THE TENNESSEE JOURNAL OF HERPETOLOGY

VOLUME 4, 2021

TENNESSEE HERPETOLOGICAL SOCIETY

ISSN 2576-9340

The Tennessee Journal of Herpetology was founded to provide an avenue of conversation about amphibians and reptiles of Tennessee. Therefore, the Tennessee Journal publishes articles and notes about amphibians, reptiles, and the people who study them.

Find more information about the journal and how to submit at:
https://www.tnherpsociety.org/tennessee-journal-of-herpetology

The Tennessee Herpetological Society is a not for profit organization dedicated to promoting the study and conservation of reptiles and amphibians worldwide, but particularly within Tennessee. We believe this can be accomplished through the education of the general public and the hard work of our members. Find out more about us at: www.tnherpsociety.org

Cover Photo by Saunders Drukker

THE TENNESSEE JOURNAL OF HERPETOLOGY

CONTENTS 2021 VOLUME 4

LANDSCAPE SELECTION OF GREEN SALAMANDERS, *ANEIDES AENEUS*, AND A MODEL OF THEIR POTENTIAL DISTRIBUTION TO GUIDE FUTURE SURVEYS IN TENNESSEE

Dustin B. Thames[a,*], Josh R. Campbell[a], Christopher S. Ogle[b], Daniel R. Istvanko[c]

[a] *Tennessee Wildlife Resources Agency, 5105 Edmondson Pike, Nashville TN USA.*
[b]*Tennessee Wildlife Resources Agency, 3030 Wildlife Way, Morristown, TN USA.*
[c]*Tennessee Wildlife Resources Agency, 464 Industrial Blvd., Crossville, TN USA.*
[*]*Corresponding Author: dustin.thames@tn.gov*

Abstract.— Green salamander, *Aneides aeneus,* populations have declined in parts of their geographic range. In response to these declines, the United States Fish and Wildlife Service has been petitioned to protect the species under the Endangered Species Act. Additional information is needed on the geographic distribution and the ecological requirements of green salamanders in Tennessee to aid regulatory decision making and conservation management. The objectives of our research were to determine what environmental characteristics best predict landscape suitability for green salamanders and to create a map of the model to guide future surveys. We compiled green salamander occurrence records from the Tennessee Wildlife Resources Agency's State Wildlife Action Plan database and from researchers currently conducting research. We used maximum entropy modeling to evaluate the relative importance of environmental characteristics across the landscape and we projected the model into geographic space to map the potential distribution of green salamanders in Tennessee. Environmental variables associated with rock outcroppings, tree canopy cover, and elevation contributed most to the model. Green salamanders select areas on the landscape with rocks at the soil surface, but not necessarily bedrock, with a high percent canopy cover at moderate elevations. Rocks at the soil surface likely represent large rocks and boulders deposited on the landscape by geological processes that are not parent material. The model can be used to guide future surveys and may make surveys more efficient at locating new populations in Tennessee. Accumulating up-to-date occurrence records will enable managers to better assess the current distribution of green salamanders in Tennessee and may improve conservation efforts.

Key Words.—conservation, ecological niche modeling, habitat suitability, occurrences

World-wide amphibian populations are declining at an alarming rate. Of the approximately 5,743 species of described amphibian, an estimated 32.5% are globally threatened, endangered, or extinct. Data is insufficient to assess the conservation status of 22.5% of the world's amphibians (Stuart et al. 2004). Green salamander (*Aneides aeneus*) populations, at least in Tennessee, arguably fall into this data insufficient category due to the cryptic nature of the species and a lack of large scale research in the state.

Green salamanders are a species of plethodontid salamander that range from southern Pennsylvania to northern Mississippi, with disjunct populations occurring in southern Indiana and along the Blue Ridge Escarpment in South Carolina, North Carolina, and Georgia (Petranka 1998). Green salamanders are imperiled in 10 of the 13 states where they occur and have declined substantially in the disjunct populations along the Blue Ridge Escarpment (Corser 2001, Waldron and Humphries 2005). Declines here are partially attributed to habitat loss caused by logging of old-growth forests (Wilson 2003). Declines have also been attributed to over collecting, epidemic disease, and climate change (Corser 2001). In 2012 the United States Fish and Wildlife Service (USFWS) was petitioned to protect green salamanders under the Endangered Species Act (Center for Biological Diversity 2012). A 90-day finding conducted by the USFWS determined that listing may be warranted and the species is currently under a status review (USFWS 2019).

In Tennessee, green salamanders are primarily found in the Eastern Highland Rim, the Cumberland Plateau, the Cumberland Mountains, and the northern Ridge and Valley physiographic regions. Sandstone bluffs along the margins of the Cumberland Plateau in Tennessee appear to be particularly important landscape features for green salamanders and are where many occurrences have been documented in the state (Tennessee Wildlife Resources Agency 2005). However, type of rock (i.e. limestone, sandstone, dolomite, etc.) has been determined to not be as important as the presence of suitable crevices (Gordon and Smith 1949).

Green salamanders are most often found in moist, narrow crevices, ≤2cm in width on average, along shaded rock outcroppings and on tree trunks near rock outcroppings (Gordon and Smith 1949, Wyatt 2010, Niemiller and Reynolds 2011). At Catoosa Wildlife Management area in Tennessee, the best predictor of green salamander presence was length of the crevice, which was a reflection of the size of the rock outcrop (Wyatt 2010). Green salamanders selected the longest available crevices. Also, there was a strong positive correlation between green salamander presence and slope below the rock outcropping and number saplings around the outcropping (Wyatt 2010). In plethodontid salamanders respiration is primarily cutaneous and they have high rates of evaporative water loss (Peterman et al. 2013). Plethodontid salamanders often select areas with specific microclimates to avoid desiccation (Wells 2007). Steep slopes below the outcroppings are indicative of ravine type landscapes that typically have cool, moist microclimates due to the terrain blocking insolation. Saplings around the outcroppings provide shade and further maintain a cool and moist microclimate (Wyatt 2010).

While several studies have examined selection of rock outcroppings and crevices by green salamanders, few have examined selection at a broader scale across the landscape. A species distribution model (SDM) conducted in North Carolina found that green salamanders select certain soil types, shallow soils, and areas with a high percent canopy cover at intermediate elevations (Hardman 2014). Shallow soils are indicative of exposed bedrock and the species is considered a rock crevice specialist, so this result corroborates other research (Smith et al. 2017). However, soil type, the most robust predictor of landscape suitability, did not correlate with exposed bedrock, so soil characteristics other than depth appear to be an important landscape attribute for green salamanders in North Carolina. It is unclear what these characteristics may be, and the author doesn't speculate. However, soil pH and moisture have been demonstrated to impact the distribution and abundance of *Plethodon cinereus* (Wyman and Hawksley-Lescault 1987, Sugalski and Claussen 1997). There is potential that these soil characteristics could also impact green salamanders. High percent canopy cover suggests that green salamanders are selecting forested areas with dense shade and cool microclimates (Hardman 2014).

