may be preferential to Green Salamanders and additionally, salamanders may not be able to access higher elevations due to limitations in dispersal (Bruce 1968).

Determining Occupancy

Detection of Green Salamanders in South Carolina was most influenced by time of day in which surveys were conducted. Unexpectedly, my results suggested that Green Salamanders were more easily detected later in the day. Therefore, I recommend surveying Green Salamanders mid-day. Although observer did not emerge as a detection covariate, I strongly recommend developing a search image of the species before conducting surveys. Green Salamanders are known to be a cryptic species and thus having a search image for the species will greatly increase an observer's odds of seeing a camouflaged salamander in its habitat. In order to develop a search image, I recommend all surveys have previous experience or gain experience by working with a trained individual.

When conducting visual encounter surveys for Green Salamanders, I recommend visiting a site three times in order to determine whether or not a site is occupied. Three visits to a site provides sufficient data to make an informative decision about a particular site. The top model suggests a 10% chance of detecting a salamander and so if a site has at least ten salamanders, an observer is likely to detect at least one of them on a single visit (if the highest detection probability is assumed). Further, I also suggest spreading visits throughout the Green Salamander's active season (Gordon 1952). Green Salamanders come out of hibernation starting in late April, breed from May – September, and finally have a period of dispersal/aggregation before hibernating in November (Gordon 1952).

Logging Management on Public Property

Of the 61 surveyed Green Salamander sites that I surveyed, there were 14 localities with < 20 m of a forested buffer between the rock outcrop site and a landscape disturbance (paved road or powerline cut). Sites without a forested buffer were expected to have fewer salamanders per site (8.82 ± 6.10) compared to sites with a forested buffer (11.66 ± 8.63). During the survey season, I documented a Green Salamander 35 m away from the nearest rock outcrop and the longest documented movement from a rock outcrop to a tree is 42 m (Waldron and Humphries 2005). In previous literature, scientists have suggested a 100-m forested buffer around rock outcrops (Petranka 1998; Wilson 2001; Waldron and Humphries, 2005). I strongly agree with this recommendation as this is a seasonally arboreal species that likely spends significant amounts of time in trees (Waldron and Humphries 2005).

REFERENCES

- Araújo, M. B., W. Thuiller, and R. G. Pearson. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* 33:1712–1728.
- Barrett, K., N. P. Nibbelink, and J. C. Maerz. 2014. Identifying priority species and conservation opportunities under future climate scenarios: Amphibians in a biodiversity hotspot. *Journal of Fish and Wildlife Management* 5:282–297.
- Blackburn, M., J. Wayland, W. H. Smith, J. H. McKenna, M. Harry, M. K. Hamed, M. J. Gray, D. L. Miller. 2015. First report of Ranavirus and *Batrachochytrium dendrobatidis* in green salamanders (*Aneides aeneus*) from Virginia, USA. *Herpetological Review* 46:357–361.
- Brodman, R. 2004. R9 species conservation assessment for the green salamander, *Aneides aeneus* (Cope and Packard). *Conservation Assessment* 1–17.
- Bruce, R. C. 1968. The role of the Blue Ridge Embayment in the zoogeography of the green salamander, *Aneides aeneus*. *Herpetologica* 24:185–194.
- Cicero, C., H. Bart, D. Bloom, R. Guralnick, M. Koo, J. Otegui, N. Rios, L. Russell, C. Spencer, D. Vieglais, J. Wiczorek,. 2010. VertNet: an online reference Available at: <http://www.vertnet.org/index.html>. Archived by WebCite at <http://www.webcitation.org/6pD7hOsZF> on 24 March 2017.
- Cope, E. D., A. S. Packard. 1881. The fauna of the Nickajack Cave. *American Naturalist* 15:877–882.
- Corser, J. D. 2001. Decline of disjunct green salamander (*Aneides aeneus*) populations in the southern Appalachians. *Biological Conservation* 97:119–126.
- Corser, J. D. 1991. The Ecology and Status of the Endangered Green Salamander (*Aneides*

- aeneus*) in the Blue Ridge Embayment of North Carolina. Master's Thesis. Duke University, Durham, North Carolina, United States of America.
- Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5:735–748.
- Fiske, I., R. Chandler. 2011. unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software* 43:1-23.
- Gibbs, J. P. 1998. Distribution of woodland amphibians along a forest fragmentation gradient. *Landscape Ecology* 13:263-268.
- Gordon, R. E. 1952. A contribution to the life history and ecology of the plethodontid salamander *Aneides aeneus* (Cope and Packard). *The American Midland Naturalist* 47:666–701.
- Hafer, M. L. A., J. R. Sweeney. 1993. Status of the green salamander in South Carolina. *Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies* 47:414–418.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.
- Lipps, Jr. G. J. 2005. A Framework for Predicting the Occurrence of Rare Amphibians: A Case Study with the Green Salamander. Master's Thesis. Bowling Green State University, Bowling Green, Ohio, United States of America.
- Locosselli, G. M., R. H. Cardim, G. Ceccantini. 2016. Rock outcrops reduce temperature-induced stress for tropic conifer by decoupling regional climate in the semiarid

- environment. *International Journal of Biometeorology* 60:639-649.
- Miloski, S. E. 2010. Movement Patterns and Artificial Arboreal Cover Use of Green Salamanders (*Aneides aeneus*) in Kanawha County, West Virginia. Master's Thesis. Marshall University, Huntington, West Virginia, United States of America.
- Moffitt, D., L. A. Williams, A. Hastings, M. W. Pugh, M. M. Gangloff, and L. Siefferman. 2015. Low prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the southern Appalachian mountains. *Herpetological Conservation and Biology* 10:123–136.
- Mount, R. H. 1975. The Reptiles and Amphibians of Alabama. Alabama, United States of America.
- Munday, P. L. 2004. Habitat Loss, resources specialization, and extinction on coral reefs. *Global Change Biology* 10:1642–1647.
- Natureserve. 2017. NatureServe Explorer: an online encyclopedia of life [web application]. Version 7.0. NatureServe, Arlington, VA. U.S.A. Available at <http://explorer.natureserve.org>. Archived by WebCite at <http://www.webcitation.org/6pEaNoD3E> on 25 March 2017.
- Noël, S., M. Ouellet, P. Galois, F. J. Lapointe. 2007. Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. *Conservation Genetics* 8:599–606.
- Owens, I. P. F. and P. M. Bennett. 2000. Ecological basis of extinction risk in birds: habitat loss versus human persecution and introduced predators. *Proceedings of the National Academy of Sciences of the U. S. A.* 97:12144–12148.
- Petranka, J. W., Eldridge M. E., Haley, K. E. 1993. Effects of timber harvesting on southern

- Appalachian salamanders. *Conservation Biology* 7:363–370.
- Petranka, J. W. 1998. Salamanders of the United States and Canada. United States of America.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439:161–167.
- Price, S. J., M. E. Dorcas. 2007. Carolina Herp Atlas: an online reference Available at <https://www.carolinaherpatlas.org/>. Archived by WebCite at <http://www.webcitation.org/6pD5Mcnyp> on 24 March 2017.
- Snyder, D. H. 1991. The green salamander (*Aneides aeneus*) in Tennessee and Kentucky, with comments on the Carolinas' Blue Ridge populations. *Journal of the Tennessee Academy of Science* 66:165–169.
- Snyder, D. H. 1983. The apparent crash and possible extinction of the green salamander, *Aneides aeneus*, in the Carolinas. *Association of Southeastern Biologists Bulletin* 30:82.
- Spickler, J. C., S. C. Sillett, S. B. Marks, and H. H. Welsh, Jr. 2006. Evidence of a new niche for a North American salamander: *Aneides vagrans* residing in the canopy of old-growth redwood forest. *Herpetological Conservation and Biology* 1:16–26.
- Travis, J. M. J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society of London* 270:467–473.
- Tockner, K., F. Schiemer, C. Baumgartner, G. Kum, E. Weigand, I. Zweimüller, J. V. Ward. 1999. The Danube restoration project: species diversity patterns across connectivity gradients in the floodplain system. *Regulated Rivers-Research and Management* 15:245–258.

- USGS. 2013. Biodiversity Information Serving Our Nation (BISON): an online reference
Available at <https://bison.usgs.gov/#home>. Archived by WebCite at
<http://www.webcitation.org/6pD6JtGpT> on 24 March 2017.
- Vallan, D. 2002. Effects of anthropogenic environmental changes on amphibian diversity in the
rain forests of eastern Madagascar. *Journal of Tropical Ecology* 18:725–742.
- Waldron, J. L. and W. J. Humphries. 2005. Arboreal habitat use by the green salamander,
Aneides aeneus, in South Carolina. *Journal of Herpetology* 39:486–492.
- Wilson, C. R. 2001. Green Salamander, *Aneides aeneus*. *Chattooga Quarterly*: Spring/Summer
2001 Edition 3–4.
- Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S.
Ron, E. La Marca, J R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001.
Population declines and priorities for amphibian conservation in Latin America.
Conservation Biology 15:1213–1223.

TABLE 1.1— Abundance covariates (and associated supporting literature) used in the single-species abundance model for green salamanders in the South Carolina Blue Ridge Mountains. I measured these variables at each site, and their relative importance was assessed in a multi-model Akaike Information Criterion (AIC) framework.