Accurate knowledge of species occurrence is essential for conservation management and regulatory decision making. For cryptic species like green salamanders, surveys aimed at documenting new occurrences are labor intensive and require adequate funding. Species distribution modeling (SDM) is a technique that uses known occurrence records, occasionally absences, and environmental data to predict the distribution of a species in geographic space. The technique has been used successfully to guide sampling effort and can ultimately make documenting new occurrences or new populations more efficient for cryptic or rare species (Rebelo and Jones 2010; Olatz et al. 2015; Fois et al. 2018). Hardman (2014) was successful in using SDMs to find new populations of green salamanders in North Carolina and the results of our research can be used to guide future survey efforts in Tennessee and will potentially increase the efficacy of the searches. With more up-to-date occurrences and the discovery of new populations, managers and regulators will have an improved knowledge of the species' actual distribution on the landscape. This can improve their ability to identify areas on the landscape where recovery efforts will be most effective and efficient

The objectives of this research were to use species distribution modeling to determine what features on the landscape in Tennessee best predict the presence of green salamanders and to create a map of the potential distribution of green salamanders to guide future survey efforts. The potential distribution represents areas on the landscape that are environmentally suitable for the species, whereas the actual distribution depends on factors such as competition, predation, and barriers to dispersal that are not accounted for in the modeling process. Based on a SDM of green salamanders from western North Carolina and the ecology of the species, we hypothesized that soil type, percent of tree canopy cover, elevation, and depth of soil to bedrock would be the primary environmental predictors of landscape suitability for green salamanders in Tennessee (Hardman 2014).

METHODS AND MATERIALS

To model the potential distribution of green salamanders in Tennessee, we compiled occurrence records from the Tennessee Wildlife Resources Agency's State Wildlife Action Plan database and from researchers currently conducting surveys. As a precautionary measure, we excluded occurrences documented before 2004. This year was selected as a cut-off because the majority of occurrences in the database were documented in 2004 or after. Loss of forest cover can negatively impact green salamander populations (Wilson 2003), so including historic occurrences where logging has occurred would adversely affect model performance. We used recent occurrences assuming they are from extant populations. Because most occurrences between these years were documented on standardized surveys of transects, we used the SDMtoolbox (Brown et al. 2017) in ArcMap (ArcMap ver. 10.6.1, ESRI, Redlands, CA, USA) to spatially filter or thin the occurrences to minimize the risk of spatial autocorrelation problems in the model. The minimum distance allowed between occurrences was set at 500 m.

We used maximum entropy modeling with presence-only data using Maxent software (Phillips et al. 2006) to model the potential distribution of green salamanders in Tennessee. Maxent uses a machine learning algorithm to compare cells in environmental raster files with occurrence records to randomly selected cells in the background without occurrences to estimate a suitability score for each cell in the grid (Phillips 2009). We selected the logistic output that assigns each cell in the grid a value between 0 (least suitable) and 1 (most suitable). In the model occupied cells were compared with 10,000 unoccupied cells in the background.

We employed a 10-fold cross validation technique in which the occurrence records are randomly partitioned into 10 equal subsets. The model is run 10 times (folds) using a different subset of the sample in each run to test the model. In this technique each occurrence record is used to both test the model and to fit the model. The technique gives an estimate of errors in the predictive performance and fitted functions of the model (Taylor et. al 2017). Maps of the model were created using the average of the model runs.

In total, eight environmental predictor variables were used in the models and acquired as raster files with a 250 m spatial resolution. The variables are related to rock outcrop habitat and microclimate and were chosen to closely replicate the SDM in North Carolina by Hardman (2014). ArcMap was used to align the cells of each raster file and they were clipped to the extent of our study area, which is the state of Tennessee. We attained a digital elevation model at 250 m resolution and used ArcMap to derive aspect and percent slope (U.S. Geological Survey and National Geospatial-Intelligence Agency 2010). We transformed aspect to northness and eastness to make it a continuous variable by first converting degrees to radians. Then for northness we calulacted the cosine of each radian and the sine of for eastness. For northness the result is a continuous variable with 1 being north and -1 being south. For eastness the result is a continuous variable with 1 being east and -1 being west. Soil type, depth of soil to bedrock, and coarse soil fragments at the surface of the soil were acquired from the International Soil Resource and Information Center (Batjes 2012). The soil type dataset is a model of the most likely soil class from the World Reference Base for Soil Resources classification system (International Union of Soil Scientists Working Group 2015). Depth of soil to bedrock is the predicted depth (cm) of soil overlaying bedrock parent material. Coarse soil fragments is the predicted volumetric percent of rock fragments >2mm in diameter at the surface of the soil including rocks and boulders. Finally, percent tree canopy cover was downloaded for both 2004 and 2016 (Dimiceli et al. 2015). Tree canopy cover for 2016 was the most recent year available when the SDM was being created. We used ArcMap to average tree canopy cover for the two years and the resulting raster with averaged values was used in the model to match as close as possible the temporal resolution of the occurrence data. These eight variables were selected to allow us to compare our model results with the results from a SDM in North Carolina (Hardman 2014). Pearson correlations between environmental variables were acceptable ($r \leq 0.72$), so collinearity was assumed to not be an issue in the model.

We used area under the curve (AUC) statistic of the receiver operating characteristic (ROC) averaged across each model run to assess the performance of the model. An AUC of 0.5 represents a model that performs no better than random and an AUC of 1.0 represents a model with excellent predictive ability. We also examined the average omission rate, which is the percentage of localities with an occurrence that the model predicts as unsuitable. To define unsuitable, we made the model binomial (i.e. unsuitable or suitable) based on a defined suitability threshold. We examined omission rates of the test data subsets at two suitability thresholds: minimum training presence and 10 percentile training presence (Taylor et. al 2017). The minimum training threshold is the lowest suitable value assigned to a training occurrence. The 10 percentile training presence is the suitability value that excludes 10 percent of the training occurrences with the lowest suitability scores.

RESULTS

We compiled 213 green salamander occurrence records (Fig. 1). After spatially rarifying the records, we ended up with 79 occurrences to build the model. The average AUC for the test subsets of the model was 0.93

± 0.02. Based on this threshold independent measure of model performance, the model has good predictive ability. The average minimum training threshold of the training data was 0.03 and the omission rate of the test data was 0.01 ± 0.05. The average 10 percentile training presence was 0.23 and the omission rate was 0.019 ± 0.15. Overall, the omission rates are relatively low and cross-validation suggests the model has strong discriminatory power.

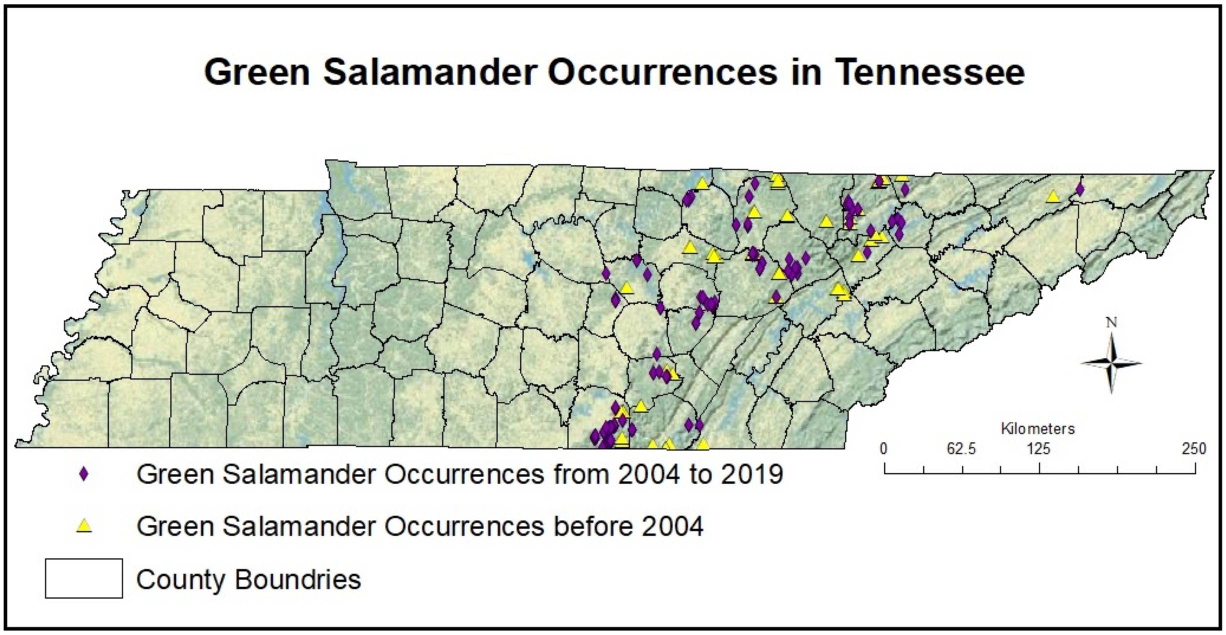


FIG. 1. Green salamander occurrence records in Tennessee from Tennessee Wildlife Resources Agency's State Wildlife Action Plan database and from researchers currently conducting research.

TABLE 1. The table displays environmental variables, and average percent contribution and standard deviation of each variable to landscape suitability for green salamanders in Tennessee across 10 cross-validated model runs.

Variable	Percent Contribution	SD
Coarse Soil Fragments	42.8	4.87
Percent Canopy Cover	16.6	4.63
Elevation	15.7	2.28
Depth of Soil to Bedrock	12.8	1.98
Soil Type	6.2	1.57
Eastness	2.4	0.46
Slope	1.8	0.56
Northness	1.6	0.39

The variable coarse soil fragments, measured in volumetric percent at the soil surface, contributes most to the model at 42.8% followed by percent canopy cover at 16.6 %. Elevation, measured in m above sea level, contributed 15.7% and depth of soil to bedrock, measured in cm, contributed 12.8% (Table 1). Landscape suitability for green salamanders has a strong positive correlation with coarse soil fragments at the soil surface up to approximately 15% coarse fragments by volume and then suitability drops slightly or plateaus (Fig. 2). Tree canopy cover also has a positive relationship with suitability up to approximately 68% then suitability drops substantially. This drop in suitability likely reflects availability. Only 2.5% of the cells in the dataset have over 77.6% tree canopy cover

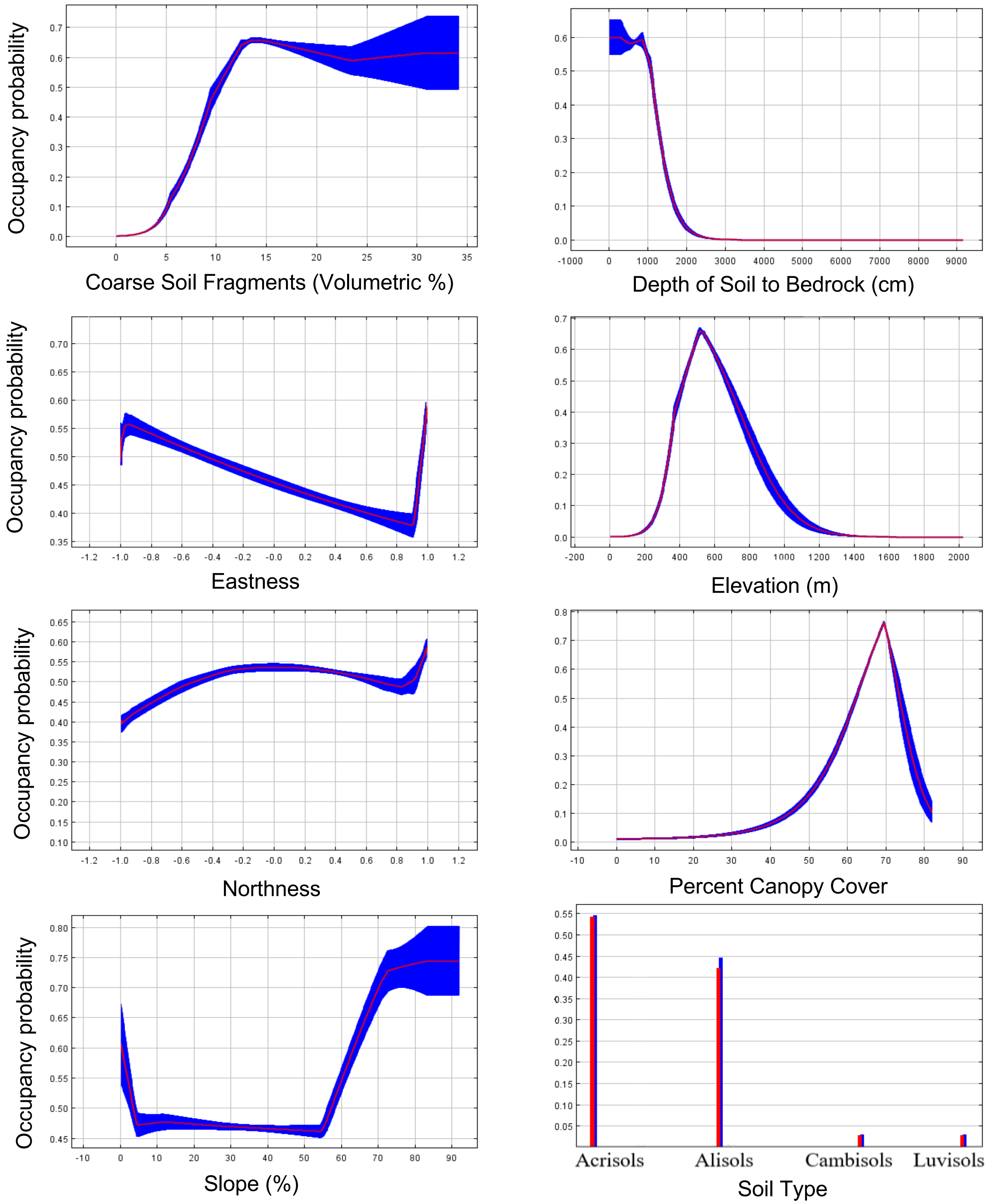
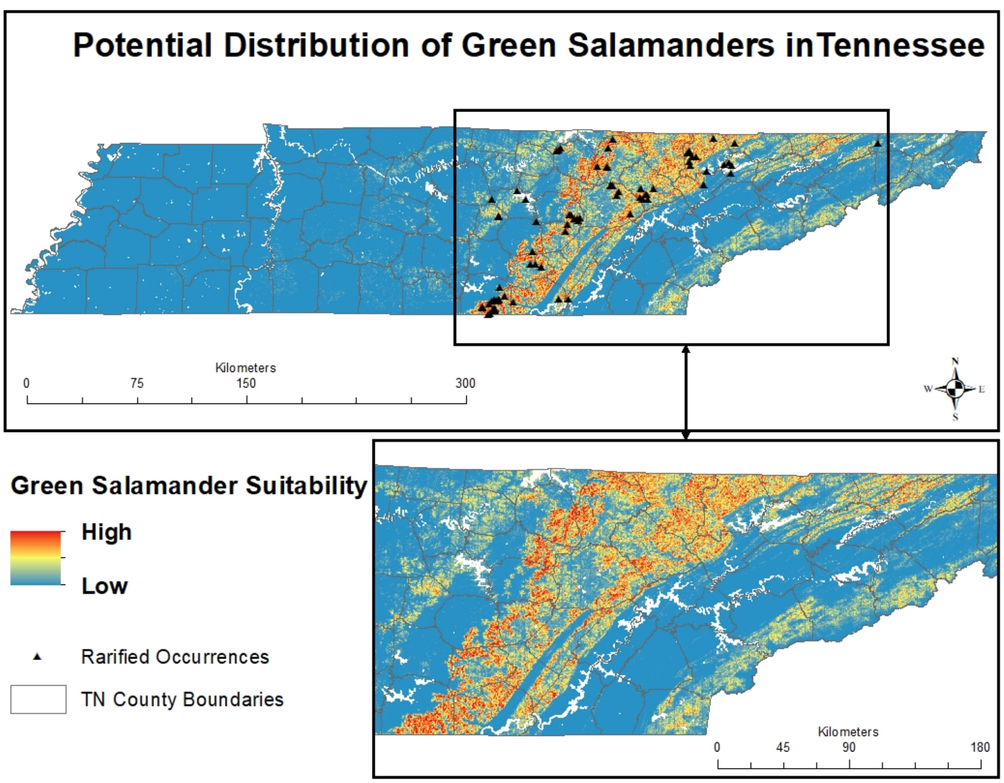