Abundance covariate	Type	Description	Source
Size	Continuous	Size of rock outcrop (m ²)	Brodman, 2004
Elev	Continuous	Average midpoint elevation (m)	Bruce, 1968; Corser, 1991; Hafer and Sweeney, 1993; Lipps, 2005
Slope	Continuous	Average midpoint slope (°)	Bruce, 1968; Corser, 1991; Hafer and Sweeney, 1993
Aspect	Continuous	Average midpoint aspect (°)	Bruce, 1968; Hafer and Sweeney, 1993
BA	Continuous	Average basal area taken from start of four transects (m ² /ha)	Spickler et al., 2006
CC	Continuous	Average percentage canopy cover taken from start of four transects (0-100)	Gordon, 1952; Spickler et al., 2006
Drain_Presc	Categorical	Presence or absence of drainage at a site	Hafer and Sweeney, 1993

Table 1.1, continued,

Dist_Water	Categorical	Drainage < or > 400m from site	Hafer and Sweeney, 1993
LC	Categorical	Type of forest (mixed forest, mixed hardwood, softwood, shrubs)	Gordon, 1952; Bruce, 1968; Waldron and Humphries, 2005
Dist	Categorical	Presence or absence of a landscape disturbance at a site (heavy, light, none)	Gordon, 1952; Snyder, 1991; Corser, 2001
BIO 1	Continuous	Annual mean temperature (World Clim)	Corser, 2001; Barrett et al., 2014
BIO 5	Continuous	Maximum temperature of the warmest month (World Clim)	Corser, 2001; Barrett et al., 2014
BIO 12	Continuous	Annual precipitation (World Clim)	Corser, 2001; Barrett et al., 2014
BIO 17	Continuous	Precipitation of the driest quarter (World Clim)	Corser, 2001; Barrett et al., 2014

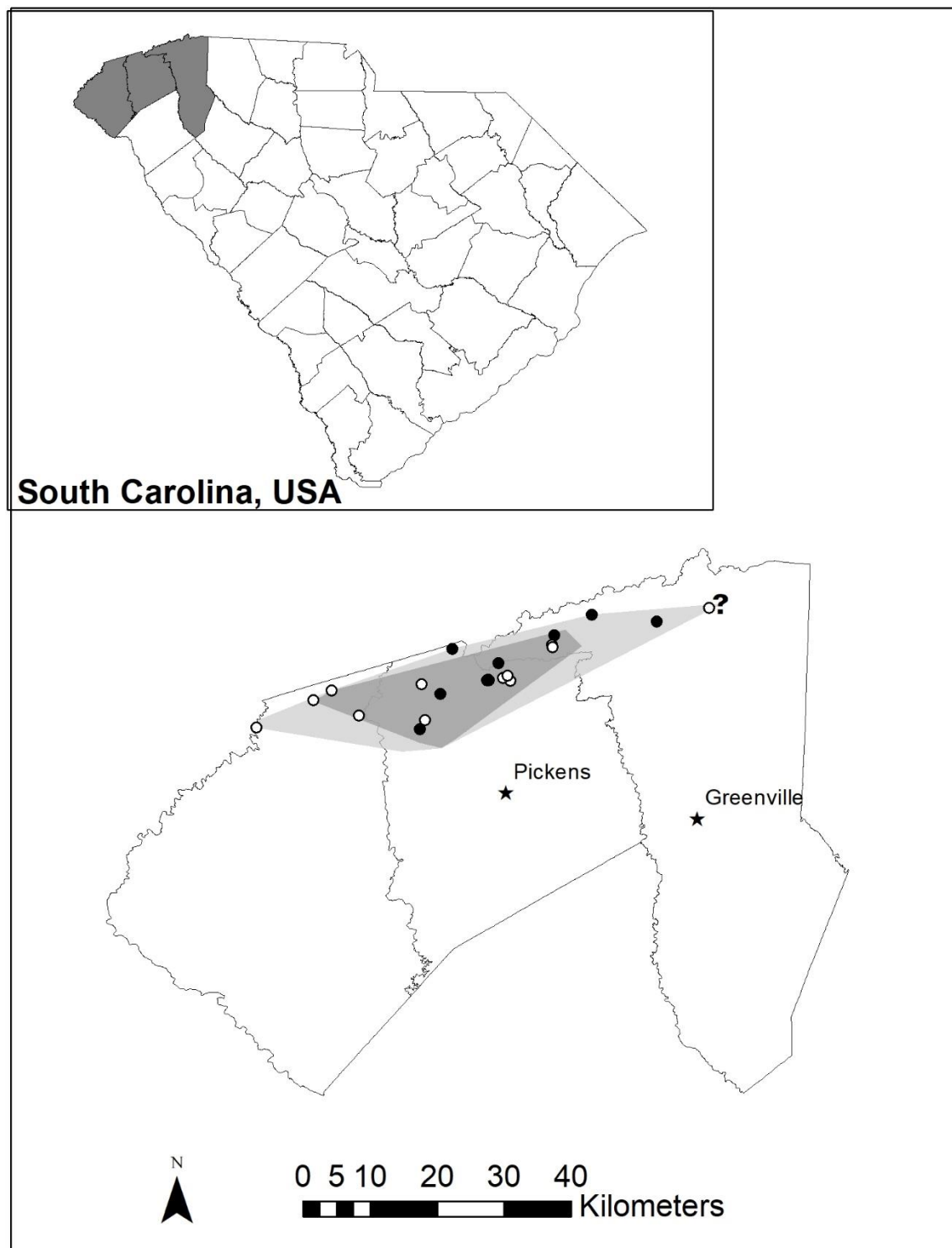
TABLE 1.2— Results of AIC analysis for 12 candidate models describing environmental covariates of Green Salamander abundance among rock outcrops in the Blue Ridge Escarpment of South Carolina. All models include time of day as the covariate of detection probability. The models below represent our final AIC comparison, which included the null model, all competitive univariate models, and all possible combinations of covariates from those univariate models. See Table 1.1 for definitions of model abbreviations; k = number of modeled parameters.

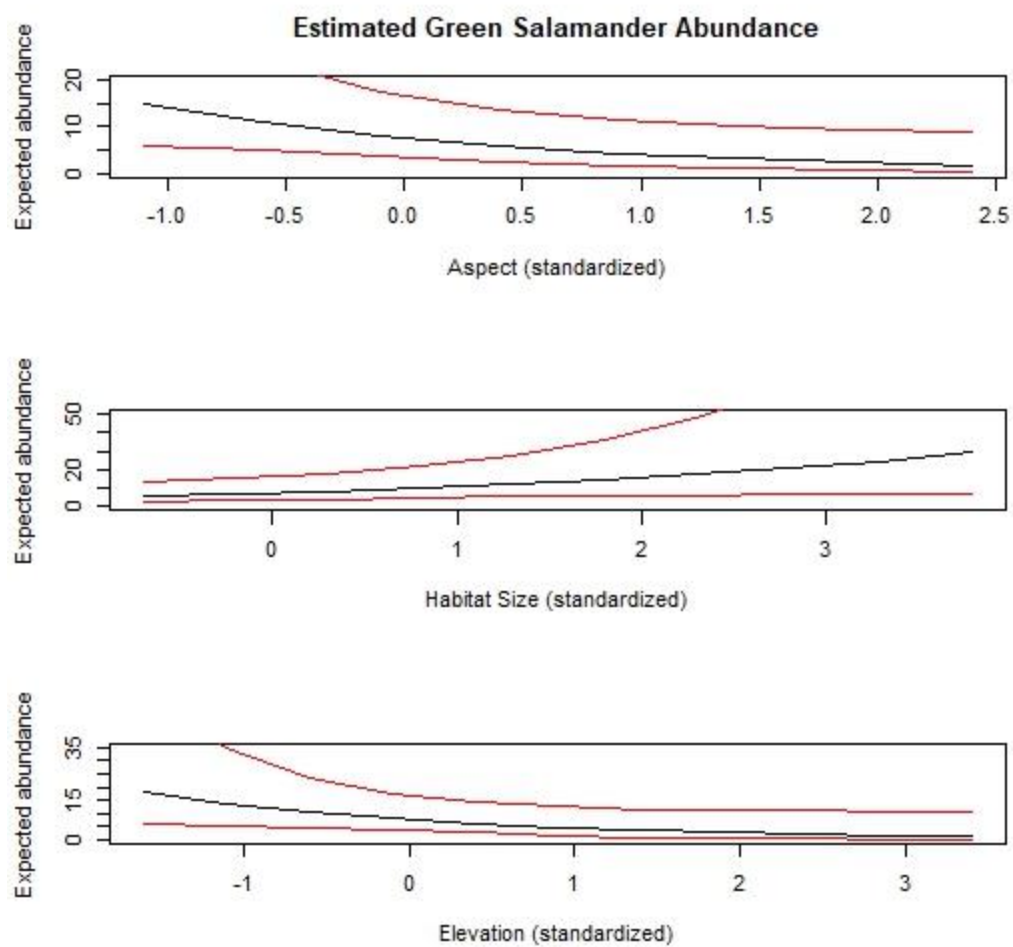
Model	k	λ (SE)	AIC	ΔAIC	AIC weight	Cum. weight
Aspect+Size+Elev	7	-0.60(\pm 0.28), 0.36(\pm 0.18), -0.54(\pm 0.29)	338.65	0.00	0.32	0.32
Aspect+Size	6	-0.67(\pm 0.29), 0.30(\pm 0.19)	340.24	1.59	0.15	0.46
Aspect+Size+Elev ²	8	-0.59(\pm 0.29), 0.33(\pm 0.18), -0.45(\pm 0.37), -0.09(\pm 0.23)	340.47	1.82	0.13	0.59
Aspect	5	-0.72(\pm 0.29)	341.27	2.62	0.09	0.68
Size+Elev	6	0.40(\pm 0.19), -0.61(\pm 0.30)	341.46	2.81	0.08	0.76
Aspect+Elev	6	-0.68(\pm 0.29), -0.39(\pm 0.29)	341.47	2.82	0.08	0.84
Aspect+Elev ²	7	-0.61(\pm 0.30), -0.17(\pm 0.35), -0.23(\pm 0.24)	342.31	3.66	0.05	0.89
Size+ Elev ²	7	0.35(\pm 0.20), -0.40(\pm 0.38), -0.22(\pm 0.27)	342.72	4.07	0.04	0.93
Size	5	0.34(\pm 0.21)	343.79	5.13	0.03	0.95