FIG. 2. Graphs showing the response of landscape suitability (y-axis) for green salamanders to changes in values of each response variable (x-axis) in the absence of other variables. The red line in the response curve represents the average from 10-fold cross-validation and the blue area is ± 1 SD.

and the maximum is 82%. Landscape suitability peaks at approximately 500 m in elevation. The elevation of the Cumberland Plateau ranges between 457 m and 549 m and sandstone bluffs generally line the margins of the plateau (Tennessee Wildlife Resources Agency 2005). Many of the occurrences in Tennessee are along the margins of the Cumberland Plateau (Fig. 2) and the relative of importance of elevation suggests this is an important landscape feature for green salamanders. However, the variables elevation and coarse soil fragments had the strongest correlation of the environmental variables (r= 0.72), so the importance of elevation may have a slight influence from this correlation. Depth of soil to bedrock has a strong negative correlation with suitability. Suitability is highest between approximately 0 cm and 300 cm soil depth to bedrock and drops precipitously with deeper soils. Surprisingly, soil type contributed relatively little to the model at 6.2%.

The Cumberland Plateau and the Cumberland Mountains physiographic regions had the largest area of high suitability based on the model results (Fig. 3). The western margin of the Cumberland Plateau is characterized by expansive sandstone bluffs and this area was identified as highly suitable generally from the southern border to the northern border of Tennessee. The northern reaches of the Ridge and Valley physiographic region also have areas with high environmental suitability. Somewhat surprisingly, even with a complete lack of occurrence records, areas along the Blue Ridge Mountains were identified as suitable.

FIG. 3. The results of a species distribution model with landscape suitability for green salamanders displayed as a continuous field projected into geographic space with the occurrences records used to build the model.



DISCUSSION

Our model of the potential distribution of green salamanders in Tennessee indicates that areas with rocks at the soil surface, not necessarily bedrock, and a high amount of tree canopy cover at moderate elevations are important landscape features for the species in Tennessee. Model results support our hypothesis that tree canopy cover and elevation would be important variables in the model. However, contrary to our hypothesis, depth of soil to bedrock and soil type contributed less to the model than expected and coarse soil fragments contributed more than expected.

In a SDM for green salamanders in North Carolina, soil category was the greatest contributing environmental variable, contributing more than depth of soil to bedrock. Hardman (2014) suggests that soil attributes other than depth may be important in their study area but doesn't speculate what the attributes could be. These soil attributes were possibly less important in Tennessee due to the abundance and connectivity of rock outcroppings along the Cumberland Plateau that are generally lacking along the Blue Ridge Escarpment. It should be noted that our model and the North Carolina model used different soil category datasets from different sources and this could explain why soil type contributed less in our model. Additionally, Hardman (2014) didn't include coarse soil fragments at the soil surface and inclusion of this variable in the model could have decreased the contribution of soil category. Hardman (2014) speculated that depth of soil to bedrock would be a more important variable along the Cumberland Plateau where expansive bluff lines are abundant and we found little evidence for this in our model. The presence of coarse soil fragments on the soil surface was a more robust predictor of suitability.

An expansive area along the Blue Ridge Mountains in Tennessee was identified as suitable in our model. The last green salamander occurrence in this physiographic region in Tennessee was documented in the Great Smoky Mountains National Park in 1929 (Neimiller and Reynolds 2011). Green Salamanders have declined dramatically in the near-by disjunct populations of the Blue Ridge Escarpment in North Carolina, South Carolina, and Georgia (Corser 2001). If populations of green salamanders did exist in the Blue Ridge Mountains of Tennessee historically, it's likely that dramatic declines occurred here as well. Of course, our model does not account for biotic interactions (i.e. competition and predation) and the species may have never been abundant in the Blue Ridge Mountains because of these interactions. Still, this model could direct future green salamander surveys in the Blue Ridge Mountains of Tennessee and help to determine if populations are extant in this physiographic region.

Several areas in Tennessee are highly suitable for green salamanders but lack occurrences records in Tennessee's State Wildlife Action Plan Database. Notably, Putnam and Overton Counties along the western escarpment of the Cumberland Plateau, Bledsoe County on either side of the Sequatchie Valley, and the boarder of Hancock and Hawkins Counties in the Ridge and Valley physiographic region have large areas of high suitability. Most of these areas are privately owned and have likely never been systematically surveyed. Based on the results of the model and our knowledge of the landscape in these areas, we speculate that green salamander populations occur in these geographic areas. Standardized surveys are needed for a more complete assessment of the actual distribution of green salamanders in Tennessee.

Forest canopy cover is an important variable in landscape selection of green salamanders and this result provides further evidences that green salamander conservation in Tennessee should focus on protecting forests surrounding rock outcrops (Wyatt 2010).

Forests regulate temperature and humidity regimes around the rock outcrops and trees in close proximity to outcrops may be additionally important for foraging during rain events (Smith et al. 2017). In Tennessee these features are particularly important along the margins of the Cumberland Plateau, the Cumberland Mountains, and northern Ridge and Valley physiographic regions in Tennessee where the landscape is most suitable for the species. A 100 m forest buffer has been suggested to protect rock outcroppings from solar radiation (Petranka 1998). Research is needed to demonstrate if this buffer size is adequate and how fragmentation of forests around and between outcroppings impacts green salamander populations.

Acknowledgments.—This research was funded by the Tennessee Wildlife Resources Agency through the State Wildlife Grants program. We thank Dr. Matthew Niemiller from the University of Alabama- Huntsville and Dr. Becky Hardman from the University of Tennessee- Knoxville for their help in the field and contributions to the larger objectives of the project. Additionally, we thank Dr. Kristen Cecala and anonymous reviewers who improved the manuscript by providing comments on previous versions.

LITERATURE CITED

Batjes, N.H. 2012. ISRIC-Wise derived soil properties on a 5 by 5 arc-minutes global grid. Available http://data.isric.org/geonetwork/srv/eng/catalog.search#/home. Accessed 5 November 2018.

Brown, J.L., J.R. Bennett and C.M. French. 2017. SDMtoolbox 2.0: the next generation Python based GIS toolkit for landscape genetic, biogeographic, and species distribution model analyses. PeerJ 5:e4095.

Center for Biological Diversity. 2012. Petition to list 53 amphibians and reptiles in the United States as threatened or endangered species under the Endangered Species Act. Available https://ecos.fws.gov/docs/petitions/92210/662.pdf. Accessed 24 September 2018.