Elev ²	6	-0.12(± 0.36), -0.39(± 0.28)	344.37	5.72	0.02	0.97
Elev	5	-0.47(± 0.30)	344.80	6.15	0.02	0.99
(.)	4	2.38(± 0.38)	345.15	6.50	0.01	1.00

FIG 1.1 —The inset map at the top represents the counties within South Carolina known to contain Green Salamander localities. From left to right the shaded polygons are Oconee, Pickens, and Greenville Counties. The main map shows the known distributional range of Green Salamanders, *Aneides aeneus*, in upstate South Carolina. The light gray polygon represents the historic range and the dark gray polygon represents the range based on sites with confirmed presence from this study. Polygons were created using minimum boundary geometry in ArcGIS. White circles represent sites that did not have suitable habitat and black circles represent sites that were not accessible due to terrain. The question mark represents the site that I did not have permission to access.

FIG 1.2—Aspect, habitat size, and elevation emerged as the best predictors of abundance for Green Salamanders (*Aneides aeneus*) in South Carolina (Table 1.2). The top panel is illustrating the effect of aspect on the estimated abundance of Green Salamanders when both habitat size and elevation are held at their mean values. The middle panel is illustrating the effect of size on the estimated abundance of Green Salamanders when both aspect and elevation are held at their mean values. The bottom panel is illustrating the effect of elevation on the estimated abundance of Green Salamanders when both aspect and habitat size are held at their mean values. All panels have 95% CI.





CHAPTER TWO

ADDING MECHANISM TO A DISTRIBUTION MODEL CHANGES PREDICTIONS OF CLIMATE VULNERABILITY FOR A PRIORITY AMPHIBIAN

INTRODUCTION

Understanding species response to global climate change will allow for more informed conservation and management decisions. Climate change has been implicated in population declines of several species (Both et al. 2006; Carpenter et al. 2008) and disruptions in behavior for others. For example, avian and anuran species have shifted their breeding seasons due to changes in temperature (Brown & Bhagabati 1999; Barbraud & Weimerskirch 2006; Kusano & Inoue 2008). Further, it has been projected that many European herpetofaunal species with limited dispersal will lose suitable habitat in the future (Araújo et al. 2006), which may lead to population declines for additional species.

A wide range of tools are available to forecast species response to climate change (Füssel & Klein 2006; Butchart et al. 2010; Sutton et al. 2015). Among the available tools, species distribution models provide spatially explicit models of vulnerability and resilience. While the maps produced from such models offer conservation practitioners guidance on where to exert efforts, there are important caveats associated with many of the distribution modeling approaches (Buckley et al. 2010; Barrett et al. 2014; Roach et al. 2017). Specifically, correlative models, which examine the association between climatic variables and species locality data (Kearney et al. 2010; Barrett et al. 2014)

assume some underlying relationship between environmental conditions and the distribution of the animal. Importantly, a physiological connection between these two variables is not explicitly evaluated. As a result, the models may do a poor job projecting habitat suitability in environments outside the range of the training data (Elith et al. 2010; Milanovich et al. 2010). Mechanistic models offer an alternative approach to distribution modeling. These models estimate animal performance (e.g., active foraging time or reproduction) across habitats using empirically-derived relationships between animal physiology and environmental conditions. Once these relationships are known, the distribution of an organism can be mapped onto a range of environmental conditions (Mathewson et al. 2017; Riddell et al. 2017). Research suggests that mechanism can enhance correlative models when predicting climatically suitable habitat for a species (Mathewson et al. 2017; Riddell et al. 2017); however, a recent review demonstrated few mechanistic models in literature (Urban et al. 2016). The rarity of these models may result from the data required to build them (i.e., experimentally-derived estimates of animal physiology under a range of environmental conditions); nevertheless, several examples of such models do exist – especially for ectotherms (Buckley 2008; Kolbe et al. 2010; Buckley et al. 2010; Riddell et al. 2015; Riddell et al. 2017).

I used green salamanders, *Aneides aeneus* (Cope & Packard 1881), to assess the influence of adding mechanistic parameters to climate-based distribution models. Green salamanders have experienced population declines in the disjunct portion of their range (North Carolina, South Carolina, and Georgia) beginning in the 1970's (Snyder 1983, Corser 2001). In addition to habitat loss, over-collection, and disease, climate change has

also been implicated as a threat to this species (Corser 2001). Several correlative models have been applied to green salamanders in all or parts of their range (Lipps 2005; Barrett et al. 2014; Hardman 2014). These correlative models have helped determine habitat associations and document new sites in areas where green salamanders are listed as “endangered.” Additionally, the mid-century and end-of-century predictions of habitat suitability from these models will aid in conservation-based decisions.

Currently, there are no mechanistic models for green salamander distribution. However, Gordon (1952) conducted preliminary experiments examining vital limits of green salamanders during water loss trials. Experiments compared physiological response to drying under one temperature (20°C) treatment with dry air conditions between green salamanders and *Plethodon metcalfi* (= *Plethodon jordani melavantris*). The study found that green salamanders withstood drying longer than *Plethodon metcalfi* and thus on average lost more water than *Plethodon metcalfi*. While the sample size was limited for this study (N=15 green salamanders, N=7 *Plethodon metcalfi*), these results suggest that green salamanders are likely able to withstand physiological challenges (such as increased temperature) better than closely related species.

For this study, I used program MaxEnt (Maximum Entropy), a method for modeling species distributions using presence-only data (Phillips et al. 2006). MaxEnt is used to estimate the species distribution by finding the largest spread (maximum entropy) of a geographic dataset containing presence data with association to environmental variables. This program has been used to model suitable habitat of many species, particularly amphibians that are globally-threatened or extinct (Baillie et al. 2004; Milanovich et al.

2010; Barrett et al. 2014; Hardman 2014; Sutton et al. 2015). I modeled suitable climatic habitat for green salamanders throughout their disjunct range. I coupled physiological data (resistance to water loss and metabolic rates) with climatic data to create a mechanistic model for green salamanders. I compared the correlative models with and without a mechanistic layer to evaluate model predictions under different climatic scenarios.

METHODS

Salamander Care and Collection

I conducted experiments on green salamanders to evaluate the thermal sensitivity of water loss rates and metabolic rates (Fig 2.1). I collected 2-6 green salamanders (avoiding nesting and gravid females as well as juveniles) from four sites in South Carolina (N=19) at night from April – May 2017. Sites were located in Table Rock State Park and Nine Times Forest (precise locality withheld due to conservation concerns). These sites were selected because previous surveys suggested animal densities were high enough to provide sufficient captures for our trials without compromising the population. Salamanders were transported in Ziploc bags (with moist leaf litter) to Clemson University and placed in an incubator (15°C). Salamanders acclimated in individual Ziploc containers with a wet paper towel for five days to ensure that physiological measurements occurred during a post-absorptive state. As part of our animal care protocol, I estimated a baseline mass (to the nearest 0.001 g) at the beginning of the

experiment to ensure that salamanders did not lose more than 10% of their baseline mass while in the laboratory. Salamanders that did not maintain a baseline mass were excluded from the experiment (see below). After the acclimation period, I measured water loss rates and metabolic rates using a flow through system. All experiments were approved by the Institute for Animal Care and Use Committee at Clemson University (AUP 2016-035), and approval for collections and experimentation were granted by the South Carolina State Park Service and the South Carolina Department of Natural Resources. After the experimental trials, all collected animals were returned to capture sites.

Flow through system and physiological measurements

I measured the thermal sensitivity of water loss rates and metabolic rates using a flow through system. Our system continuously exposed salamanders to highly controlled temperature and humidity environments and simultaneously measured their physiology. I controlled the environmental temperature using a programmable incubator (Percival VL36). The system used a sub-sampler (SS-4; Sable Systems International (SSI)) to push air through a dewpoint generator (DG-4; SSI) controlling the vapor pressure deficit (VPD; the difference between the amount of moisture in the air and the amount of moisture the air can hold). A flow manifold (MF-8; SSI) was then used to divide the airstream into the individual cylindrical acrylic chambers. The chambers (16cm x 3.5cm; volume ~ 153 mL) contained an individual green salamander placed on hardwire mesh to expose its surface to the airstream (simulating posture during activity). I cycled air between each chamber three times every ten minutes using a multiplexer (M8; SSI). The

airstream was then sampled using a vapor analyzer (RH-300; SSI) which measured the change in water vapor pressure (kPa). Then, the air was scrubbed of water vapor and carbon dioxide using Drierite (W. A. Hammond Drierite Co. Ltd.) and soda lime, respectively. After scrubbing, I measured the partial pressure of oxygen using Oxzilla (SSI).

I moved the individual Ziploc containers to an environmental chamber set to a regulated experimental temperature (12°C, 18°C, 24°C) two hours prior to measuring water loss rates. These temperatures were chosen to reflect the temperatures that green salamanders would experience in nature during their active season (April - October; Gordon 1952). I calculated skin resistance to water loss of green salamanders using a combination of one of the three treatment temperatures (12°C, 18°C, 24°C) and a single VPD (0.5 kPa). This VPD was chosen because it is ecologically relevant for terrestrial salamanders. I randomized the temperature treatments with respect to night of experiment to avoid acclimation effects. Physiological traits were measured between 1900 and 100 EST to reduce influence of circadian rhythm of metabolism. Salamanders were allowed to acclimate to the flow through chambers for 30 minutes to adjust to their new surroundings. To ensure animals were resting, I did not include any measurements in our analyses with spikes or irregularities in vapor pressure that are indicative of activity. I measured water loss rates and metabolic rates separately because metabolic rates were too low to detect using the flow rates from the water loss measurements.

Thermal sensitivity of metabolism

Energy balance for an ectotherm depends upon the temperatures that the organism experiences. I used the same flow-through system in the laboratory to measure volume of oxygen consumption (VO_2) to estimate energetic costs for the mechanistic distribution models. I reduced the flow rate to 50 mL/min allowing for increased resolution of the oxygen depletion curves during cooler temperature treatments when salamanders exhibit very low metabolic rates. I held VPD (0.5 kPa or 64% -83% relative humidity) constant across treatments. Using Oxzilla (SSI), I measured partial pressure of oxygen to measure volume of oxygen consumption at four experimental temperatures (6°C, 12°C, 18°C, 24°C) each of which occurring on a single evening. I wanted to measure volume of oxygen consumption at as broad of a range as possible, so I included a fourth, lower, temperature treatment that was not used in the water loss trials due to limitations with the equipment. I randomized the order of each experimental temperature to avoid acclimation effects. I excluded two individuals from the metabolic trials because they failed to return to baseline mass after the water loss experiment. Partial pressures of gases were converted into meaningful physiological values using a series of established calculations.

Calculations for Skin Resistance to Water Loss

I measured skin resistance to water loss using a suite of calculations presented in Riddell et. al. (2015). First, I converted the water vapor pressure (e ; kPa) to water vapor density (ρ_v ; g/m³) using the following equation:

$$\rho_v = \frac{e}{(T \cdot R_v)} \quad (1)$$

where T is temperature in Kelvin (K) and R_v is the gas constant for water vapor (461.5 J·K⁻¹·kg⁻¹). I then converted the vapor density to evaporative water loss (EWL; mg/hr) using:

$$EWL = \rho_v \cdot FR \cdot \frac{1}{60} \quad (2)$$

where FR is the flow rate of the air stream (mL/hr) and 1/60 is a conversion factor for mg/min. Next, I calculated cutaneous water loss (CWL ; g·cm²·sec⁻¹) by dividing the rate of water loss by the surface area of each salamander. The surface area (cm²) was estimated by an empirically derived formula for the family Plethodontidae, where surface area = 8.42 x mass (g)^{0.694} (Whitford & Hutchison 1967). I used CWL to calculate total resistance to water loss, r_T (sec/cm) as:

$$r_T = \frac{r}{CWL} \quad (3)$$

where ρ is the vapor density gradient (g/cm³).

I then used a series of biophysical equations, described in detail by Riddell et al. (2017), to estimate the resistance of boundary layer assuming free convection conditions.

Boundary layer resistance (r_b) is required to calculate skin resistance to water loss (r_i),

and once I estimated r_b , I calculated skin resistance using:

$$r_i = r_T - r_b \quad (4)$$

where r_T is the total resistance (sec/cm) and r_b is the boundary layer resistance (sec/cm). This physiological trait was then used in the physiologically-structured species distribution model to estimate activity and energy budgets (see below).

Estimation of environmental data for mechanistic SDM

Mechanistic models predict activity budgets and energetic costs based upon the temperature and humidity values that the focal organism experiences in their habitat. Similar mechanistic SDMs have used *microclim* to estimate the relevant temperatures experience by terrestrial salamanders; however, I estimated relevant air temperatures from bioclimatic layers for green salamanders for two reasons. Firstly, green salamanders are typically active at night on the surface of large boulders; thus, the temperatures that they experience are likely closer to air temperature 1-2 m off the ground. Secondly, the spatial layers derived from the physiologically-structured model were integrated into a correlative framework to evaluate the role of mechanism in predictions of habitat suitability under climate change. By using the same climatic layers, our study ensures that the correlative and mechanistic models are not producing different results simply due to the source of data. With the bioclimatic layers, I estimated hourly variation in temperature from monthly minimum and maximum temperatures using standard protocols described in Campbell and Norman (1998). Similar to previous mechanistic models, I estimated vapor pressure deficits under the established pattern that minimum nightly temperatures approach the dew point temperature (Riddell et al. 2017).

Foraging-energetic model in mechanistic SDM

Physiologically-structured SDMs predict activity and energy balance based upon thermal sensitivities of traits and the typical activity patterns of the focal organism. I simulated nightly activity for each location based upon the average hourly temperature

and humidity data over the entire year (described above). Salamander activity was restricted to nighttime conditions (2100 – 0600), and activity only occurred when temperatures were suitable (5°C - 25°C). While active, I estimated body temperatures by accounting for the effect of evaporative cooling using humid operative temperatures (T_{eh}) (Campbell and Norman 1998). I estimated T_{eh} using:

$$T_{eh} = T_a + \frac{g^*}{s + g^*} \left(\frac{R_{abs} - \epsilon_s \sigma T_a^4}{c_p g_{Hr}} - \frac{D}{g^* p_a} \right)$$

where T_a is ambient temperature, s is the slope of saturation mole fraction function, R_{abs} is the absorbed short- and long-wave radiation, γ^* is the apparent psychrometer constant, ϵ_s is the emissivity of the salamander (0.96), sigma is the Stefan-Boltzmann constant, c_p is the specific heat of air at constant pressure, g_{Hr} is the sum of boundary layer and radiative conductance, D is the vapor pressure deficit of the air, and p_a is the atmospheric air pressure (Campbell & Norman 1998). I used these temperatures to estimate energetic costs from volume of oxygen consumption while active for every hour. I calculated energetic costs based upon the thermal sensitivity of $V O_2$ using the following equation:

$$\log VO_2 = (0.046 \cdot T) + (0.59 \cdot \log(mass)) + 0.86$$

where VO_2 is volume of oxygen consumption ($\mu\text{L}/\text{min}$), T_b is body temperature ($^{\circ}\text{C}$), and $mass$ refers to the mass (g) of the green salamander. I then assumed a conversion factor of 20.1 J/mL to convert $V O_2$ to standardized units of energy.

Salamanders frequently retreat to their microhabitat to avoid poor climatic conditions. While inactive, I assumed that salamanders retreated to 20 cm below the surface of granite boulders. I assumed this depth because it represents the maximum

depth at which temperatures approached average temperatures for a given month. I estimated damping depths based upon the typical properties of granite (Cho, Kwon, and Choi 2009). I then used the damping depth to determine the temperatures that salamanders experience during inactivity inside a granite boulder throughout the day. I assumed that salamanders were not able to forage during times of inactivity. Salamanders ceased activity upon reaching their dehydration threshold, experiencing temperatures beyond their preferred range, or during the daytime. I selected a range of dehydration thresholds (3.5%, 7%, and 10%) at which salamanders ceased activity based upon empirically-observed values for plethodontids (Feder and Londos 1984). Simulations were run in an iterative process with each dehydration threshold. I also ran simulations across various body sizes reflected in our physiological experiments (2 g, 3 g, 4 g), humidity scenarios (+25% and -25% value of VPD), and skin resistance to water loss (average $r_i = 7.8$ and maximum $r_i = 14.0$) to determine the sensitivity of our predictions to input parameters. I ran our simulations for every possible combination of body size, dehydration threshold, humidity scenario, and skin resistance to water loss to estimate activity budgets and energy balance throughout the year. These physiologically-derived layers were averaged together to integrate into the correlative framework.

Correlative Species Distribution Model

I used MaxEnt to assess correlations between climatic factors and presence data because it is known to perform as well or better than other tools during a comprehensive model evaluation (Elith et al. 2006). It is commonly used to generate distribution models

of climate vulnerability (Pearson et al. 2007; Loarie et al. 2008; Puschendorf et al. 2009; Bradley et al. 2010). I focused on the disjunct population of green salamanders (North Carolina, South Carolina, and Eastern Georgia). Recent genetic studies have revealed that this disjunct population, not including the Hickory Nut Gorge region of North Carolina, is an evolutionary significant unit from the mainland population (J. J. Apodaca, personal communication). To create the spatial boundaries of our model, I used minimum bounding geometry in ArcGIS based on known locality points for the species. I created a 25-km buffer around this disjunct range. I extrapolated data on green salamander movement and predicted that green salamanders could potentially to disperse ~15-km in 33 years if projecting to 2050 (Gordon 1952; Canterbury 1991; see Appendix A). The remaining 10-km accounts for the possibility that the current range extends beyond currently cataloged localities.