Corser, J.D. 2001. Decline of the green salamander (*Aneides aeneus*) populations in the southern Appalachians. Biological Conservation 97:119–26.

Dimiceli, C., M. Carroll, R. Sohlberg, D.H. Kim, M. Kelly, and J.R.G. Townshend. 2015. MOD44B MODIS/Terra Vegetation Continuous Fields Yearly L3 Global 250m SIN Grid V006. Available https://lpdaac.usgs.gov/dataset_discovery/modis/modis_products_table/mod44b_v006. Accessed 5 November 2018.

Fois, M., A. Cuena-Lombrana, G. Fenu, and G. Bacchetta. 2018. Using Species distribution models at local scale to guide the search of poorly known species: Review, methodological issues and future directions. Ecological Modeling 385:124–32.

Gordon R.E. and R.L. Smith. 1949. Notes on the life history of the salamander Aneides aenus. Copeia 1949:173–75.

Hardman, R.E. 2014. Modeling occurrence of the green salamander, Aneides aeneus, in the Blue Ridge Escarpment. M.S. thesis, Western Carolina University, Cullowhee, North Carolina, USA.

International Union of Soil Scientists Working Group. 2015. World Reference Base for Soil Resources. World Soil Resources Reports 106. Rome, Italy.

Liu, C., G. Newell, and M. White. 2016. On the selection of thresholds for predicting species occurrence with presence-only data. Ecology and Evolution 6:337–348.

Niemiller, M.L. and R.G. Reynolds. 2011. The amphibians of Tennessee. The University of Tennessee Press, Knoxville, Tennessee, USA.

Olatz, A., L. Cantu-Salazar, G. San Martin, G. Biver, L. Brotons, and N. Titeux. 2015. Reconciling expert judgement and habitat suitability models as tools for guiding sampling of threatened species. Journal of Applied Ecology 52:1608–616.

Peterman, W., J.L. Lcoke, R.D. Semlitsch. 2013. Spatial and temporal patterns of water loss in heterogeneous landscapes: using plaster models as amphibian analogues. Canadian Journal of Zoology. 91:135–140.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C., USA.

Phillips S.J., R.P. Anderson, and R.E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modeling. 190:231–59.

Phillips, S.J. 2009. A brief tutorial on Maxent. Lessons in Conservation 3:108–35.

Rebelo, H., and G. Jones. 2010. Ground validation of presence-only modeling with a rare species: a case study on barbastrelles Barbastrella barbastrellus (Chiroptera: Vespertilionidae). Journal of Applied Ecology 47:410–420.

Smith, W.H., S.L. Slemp, C.D. Stanley, M.N. Blackburn, and J. Wayland. 2017. Rock crevice morphology and forest contexts drive microhabitat preferences in the green salamander (Aneides aeneus). Canadian Journal of Zoology 95:353–358.

Stuart, S.N., et al. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Sugalski, M.T., and D.L. Claussen. 1997. Preference for soil moisture, soil pH, and light intensity by the salamander, *Plethodon cinereus*. Journal of Herpetology 31:245–250.

Taylor, A.T., M. Papes, and J. M. Long. 2017. Incorporating fragmentation and non-native species into distribution models to inform fluvial fish conservation. Conservation Biology 32:171–182.

Tennessee State Wildlife Action Plan Team. 2015. Tennessee State Wildlife Action Plan. Tennessee Wildlife Resources Agency. Nashville, Tennessee, USA.

Tennessee Wildlife Resources Agency. 2005. Tennessee's comprehensive wildlife conservation strategy. Tennessee Wildlife Resources Agency. Nashville, Tennessee, USA.

United States Fish and Wildlife Service. 2019. Conserving South Carolina's at-Risk species: Species facing threats to their survival. Available https://www.fws.gov/southeast/pdf/fact-sheet/green-salamander.pdf. Accessed 5 December 2019.

U.S. Geological Survey and National Geospatial-Intelligence Agency. 2010. Global multi-resolution terrain elevation data 2010. Available https://topotools.cr.usgs.gov /gmted_viewer/. Accessed 5 November 2018.

Waldron, J.L., and W.J. Humphries. 2005. Arboreal habitat use by the green salamander, *Aneides aeneus*, in South Carolina. 39:486–492.

Wells K.T., 20007. The ecology and behavior of amphibians. The University of Chicago Press, Chicago, IL, USA.

Wilson, C.R. 2003. Woody and arboreal habitats of the Green Salamander (*Aneides aeneus*) in the Blue Ridge Mountains. Contemporary Herpetology 2.

Wyatt, S.B. 2010. Status and species-habitat relationships of the green salamander, *Aneides aeneus*, at Myatt Creek in Catoosa Wildlife Management Area. M.S. thesis, Tennessee Technological University, Cookeville, Tennessee, USA.

Wyman R.L., and D.S. Hawksley-Lescault. 1987. Soil Acidity affects distribution, behavior, and physiology of the salamander *Plethodon cinereus*. Ecology 68:1819–27.

SURVEY OF WOODLAND SALAMANDERS IN SOUTHERN GREENE COUNTY, TENNESSEE

Robert L. Morgan III*, and S. Conor Keitzer

Tusculum University, 60 Shiloh Road, Greeneville, TN 37745
**Corresponding Author e-mail: rmorga5@clemson.edu*

Abstract.—Salamanders in northeast Tennessee face a number of potential threats, including climate and land use change. However, the current status and distribution of species in much of this area, which is important fundamental information for effective species management, are not well documented in the literature or public data sources. To help increase knowledge of salamander species in this area, we conducted salamander transect surveys (n = 246) from May 2017 to October 2019 across 70 transects within the Cherokee National Forest (CNF) of southern Greene County, Tennessee. Transects covered a wide range of elevations (500 – 965 m) and forest types and were located in five different publicly accessible areas. We observed 11 different species of salamanders, with an additional 10 species of herpetofauna documented. The Carolina Mountain Dusky Salamander (*Desmognathus carolinensis)*, Eastern Red-backed Salamander (*Plethodon cinereus*), and Northern Slimy Salamander (*P. glutinosus*) were the most common species observed. We also found the Yonahlossee Salamander (*P. yonahlossee*) further west than it had previously been documented in Tennessee. From a standpoint of conservation planning, we found that higher elevation locations (> 700 m) were more diverse, highlighting the need for protection of high elevation forested ecosystems, which harbor much of the salamander diversity found in the southern Appalachian Mountains.

*Key Words.—*conservation, distribution, diversity, elevation effect, natural history, Yonahlossee salamander

The southern Appalachian Mountains are well known as a global hotspot for salamander diversity. Currently, there are about 55 species of salamanders found in the southern Appalachians, with new species still being discovered (Mitchell et al 1999, Patton et al 2019). Abundant moisture and mild temperatures, coupled with a wide gradient of elevation and a long evolutionary history, has enabled a vast array of species to arise in this region (Petranka, 1998, Niemiller and Reynolds 2011). However, this unique diversity is currently under threat, with apparent population declines documented for many species in the region (Caruso and Lips 2012).

While apparent declines in salamander populations can be attributed to several factors, two of the greatest threats salamanders in the southern Appalachian Mountains face is the impact of land use and global climate change (Milanovich et al. 2010). Salamanders require temperate and moist environments to survive, but these habitat requirements and limited dispersal ability mean that salamanders are highly vulnerable to both of these threats. In recent decades, the southern Appalachian region has seen increased human population growth and associated land conversion. In fact, since 2010, the population of the south-central region of Appalachia (inclusive of Greene County, TN) has grown in population by about

4%, which is only 1.8% lower than the national average for the same duration (Appalachian Regional Commission 2020). As the southern Appalachian region continues to modernize, increasingly more demand will be placed on the region's forested ecosystems. While a sizable portion of the southern Appalachian Mountains are state or federally owned, the remaining "uninhabited" private land is being converted to accommodate the growing population and influx of visitors. This increases fragmentation, which may affect genetic diversity and flow. As salamanders are less mobile than some taxonomic groups (i.e. *Avian*, *Canine*, or *Ungulates*), the barriers and loss of habitat that land development creates have huge implications on salamander populations.

In addition to changes in land use, global climate change has the potential to severely impact the salamander diversity of the region. There are multiple lines of evidence that suggest salamanders are already experiencing the effects of climate change. Past surveys within the Great Smokey Mountains National Park (GSMNP) found an approximately 39% decline in *Plethodon* salamander populations (Caruso and Lips 2012). These declines are troubling because they occurred in GSMNP, an area protected from most of the human disturbances that would affect salamanders. Despite this grim finding, some research suggests that salamanders have an increased ability to adapt to these future alterations. Recent findings suggest that extinction rates of salamanders likely could be reduced by about 72%, as a result of physiological adaptation and a change in behavioral patterns (Riddell et al. 2018). Additional evidence suggests that salamander diversity in the southern Appalachian Mountains has benefited from forest regrowth and modern management practices (Moskwik 2014). Forest regrowth has lowered the temperature of certain sites, therefore buffering salamanders from climate alterations. Thus, while climate change and land use change are potential threats, how salamanders have or will respond to those threats in the region is uncertain.

Outside of areas like Roan Mountain or Great Smoky Mountains National Park, relatively little information about the current distribution and status of salamanders is available for northeast Tennessee. This baseline data is greatly needed to help identify threatened populations and possible drivers of declines (Caruso and Lips 2012). To better understand the natural history and distribution of salamander species in northeast Tennessee, we conducted salamander surveys within the Cherokee National Forest (CNF) of Greene County, TN (Figure 1) over three summer field seasons from May 2017 to October 2019. These surveys are part of a long-term monitoring project of salamander populations in the region, but here we report on the first three field seasons.

METHODS AND MATERIALS

Survey Sites. – From May 2017 to October 2019, transect surveys (n = 246) were conducted on 70 established transects in 5 focal areas located in southern Greene County, TN. These included: Round Knob, Greene Mountain, Old Forge, Jennings Creek, and Squibb Creek (Table 1 and Figure 1). Survey elevations ranged from a low of 500 m at Squibb Creek to a high of 965 m at Round Knob. Most transects were in Cove Hardwoods (n = 52), but Oak-Conifer forests were also sampled (n = 18). All survey locations were within the Southern Sedimentary Ridges Ecoregion of the Blue Ridge Mountains of East Tennessee (See Figure 1). This ecoregion is best characterized by slopes with a steep elevation gradient, along with a combination of mixed and northern hardwood forest cover types dependent of elevation (Niemiller and Reynolds 2011).

TABLE 1. Survey locations with corresponding elevation, forest type, and transect totals.

Survey Site	Elevation Range (m)	Forest Type	Number of Transects
Round Knob	875-965	Cove Hardwood	29
Greene Mountain	740-770	Oak-Conifer	8
Old Forge	570-600	Oak-Conifer	10
Squibb Creek	530-670	Cove Hardwood	13
Jennings Creek	500-645	Cove Hardwood	10

Transects. – Transects measured 30 meters in length, and were established parallel to the slope contour to maintain a consistent elevation across the transect. Our team (2-6 surveyors) would turn over natural cover objects (i.e. rocks, logs, and leaf litter) within 1 meter of either side of the transect to capture salamanders. In addition to recording species capture totals, we collected total length, snout-vent length, and weight on every salamander before they were safely returned to their capture location. Transects were sampled randomly at various times (day & night) and climatic conditions (wet & dry) following a robust sample design (Pollock 1982). The number of sampling events was varied given logistical challenges, but transects were usually surveyed ≥ 3 times in a given field season (typically May – August). In August 2018, cover boards were added to several transects (n = 23). Our cover boards were locally sourced rough sawn pine lumber cut out into 30 x 30 cm squares, and about 2.5 cm thick. Cover boards were placed 1 meter apart along the length of the transect, with each transect having 30 boards deployed. Round Knob (n = 10), Greene Mountain (n = 7), and Old Forge (n = 6) are the only locations where cover board transects were added. These locations were selected for cover board inclusion due to their proximity to established trail systems, which made transport of supplies feasible for our small team. Cover board surveys began in May 2019, after winter acclimatization. We calculated both Species Richness and Simpson's Index of Diversity for each focal area.

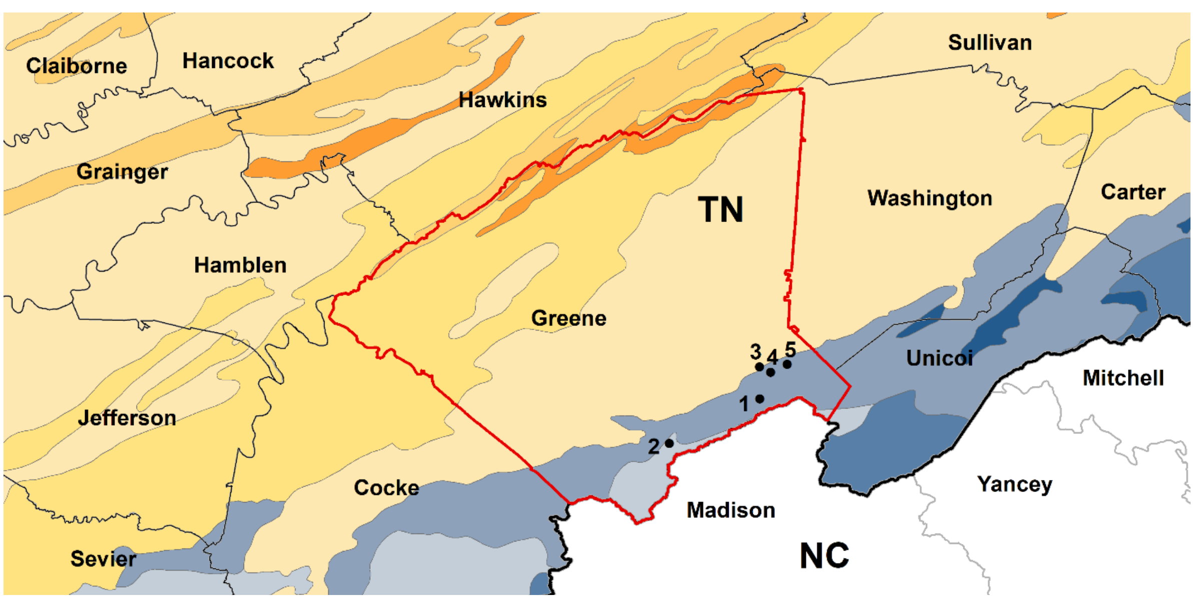


FIG. 1. Survey sites were within the Southern Sedimentary Ridges Ecoregion of Greene County, TN. (1) Round Knob, (2) Greene Mountain, (3) Old Forge, (4) Jennings Creek, and (5) Squibb Creek. Figure was adapted from Griffith et al. 1998.