I collected green salamander presence data from the South Carolina Department of Natural Resources, Georgia Department of Natural Resources, North Carolina Wildlife Resources Commission and publically-accessible online databases (Price and Dorcas 2007; Cicero et al. 2010; USGS 2013). I also gathered new sites in South Carolina from a recent extensive habitat association survey (Chapter 1). All points were uploaded into ArcMap 10.3. I reduced clusters of points (and thus removing potential sampling biases such as repeated sampling from easily-accessible sites) by using a random point generator in ArcMap. Because the average north-south distance of rock outcrop of sites in South Carolina was 31-m (Chapter 1), I randomly removed points in clusters that were less than 31m apart.

I selected seven bioclimatic variables (BIO 1-2, 4, 8-9, 12, 15; Table 2.1), from a list of 19 (Hijmans et al. 2005) based on low pairwise correlations between variables. Although there is a high pairwise correlation value between variables BIO1 and BIO15 (0.74), I wanted to capture two dimensions of precipitation in the analysis. This approach was first used by Rissler and Apodaca (2007) for west coast congener species, *Aneides fahaka* and used has since been used several times to generate distribution models for amphibians (Milanovich et al. 2010; Barrett et al 2014; Sutton et al 2015). WorldClim derives these bioclimatic variables from a 30 year (1960-1990) dataset of monthly averages compiled of temperature and rainfall data at a spatial resolution of $\sim 1\text{km}^2$ (Hijmans et al. 2005). I intersected these climatic variables with both green salamander presence points and background points in ArcMap (ArcGIS 10.3.1, ESRI). I generated background points by randomly placing $\sim 2,000$ herpetofaunal presence points (*Plethodon yonhalosse*, *Plethodon teyahalee*, *Plethodon metcalfi*, *Plethodon jordani*, *Terrapene carolina*, *Chrysemy picta*, *Pantherophis obsoletus*, *Diadophis punctatus*, and *Storeria dekayi*) collectively distributed through the entire buffered disjunct range of the green salamander.

I used two different Global Climate Models (GCM), with one Representative Concentration Pathway (RCP) each. I downloaded two widely used GCM's from WorldClim: HadGEM2-CC (Hadley) and CCSM4 (CCSM). Model selection was based on hindcast accuracy in the northern hemisphere (Overland et al. 2011) and availability of projected data of the 8.5 RCP. I included two GCMs as the Hadley GCM tends to predict wetter future species distribution models while the CCSM4 GCM tends to predict dryer

future species distribution models (CIESIN 2000). I included the 8.5 RCP trajectory to provide a perspective representing rapid increase in greenhouse gas emission. MaxEnt produces species distribution models with climatic suitability (ranging from 0-1 representing low to high habitat suitability). I used two thresholds (strict and moderate) to generate distributional range shifts in projected suitable habitat within the disjunct range of green salamanders. I used the fixed cumulative value 10 (F10; a threshold resulting in 10% omission of training data), and the equal training sensitivity plus specificity (ETSP; threshold that balances the probability of missing suitable sites with the probability of assigning suitability to a site where the species is absent).

Integration of mechanistic and correlation models

I created a suite of climatic niche models for green salamanders under current and future climatic conditions. Both correlative and mechanistic data were used within an inductive, presence-only modeling approach MaxEnt (Phillips et al. 2006). Correlative-only models were built using only climatic variables, whereas our correlative + mechanistic models contained climatic variables and two experimentally-derived mechanistic layers: activity and energetic costs. I compared model predictions using a variety of methods. Firstly, I compared the number of cells containing suitable habitat that were lost or gained after mechanism was added to the correlative model. I then conducted a correlation analysis to see if environmental variables correlated with differences in predictive ability between model types. Lastly, I tested the null hypothesis that resistance to water loss was not different among temperature treatments using an

analysis of covariance (ANCOVA). To test the assumption of normality I used a Shapiro Wilk test and QQ plot. I found that one category of the data was significantly not normally distributed, however, after attempting multiple transformations on the data, I could not meet this assumption of normality but still proceeded with the analysis. I also evaluated the significance of the interaction between body mass and temperature on resistance to water loss, as an ANCOVA assumes an absence of such interaction. The interaction term was not statistically significant ($p=0.29$). I tested the differences between groups by using a Tukey test. I also evaluated the effect of the four temperature treatments on volume of oxygen consumption ($\mu\text{L}/\text{min}$) using linear regression.

RESULTS

The ANCOVA suggested that both temperature and body mass were significant (Table 2.2). At the highest temperature treatment (24°C), green salamanders increased their skin resistance to water loss, r_i (Fig 2.2). The results of the Tukey test demonstrated that the highest treatment was significantly different than the lower two treatments. There was no significant difference in r_i between the lower temperature treatments, 12°C and 18°C (Fig 2.2). Metabolic rates, illustrated by volume of oxygen consumption, increased with temperature treatments ($p < 0.0001$, Table 2.3, Fig 2.3). There was a small, but significant interaction between salamander mass and temperature ($p = 0.02$; Table 2.3), such that larger animals increased oxygen consumption at higher temperature more than smaller animals.

I developed 12 species distribution models for green salamanders in their disjunct range (Fig 2.4). The Hadley GCM models predicted the most suitable habitat followed by CCSM4 GCM models, and current models (Table 2.4). When mechanism was added to the current correlative model, there is virtually no change in the number of cells with suitable habitat (Table 2.4). On the other hand, mechanism reduces 79.9% of cells containing suitable habitat in the CCSM4 model and 44.7% of cells containing suitable habitat in the Hadley model (Table 2.4). Among all model runs, the F10 threshold predicted 1.94 ± 0.67 times more suitable habitat than the ETSP model.

For the three correlative only models, BIO4, BIO15 and BIO12 accounted for 43.6%, 22.2%, and 22.2% of the variation, respectively. For the three correlative + mechanistic models, BIO4, BIO15 and BIO12 accounted for 41.4%, 22.4%, and 20.4% of the variation, respectively. None of the environmental variables I evaluated (bioclimatic and elevation) correlated with the difference values between correlative + mechanistic and correlative-only models (with correlation values ranging from -0.15 to 0.15). That is, I were unable to identify any environmental conditions that would predict where one model type would differ from the other.

DISCUSSION

There have been multiple approaches described in the literature for forecasting climate change vulnerability, including correlative models and mechanistic models (Milanovich et al. 2010; Kearney et al. 2010; Barrett et al. 2014; Briscoe et al. 2016; Mathewson et al. 2017). Such models can be used to construct species distribution

models in order to predict climatically suitable habitat for potentially vulnerable species. Correlative models can be built for a variety of taxa because minimal amounts of data are required, however, they have a number of untested assumptions. For example, correlative models exclude mechanism so they do not account for an organism's biology or physiology. Also, correlative models are heavily reliant on the quality and spatial resolution of the environmental data (i.e. GCMs, RCPs) used, so it may be beneficial to evaluate multiple measures of habitat and used multiple scenarios for future forecasts. With the incorporation of animal performance, mechanistic models have been shown to change species distribution models, however, they can also be data intensive (Briscoe et al. 2016; Mathewson et al. 2017; Riddell et al. 2017). Our study evaluated the effect of incorporating mechanistic layers into a correlative species distribution model framework. Our results suggest that under current climatic conditions, correlative models and correlative + mechanistic models projected nearly congruent suitable habitat for green salamanders, while under future climatic conditions mechanism reduced suitable habitat for green salamanders (Table 2.4). Range-wide correlative models show some resiliency in parts of the disjunct range of the green salamander (Barrett et al. 2014); however, the addition of physiology in our species distribution models picked up on limitations of the species not captured by a correlative-only model. Nevertheless, in all model runs some suitable habitat is projected to remain for the species in 2050. Mechanistic models do not necessarily yield more restrictive forecasts for species when compared to current climatic conditions. Green salamanders may present an interesting case because of their association with rock outcrops, a behavior not shared with congener plethodontids

(Gordon 1952; Jaeger 1971; Mount 1975). Green salamanders may have interspecific aggregation adaptations within rock outcrops allowing them to be more resistant to harsh conditions (Cupp Jr. 2017), and additionally, keeping them from dispersing long distances. In other words, green salamanders may be able to tolerate the warmer and dryer temperatures of granite and may not need to disperse as much as other salamanders reliant on moist soil (Jaeger 1971).

The laboratory experiments demonstrated that green salamanders increase their skin resistance to water loss when exposed to higher temperatures. These finding may shed some light on Gordon's (1952) preliminary laboratory study, showing green salamanders were tolerant to warm and dry conditions. Green salamander metabolism increased with temperature which is consistent with data from other ectotherms (Bartholomew & Tucker 1963; Johnston & Dunn 1987).

In all models, the most variation was explained by BIO4 (temperature seasonality), BIO15 (precipitation seasonality), and BIO12 (annual precipitation). These results suggest that environmental seasonality and precipitation play a major role in green salamander distribution. Green salamanders have well-documented annual cycles, categorized into four stages: the breeding period (late May to late September), dispersal and aggregation (late September to November), hibernation (November to late April), and post-hibernation aggression and dispersal (late April to late May) (Gordon 1952). Because of these stages, it is likely that green salamanders have different seasonal requirements for environmental variables. Further, green salamanders are known to use and breed in moist rock crevices (Gordon 1952), and moisture appears to be a limiting

factor in the distribution of other *Aneides* species (Rosenthal 1957; Spickler et al. 2006; Haan et al. 2007). Interestingly, both the CCSM4 and the Hadley GCMs appear to be predicting a wetter future within the BRE (Table 2.5). These wetter projections may explain why I saw habitat gains in the future models when compared to current models. Additionally, correlative models may have done a poor job projecting outside of their range as it is likely that some values for the bioclimatic variables in the 2050 maps were not present in the current maps. In some cases, there were cells in the 2050 models with climatic values > 2 standard errors from the mean of the current values.

Knowing when to use a particular distribution modeling can contribute to cost effective conservation. Under current climate conditions, several studies for endotherms have demonstrated that correlative models can accurately predict a species distribution and show minimal changes when mechanistic layers were incorporated (Kearney et al. 2010; Briscoe et al. 2016; Mathewson et al. 2017). Our study provides further evidence that this approach is likely true for ectotherms as well (Buckley et al. 2010). Collectively, these studies suggest correlative-only models are probably sufficient for many species under current climate conditions. Justification for a correlative-only approach is valuable given the data-intensive nature of mechanistic models. On the other hand, there is evidence suggesting that mechanism may be important for future projections of species distributions models. Several studies have shown that mechanism-based forecasts diverge from those made when using correlative-based models (Briscoe et al. 2016; Mathewson et al. 2017). Our correlative + mechanistic model supports this trend and thus, I encourage the use of mechanism (whether physiology, behavior, etc.) in species

distribution models for projections assessing climate change vulnerability if resources are available. With that said, it is unclear whether or not mechanistic models are more accurate and so there may be an argument to use a more conservative (i.e. restrictive) threshold. Lastly, our models specifically looked at climatically suitable data only. In order to make management decisions about where to conserve green salamander habitat, it would be beneficial to incorporate landscape data (i.e. rocky outcrops, elevation) to make the most informed decisions.

I used a different approach to creating mechanistic models than others in the literature who have used Niche Mapper to create their models (Kolbe et al 2010; Briscoe et al. 2016; Mathewson et al. 2017). Niche Mapper is a program created specifically for mechanistic models, however, its final output models forecast fundamental niches of the animals because mechanistic models are independent of presence data (Kearney and Porter 2004; Porter and Mitchell 2006; Kolbe et al. 2010). The future forecasted correlative + mechanistic models I created with program MaxEnt diverged from purely correlative models which has been commonly found in other studies (Buckley et al. 2010; Kearney et al. 2010; Briscoe et al. 2016; Mathewson et al. 2017). MaxEnt forecasts models within a correlative framework and thus the output forecasts the realized niche of an animal (Kearney 2006; Kolbe et al. 2010). Here I suggest an alternative approach to mechanistic model-making that may narrow down an animal's fundamental niche to a realized niche. This approach could potentially solve problems with both mechanistic models (i.e. honing in on biotic processes that drive species presence) and correlative

models (i.e. incorporating organismal performance, estimating outside of the training data).

When Green Salamander physiology was incorporated into climatic suitability models, future forecasts predict that suitable habitat for Green Salamanders will be greatly reduced. According to the strict threshold models, the global circulation models are predicting much of the suitable habitat for Green Salamanders to be on both National Forest and state park land in 2050. However, some of the land predicted to be suitable for Green Salamanders is outside of the protection of public land. Land acquisition in areas predicted to have suitable Green Salamander habitat (but are outside of public lands) will help conserve this species for years to come. Periodic monitoring of occupied sites that fall outside of long-term forecasts of suitability may offer an early-warning signal related to climate vulnerability.

REFERENCES

- Araújo MB, Thuiller W, and Pearson RG. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* **33**: 1712-1728.
- Baillie JEM, Hilton-Taylor C, Stuart SN, editors. 2004. 2004 IUCN Red List of Threatened Species. A Global Species Assessment. IUCN, Gland, Switzerland and Cambridge, UK. xxiv + 191 pp.
- Barbraud C, Weimerskirch H. 2006. Antarctic birds breed later in response to climate change. *Proceedings of the National Academy of Sciences (USA)* **103**:6248–6251.
- Barrett K, Nibbelink NP, Maerz JC. 2014. Identifying priority species and conservation opportunities under future climate scenarios: Amphibians in a biodiversity hotspot. *Journal of Fish and Wildlife Management* **5**:282-297.
- Bartholomew GA, Tucker VA. 1963. Control of changes in body temperature, metabolism, and circulation by the agamid lizard, *Amphibolurus barbatus*. *Physiological Zoology* **xxxvi**: 199-218.
- Both C, Bouwhuis S, Lessells CM, Visser ME. 2006. Climate change and population declines in a long-distance migratory bird. *Nature* **441**:81–83.
- Bradley B, Wilcove D, Oppenheimer M. 2010. Climate change increases risk of plant invasion in the Eastern United States. *Biological Invasions* **12**: 1855–1872.
- Briscoe NJ, Kearney MR, Taylor C, Wintle BA. 2016. Unpacking the mechanisms captured by a correlative SDM to improve predictions of climate refugia. *Global Change Biology* **22**: 2425–2439.

- Brown JL, Li S, Bhagabati N. 1999. Long-term trend toward earlier breeding in an American bird: A response to global warming? *Proceedings of the National Academy of Sciences (USA)*, **96**: 5565-5569.
- Buckley LB, Urban MC, Angilletta MJ, Crozier LG, Rissler LJ, and Sears MW. 2010. Can mechanism inform species' distribution models? *Ecology Letters* **13**:1041–1054.
- Buckley LB. 2008. Linking traits to energetics and population dynamics to predict lizard ranges in changing environments. *American Naturalist* **171**:E1–E19.
- Butchart SHM, et al. 2010. Global biodiversity: Indicators of recent declines. *Science* **328**: 1164-68.
- Campbell GS, Norman JM. 1998. An introduction to environmental biophysics. *Springer*.
- Canterbury RA. 1991. Ecology of the Green Salamander, *Aneides aeneus* (Cope and Packard), in West Virginia. Master's Thesis, Marshall University, Huntington, WV.
- Carpenter KE, et al. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**: 560-563.
- Cho WJ, Kwon S, Choi JW. 2009. The thermal conductivity for granite with various water contents. *Engineering Geology* **107**: 167-171.
- Cicero C, et al. 2010. VertNet: an online reference Available at: <http://www.vertnet.org/index.html>. Accessed March 2017.
- CIESIN. 2000. Center for International Earth Science Information Network Climate Models. http://metroeast_climate.ciesin.columbia.edu/climodels.html Accessed

- June 2017.
- Cope ED, Packard AS. 1881. The fauna of the Nickajack Cave. *American Naturalist* **15**:877–882.
- Corser JD. 2001. Decline of disjunct green salamander (*Aneides aeneus*) populations in the southern Appalachians. *Biological Conservation* **97**:119–126.
- Cupp Jr. PV. 2017. *Aneides aeneus* (Green Salamander) interspecific aggression. *Herpetological Review* **48**: 153.
- Elith J, Kearney M, Phillips S. 2010. The art of modelling range-shifting species. *Methods in Ecology and Evolution* **1**: 330-342.
- Elith J, et al. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**:129–151.
- Füssel HM, Klein RJT. 2006. Climate change vulnerability assessments: an evolution of conceptual thinking. *Climatic Change* **75**: 301–329.
- Gordon RE. 1952. A contribution to the life history and ecology of the plethodontid salamander *Aneides aeneus* (Cope and Packard). *The American Midland Naturalist* **47**: 666-701.
- Haan SS, Desmond MJ, Gould WR, Ward Jr JP. 2007. Influence of habitat characteristics on detected site occupancy of the New Mexico endemic Sacramento Mountains salamander, *Aneides hardii*. *Journal of Herpetology* **41**: 1-8.
- Hardman RH. 2014. Modeling occurrence of the green salamander, *Aneides aeneus*, in the Blue Ridge Escarpment. Master's Thesis. Western Carolina University, Cullowhee, NC.

- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.
- Jaeger RG. 1971. Moisture as a factor influencing the distributions of two species of terrestrial salamanders. *Oecologia* **6**: 191-207
- Johnston IA, Dunn J. 1987. Temperature acclimation in ectotherms with particular reference to teleost fish. *Symposia of the Society for Experimental Biology* **41**: 67-93.
- Kearney MR, Wintle BA, Porter WP. 2010. Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. *Conservation Letters* **3**: 203-213.
- Kearney M, Porter WP. 2004. Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* **85**: 3119–3131.
- Kearney M. 2006. Habitat, environment, and niche: what are we modeling? *Oikos* **115**: 186-191.
- Kolbe JJ, Kearney M, Shine R. 2010. Modeling the consequences of thermal trait variation for the cane toad invasion of Australia. *Ecological Applications* **20**:2273–2285.
- Kusano T, Inoue M. 2008. Long-term trends toward earlier breeding of Japanese amphibians. *Journal of Herpetology* **42**: 608-614.
- Lipps Jr. GJ 2005. A framework for predicting the occurrence of rare amphibians: a case study with the green salamander. Master's Thesis. Bowling Green State

- University, Bowling Green, OH.
- Loarie SR, Carter BE, Hayhoe K, McMahon S, Moe R, Knight CA, Ackerley DD. 2008: Climate change and the future of California's endemic flora. *PLoS ONE* **3**: e2502.
- Mathewson PD, Moyer-Horner L, Beever EA, Briscoe NJ, Kearney M, Yahn JM, Porter WP. 2017. Mechanistic variables can enhance predictive models of endotherm distributions: the American pika under current, past, and future climates. *Global Change Biology* **23**: 1048-1064.
- Milanovich JR, Peterman WE, Nibbelink NP, Maerz JC. 2010. Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. *PLoS ONE* **5**: e12189.
- Mount RH. 1975. *The Reptiles and Amphibians of Alabama*. Alabama, United States of America.
- Overland JE, Wang M, Bond NA, Walsh JE, Kattsov VM, Chapman WL. 2011. Considerations in the selection of global climate models for regional climate projections: The Arctic as a case study. *Journal of Climate* **24**: 1583–1597.
- Pearson RG, Raxworthy CJ, Nakamura M, Townsend Peterson A. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34**: 102–117.
- Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modeling* **190**: 231-259.
- Porter WP, Mitchell JW. 2006. Method and system for calculating the spatial-temporal effects of climate and other environmental conditions on animals.