RESULTS

Over the three field seasons from May 2017 to October 2019, 401 salamanders were encountered within the transects from 11 different species. Observed species varied based on elevation, with the Blue Ridge Two-lined Salamander (*Eurycea wilderae*), Eastern Red-backed Salamander (*Plethodon cinereus*), and Northern Slimy Salamander (*P. glutinosus*) being found over the widest range (> 375 m) in elevation (Table 2). Additionally, 10 other species of Herpetofauna were observed while sampling the transects (Table 3). The Round Knob survey sites had the highest species richness (*R = 8*), followed by Jennings Creek and Greene Mountain (*R* = *5*). Simpson's Index of Diversity showed that 3 of the 5 survey sites were relatively diverse (*D > 0.60*), with Old Forge and Squibb Creek showing less diversity (*D < 0.60*) (Table 4). Across all survey sites, the Northern Slimy Salamander, Eastern Red-backed Salamander, and the Carolina Mountain Dusky Salamander (*Desmognathus carolinensis)* were encountered with the most frequency (Table 5).

TABLE 2. Elevation species were encountered during our surveys (Observed Range), and their suggested literature ranges (Niemiller and Reynolds 2011, Petranka 1998). Note that our surveys ranged from 500 to 965 m.

Common Name	**Scientific Name**	**Min & Max Observed Elevation (m)**	**Literature Elevation (m)**
Black Bellied Salamander	*Desmognathus quadramaculatus*	880 – 906	490 – 1680
Blue Ridge Two-lined Salamander	*Eurycea wilderae*	501 – 962	< 2000
Carolina Mountain Dusky Salamander	*Desmognathus carolinensis*	597 – 962	300 – 2000
Eastern Newt	*Notophthalmus viridescens*	583 – 758	no data
Eastern Red-backed Salamander	*Plethodon cinereus*	583 – 958	< 1500
Northern Gray-cheeked Salamander	*Plethodon montanus*	932 – 947	> 800
Red Salamander	*Pseudotriton ruber*	637 – 752	< 1500
Seal Salamander	*Desmognathus monticola*	606 – 906	< 1500
Spotted Salamander	*Ambystoma maculatum*	739 – 739	< 600
Northern Slimy Salamander	*Plethodon glutinosus*	577 – 958	< 1500
Yonahlossee Salamander	*Plethodon yonahlossee*	938 – 938	440 – 1740

TABLE 3. Additional herpetofauna observed within transects 2017-2019.

Common Name	Scientific Name
American Toad	*Anaxyrus americanus*
Eastern Box Turtle	*Terrapene carolina*
Fowler's Toad	*Anaxyrus fowleri*
Gray Ratsnake	*Pantherophis spiloides*
Green Frog	*Lithobates clamitans*
Racer	*Coluber constrictor*
Pickerel Frog	*Lithobates palustris*
Ring-necked Snake	*Diadophis punctatus*
Spring Peeper	*Pseudacris crucifer*
Wood Frog	*Lithobates sylvaticus*

TABLE 4. Species Richness and Simpson's Index of Diversity per site.

Survey Site	Elevation Range (m)	Species Richness	Simpson's Index of Diversity
Round Knob	875-965	R=8	D=0.67
Greene Mountain	740-770	R=5	D=0.70
Old Forge	570-600	R=2	D=0.50
Squibb Creek	530-670	R=3	D=0.53
Jennings Creek	500-645	R=5	D=0.62

TABLE 5. Species survey proportional data 2017-2019, with the number of survey areas observed.

Species	Observation (%)	Total Areas Observed
Northern Slimy Salamander	44.1	4
Carolina Mountain Dusky Salamander	26.9	2
Eastern Red-backed Salamander	10	3
Seal Salamander	7.7	2
Blue Ridge Two-lined Salamander	5.7	3
Northern Gray-cheeked Salamander	1.5	1
Red Salamander	1.2	2
Black Bellied Salamander	1	2
Eastern Newt	0.7	1
Yonahlossee Salamander	0.7	1
Spotted Salamander	0.2	1

Discussion

Our findings show that Greene County, which is part of the Bald Mountain Range, is home to a robust assortment of salamander species. Across our sites we documented salamanders from four different Genera: *Plethodon, Desmognathus, Eurycea,* and *Ambystoma.* The most commonly encountered species were the Northern Slimy Salamander, Eastern Red-backed Salamander, and the Carolina Mountain Dusky Salamander. Combined, these three species represented 81% of our total captures. The least common species in these surveys were the Yonahlossee Salamander (*P. yonahlossee)* and the Spotted Salamander (*Ambystoma maculatum)*. Combined, these species accounted for about 1% of our total captures. These species were also apparently limited in their distribution, with each observed at only a single transect (Yonahlossee Salamander at Round Knob & Spotted Salamander at Greene Mountain).

We found the Yonahlossee Salamander (2 Adults, 1 Juvenile) in a Round Knob transect, at an elevation of 938 m, which as far as we are aware, is the first confirmed occurrence for the species in Greene County (Tennessee Natural History Program 2020). Within Tennessee, the Yonahlossee salamander is non-legally ranked as S2, or "very rare and imperiled within the state" (Tennessee Natural History Program 2020). Given its southwesterly location, this occurrence should be further assessed for protection and to determine if this is an isolated population or part of a larger population in nearby counties. A second interesting observation involved the Spotted Salamander. On 30 May 2018, we documented a Spotted Salamander within a short distance of a natural Fen wetland at one of our Greene Mountain transects. The salamander was found at an elevation of 739 m, which was higher than what has been previously suggested (< 600 m) for the species in Tennessee (Niemiller and Reynolds 2011). Currently, restoration work is being conducted on this Fen. Our observation further highlights the value of this project, and should inform management practices moving forward.

The most diverse public area we surveyed in regards to species richness was Round Knob (most species and 2nd highest diversity index). This cove-hardwood forest was one of the higher elevation areas we surveyed, which may partially explain its more diverse salamander assemblage. This is also the only area we found two of the rarer species, the Yonahlossee and the Northern Gray-cheeked Salamander (*P. montanus*). This suggests that Round Knob may be a particularly valuable area for salamander conservation and forest protection moving forward.

Collectively, these findings further highlight the conservation value of the southern Appalachians for salamander diversity. Much of these forested areas are part of protected state and federal lands, where more protection can be afforded compared to private land. In Greene County and the whole of northeast Tennessee continued protection of these lands, as well as increasing conservation acreage will be critical moving forward to ensure the long-term viability of salamanders and other amphibians in the face of uncertain global change.

Acknowledgements.—A number of Tusculum University students contributed to this research and we would like to thank Brandon Ball, Taylor Boles, Veronica Brown, Kelly Donnelly, John E. Durr, Tarah Helms, Kyler Johnson, Patrick Jones, Nathaniel Robinette, Megan Southerland, and Trevor Kahland for helping to establish transects and survey for salamanders. We would like to also thank the Tusculum University Department of Natural Sciences for access to their laboratory spaces and scientific instruments. Finally, we would like to thank the Appalachian College Association for providing financial support for a major portion of our research efforts. We greatly appreciate all who reviewed and provided valuable comments on this manuscript as well. These surveys were conducted under a Tennessee Wildlife Resources Agency scientific collection permit (#1673) with additional permission from the

Cherokee National Forest, Unaka Ranger District. We sincerely thank these agencies for their support and assistance.

LITERATURE CITED

Appalachian Regional Commission (2020, June). Appalachia's Population. Retrieved from https://www.arc.gov/appalachian_region/FactSheets.asp

Caruso, N.M., and K.R. Lips. 2012. Truly enigmatic declines in terrestrial salamander populations in Great Smokey Mountains National Park. Diversity and Distributions 2012:1-11.