- <https://www.google.com/patents/US7155377>
- Price SJ, Dorcas ME. 2007. Carolina Herp Atlas <https://www.carolinaherpatlas.org/>. Accessed March 2017.
- Puschendorf R, Carnaval AC, VanDerWal J, Zumbado-Ulate H, Chaves G, Bolaños F, Alford RA. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions*. **15**: 401-408.
- Riddell EA, Apanovitch E, Odum J, Sears MW. 2017. Physical calculations of resistance to water loss improve predictions of species range models. *Ecological Monographs* **87**: 21-33.
- Riddell EA, Sears MW. 2015. Geographic variation of resistance to water loss within two species of lungless salamanders: implications for activity. *Ecosphere* **6**: art 86.
- Rissler LJ, Apodaca JJ. 2007. Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology* **56**: 924–942.
- Roach NS, Hunter EA, Nibbelink NP, Barrett K. 2017. Poor transferability of a distribution model for a widespread coastal marsh bird in the southeastern United States. *Ecosphere* **8**:e01715.
- Rosenthal GM. 1957 The role of moisture and temperature in the local distribution of the plethodontid salamander *Aneides lugubris*. *University California Publications in Zoology* **54**: 371-420.
- Snyder DH. 1983. The apparent crash and possible extinction of the green salamander,

- Aneides aeneus*, in the Carolinas. Association of Southeastern Biologists Bulletin **30**:82.
- Spickler JC, Sillett SC, Marks SB, Welsh Jr. HH. 2006. Evidence of a new niche for a North American salamander: *Aneides vagrans* residing in the canopy of old-growth redwood forest. Herpetological Conservation and Biology **1**: 16-26.
- Sutton WB, Barrett K, Moody AT, Loftin CS, deMaynadier PG, Nanjappa P. 2015. Predicted changes in climatic niche and climate refugia of conservation priority salamander species in the northeastern United States. Forests **6**: 1–26.
- Urban MC. *et al.* 2016. Improving the forecast for biodiversity under climate change. Science **353**: pii: aad8466.
- USGS. 2013. Biodiversity Information Serving Our Nation (BISON) <https://bison.usgs.gov/#home>. Accessed on March 2017.
- Whitford WG, Hutchison VH. 1967. Body size and metabolic rate in salamanders. Physiological Zoology **40**:127–133.

Table 2.1—List of bioclimatic variables (www.worldclim.org/bioclim) used in all species distribution models for green salamander (accessed in 2017).

Variable	Definition
BIO 1	Annual mean temperature
BIO 2	Mean diurnal range (mean of monthly (max temp - min temp))
BIO 4	Temperature seasonality (standard deviation * 100)
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 12	Annual precipitation
BIO 15	Precipitation seasonality (coefficient of variation)

Table 2.2—Results of ANCOVA testing the effects of temperature and green salamander body mass on skin resistance to water loss (r_i).

Variable	Df	SS	MS	F	p
Body mass	1	67.27	67.27	35.57	<0.005
Temperature	2	334.80	167.40	88.50	<0.005

Table 2.3—Analysis of deviance table (Type II Wald F test with Kenward-Roger degrees of freedom) of the mixed effects model on the influence of log mass and temperature on volume of oxygen consumption (VO_2).

Predictor	<i>F</i>	<i>df</i>	<i>fixed effect</i>	<i>SE</i>	<i>p</i>
log mass	16.6	1	-0.0240	0.311	<0.0001
temperature	367	1	0.0329	0.006	<0.0001
log mass x temperature	5.14	1	0.0397	0.017	0.02

df = degrees of freedom, *SE* = standard error

Table 2.4—The amount of suitable habitat predicted for the two distribution model types applied to green salamanders in the Blue Ridge Mountains and the difference between model predictions. The correlative models only included climatic variables, and correlative + mechanistic model included the climatic variables plus two experimentally-derived layers of physiological performance. I used our strict threshold, equal training sensitivity plus specificity (ETSP), to compare habitat suitability amongst current and future models.

Model	Correlative	Correlative +	Cells	Habitat
		Mechanistic	Gained/Lost	Gained/Lost (ha)
Current	682	575	-107	-9.63
CCSM4	998	3	-995	-89.55
Hadley	3,566	110	-3,456	-311.04

Table 2.5—Comparison of average current and future temperature and precipitation variables from the modeled extent of green salamanders in the Blue Ridge Mountains.

Variable	Current Bioclim Average (±SE)	CCSM4 Bioclim Average (±SE)	Hadley Bioclim Average (±SE)
Temperature			
seasonality			
(standard deviation)	70.42 ± 58.28	73.51 ± 58.05	76.35 ± 55.46
(°C)			
Annual			
precipitation	1758.90 ± 99.38	1928.51 ± 109.75	1866.33 ± 105.78
(mm)			
Precipitation			
seasonality			
(coefficient of variation)	7.47 ± 0.89	9.30 ± 1.22	6.54 ± 1.27
(mm)			

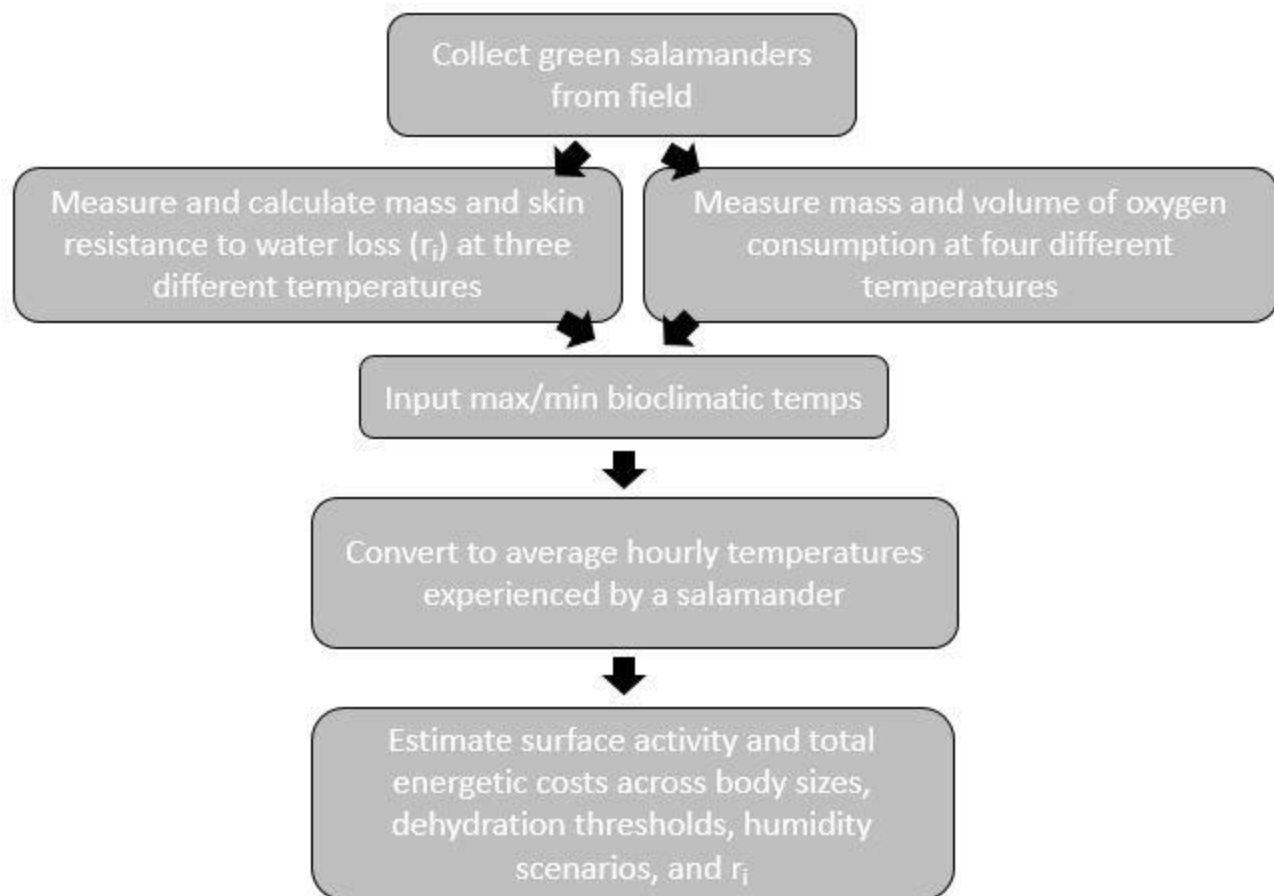
FIG 2.1 —Flowchart illustrating the inputs, process, and outputs of mechanistic layers (activity and energetic costs).

FIG 2.2 —Skin resistance to water loss of green salamanders at three different treatment temperatures (°C).

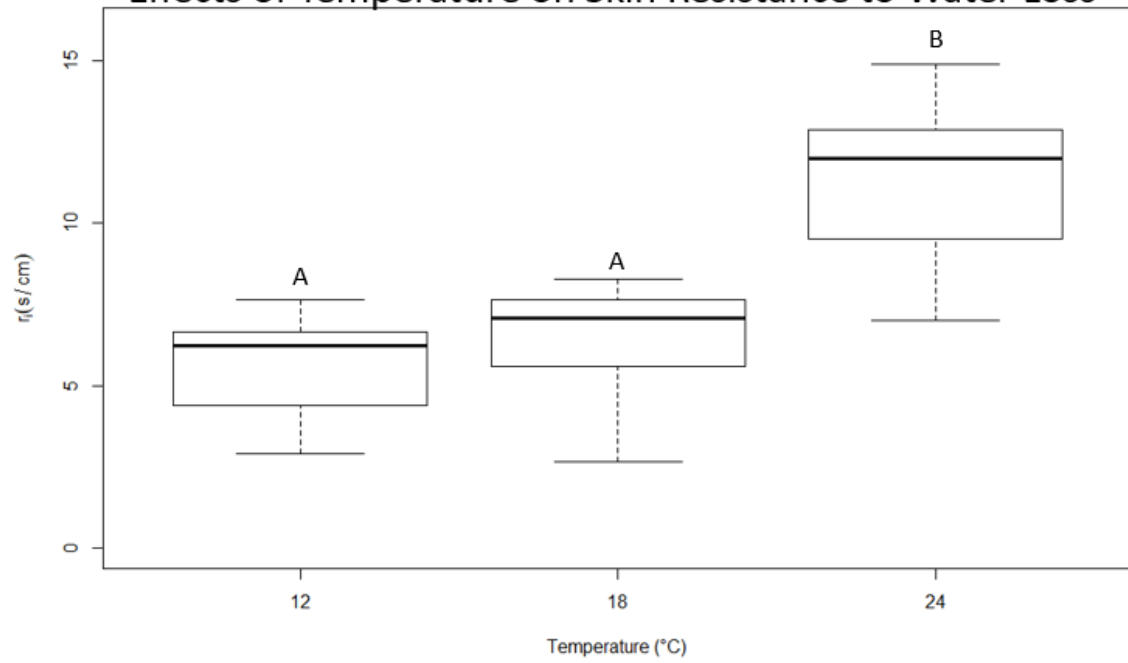
FIG 2.3 —Volume of oxygen consumption for green salamanders at four different treatment temperatures (°C).

Fig 2.4— Projected suitable habitat for green salamanders throughout their disjunct range for current and 2050 different climatic scenarios. Models predict suitable habitat for green salamanders under different scenarios by year (current or 2050), global circulation models (Hadley or CCSM4), one Representative Concentration Pathway (RCP 8.5), and model threshold (F10 and ETSP). A) The disjunct range of green salamanders located in North Carolina, South Carolina, and Georgia, USA. B) Species distribution models categorized by current or future climatic envelopes and then broken down by threshold

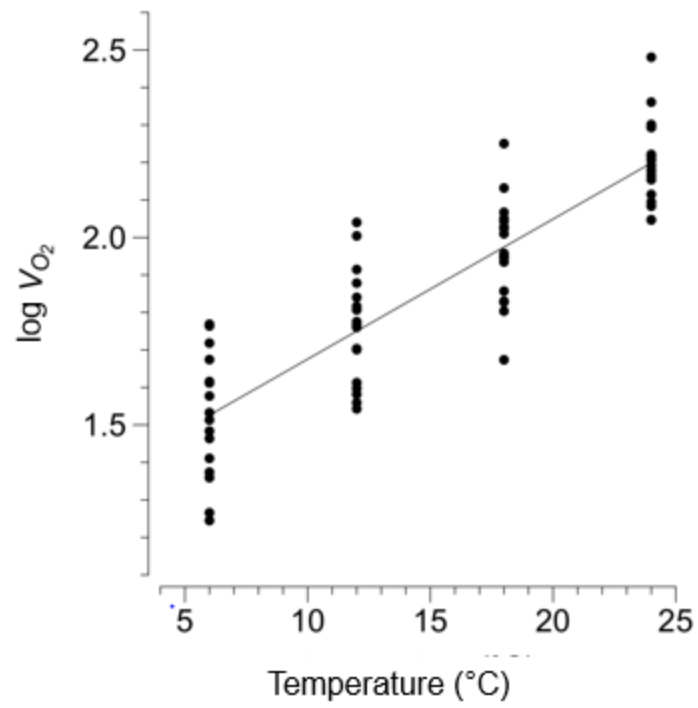
(moderate or strict). Light blue represents suitable habitat in the correlative only model, orange represents suitable habitat in the correlative + mechanistic model, dark blue represents overlapping suitable habitat by the two models (correlative and correlative + mechanistic), and black represents unsuitable habitat.



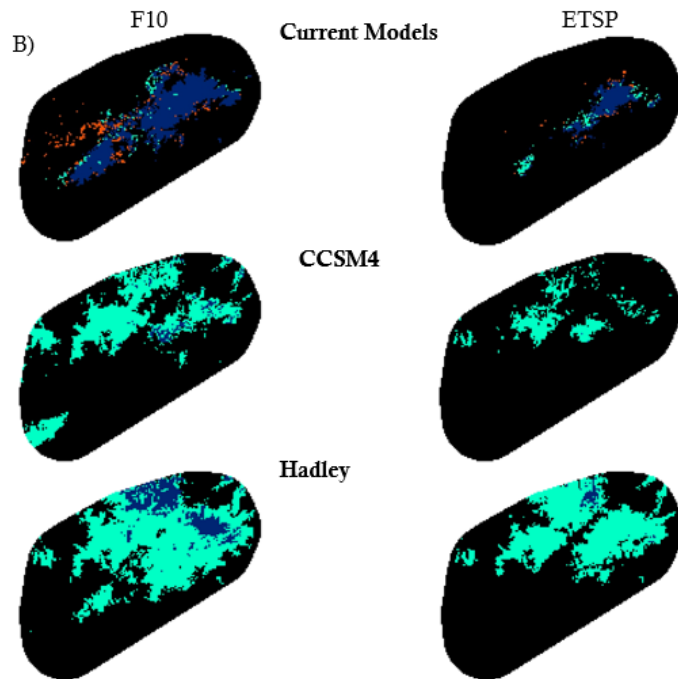
Effects of Temperature on Skin Resistance to Water Loss



Effects of Temperature on Volume of Oxygen Consumption



A)



APPENDICES

Appendix A

Estimated Green Salamander Dispersal for the Year 2050

Source	Movement (m)	Time(d)	Avg_move_day (m)	Avg_move_yr (m)	Avg_move_33yr (m)	Avg_move_33yr (km)
Gordon 1952	76.2	19	4.010526316	1463.842105	48306.78947	48.30678947
Gordon 1952	76.2	26	2.930769231	1069.730769	35301.11538	35.30111538
Gordon 1952	91.4	91.4	2.995076586	1093.202954	36075.69748	36.07569748
Gordon 1952	0.6	26	0.023076923	8.423076923	277.9615385	0.277961538
Gordon 1952	0.9	273.75	0.003287671	1.2	39.6	0.0396
Gordon 1952	1.8	10	0.18	65.7	2168.1	2.1681
Gordon 1952	0.6	21	0.028571429	10.42857143	344.1428571	0.344142857
Gordon 1952	0.6	18	0.033333333	12.16666667	401.5	0.4015
Gordon 1952	0.6	28	0.021428571	7.821428571	258.1071429	0.258107143
Gordon 1952	3.7	21	0.176190476	64.30952381	2122.214286	2.122214286
Gordon 1952	0.6	1	0.6	219	7227	7.227
Canterbury 1991	25.6	182.5	0.140273973	51.2	1689.6	1.6896
Canterbury 1991	6.4	334.584	0.019128231	6.98180427	230.3995409	0.230399541
Canterbury 1991	7.6	8	0.95	346.75	11442.75	11.44275
Canterbury 1991	46.3	35	1.322857143	482.8428571	15933.81429	15.93381429
Canterbury 1991	46.3	243.334	0.190273451	69.44980973	2291.843721	2.291843721
Canterbury 1991	33.8	60.8334	0.555615829	202.7997778	6692.392666	6.692392666
Canterbury 1991	6.4	14	0.457142857	166.8571429	5506.285714	5.506285714
Canterbury 1991	27.7	14	1.978571429	722.1785714	23831.89286	23.83189286
Canterbury 1991	16.8	4	4.2	1533	50589	50.589
Canterbury 1991	49.4	10	4.94	1803.1	59502.3	59.5023
					AVERAGE TOTAL	14.77297652