Cecala, K. K., J. C. Maerz., B. J. Halstead., J. R. Frisch., T. L. Gragson., J. Hepinstall-Cymerman., D. S. Leigh., C. R. Jackson., J. T. Peterson., and C. M. Pringle. 2018. Multiple drivers, scales, and interactions influence southern Appalachian stream salamander occupancy. Ecosphere 9(3): e02150. 10.1002/ecs2.2150

Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions. 9:89-98.

Garvey, J.E., E.A. Marschall, and R.A. Wright. 1998. From star charts to stoneflies: detecting relationships in continuous bivariate data. Ecology 79:442-337.

Griffith, G.E., J.M. Omernik., and S.H. Azevendo. 1998. Ecoregions of Tennessee. U.S. Geological Survey. Reston, Virginia.

Milanovich, J.R., W.E. Peterman., N.P. Nibbelink., and J.C. Marez. 2010. Projected Loss of a Salamander Diversity Hotspot as a Consequence of Projected Global Climate Change. PLoS One 5:8.

Mitchell, J.C., T.K. Pauly., D.I. Withers., S.M. Roble., B.T. Miller., A.L. Brasnel., P.V. Cupp., and C.S. Hobson. 1999. Conservation Status of the Southern Appalachian Herpetofauna. Virginia Journal of Science 1999:50-1

Moskwik, M. 2014. Recent elevational range expansions in plethodontid salamanders (Amphibia: Plethodontidae) in the Southern Appalachian Mountains. Journal of Biogeography 41:1957-1966.

Niemiller M.L., R.G. Reynolds. 2011. The Amphibians of Tennessee. The University of Tennessee Press, USA.

Patton, A., J.J. Apodaca., J.D. Corser., C.R. Wilson., L. A. Williams., A. D. Cameron., and D.B. Wake. 2019. A New Green Salamander in the Southern Appalachians: Evolutionary History of *Aneides aeneus* and Implications for Management and Conservation with the Description of a Cryptic Microendemic Species. Copeia, 107:4

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, USA.

Pollock, K.H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management 46:757-760.

Riddell, E.A., J.P. Odom., J.D. Damm., and M.W. Sears. 2018. Plasticity reveals hidden resistance to extinction under climate change in the global hotspot of salamander diversity. Science Advances 4: eaar5471.

Tennessee Dept. of Environment and Conservation. n.d. Natural Heritage Program. Rare Species Data Viewer. Available https://www.tn.gov/environment/program-areas/na-natural-areas/na-natural-heritage-inventory-program.html. Accessed 26 February 2020.

DESMOGNATHUS CONANTI **(Spotted Dusky Salamander). NESTING UNDER MANMADE COVER OBJECT.** At 1550 h on 17 August 2019, I overturned a manmade cover object colloquially called a "cookie" (a small circular piece of wood designed to aid in herpetological surveys) in a muddy streambank near a pond in a mesic hardwood forest in Knox County, Tennessee (35.956816 4°N, -83.868139°W) and found a female *Desmognathus conanti* guarding a clutch of 18 eggs. Also under the cover object were three conspecific adult *Desmognathus conanti* and one adult *Eurycea cirrigera*. The cover object was about 30cm in diameter and loosely embedded in the ground. In the direct vicinity were multiple natural cover objects such as small logs, rocks, and moss. In an assessment of the nesting cover object choice of *Desmognathus fuscus* (Hom. 1988. Journal of Herpetology. 22: 247-249) suggests that females will preferentially lay eggs under moss while also laying eggs under logs and rocks. To the best of my knowledge this is the first recorded observation of a salamander in the genus *Desmognathus* using a manmade cover object as a nest site. It is also interesting to note that, though they often had adult *Desmognathus*, other cover objects in the direct vicinity had no nests. This may suggest that manmade cover objects could be preferentially chosen by female *Desmognathus* when available, though more research would be necessary to confirm this hypothesis.

Submitted by **BRYCE S. WADE** University of Tennessee, Knoxville, TN, 37996 USA.

FIG. 1. A female *Desmognathus conanti* guarding her brood of eggs.

***PLETHODON WELLERI* (Weller's Salamander). EARLY COURTSHIP.** At 1428 h on 16 March 2020, I overturned a trailside log along the Appalachian Trail between Indian Grave Gap and Beauty Spot (36.112952, -82.359852, elevation 1010 m) to reveal two *Plethodon welleri* in close contact. One individual was a gravid female while the other was a male with well-developed nasolabial projections, a temporary physical trait the male salamander develops during the breeding season (Organ 1960). These two individuals were likely a mating pair given the enhanced secondary sexual characteristics and position; however, I was unable to confirm the presence of a mental gland on the male or the presence of eggs in the female due to concerns of disturbing the pair. *Plethodon welleri* are considered to be both a fall and spring breeding species with evidence of courtship documented in April and October from both Mt. Rogers, NC and under lab conditions (Organ 1960. Copeia 1960(4): 287-297; Thurow 1963. University of Kansas Publications 44:87-108). To my knowledge this is the earliest recorded breeding event for *Plethodon welleri* and may stem from an unseasonably mild and wet winter. As anthropogenic climate change progresses, anomalously mild winter conditions will likely become increasingly common and may lead to the continued documentation of earlier courtship in *P. welleri*.

BRYCE S. WADE, University of Tennessee, Knoxville, 2100 Highland Ave. Knoxville, TN 37916 USA.

Fig. 1. A breeding pair of *Plethodon welleri* from northeast Tennessee.

DIADOPHIS PUNCTATUS (Ring-Necked Snake). **COLORATION.** On 27 October 2019, we discovered a juvenile *Diadophis punctatus* underneath a discarded scrap of wood at Sandy Creek Nature Center in Clarke County, Georgia (33.9889°N, 83.3719°W; WGS 84), and we deposited a photo voucher on HerpMapper (HM 290368). This individual lacked nearly all the yellow pigment typical of *D. punctatus* in the region, with the venter and nuchal collar instead being a pale white (Fig. 1). This coloration appeared to be permanent, as the snake showed no signs of impending ecdysis (e.g., opaque eyes or lightened dorsal coloration). Other individuals of this species that we have encountered at this location exhibited typical coloration, and relatively few reports of similar coloration abnormalities are present in published literature. Jolley et al. (1983. Herpetol. Rev. 14:119–120) noted a *D. p. arnyi* from Missouri that likewise lacked "nearly all orange, yellow, and red pigmentation", but described its nuchal collar as "obscure" and its venter as "light metallic gray". These authors referred to this condition as "acarotenoidic", although it might be more commonly referred to as "axanthic"—a general term used to describe the absence of yellow (and sometimes red or orange) pigments. Brust et al. (2020. Herpetol. Rev. 51:142) described an axanthic *D. p. edwardsi* from West Virginia which very closely matches the appearance of the individual we observed.

Submitted by **KEVIN G. HUTCHESON** (e-mail: kgh58311@uga.edu) and **TODD W. PIERSON**, University of Georgia, Athens, Georgia 30602, USA.

FIG 1. An axanthic *Diadophis punctatus* from northern Georgia. PHOTO BY TODD W. PIERSON.

NERODIA SIPEDON (Northern Water Snake). **SHEDDING**. In the summer of 2019, we collected 22 Northern Watersnakes from Cheatham, Putnam, and Rutherford counties in Tennessee (TWRA Scientific Collection Permit #1907). Snakes were placed into captivity at Middle Tennessee State University (IACUC approval 19-3012). The enclosures provided for these animals consisted of 66.24 L plastic storage totes (66 × 34 × 41 cm) with ventilation holes, hide box, climbing branch, and water dish. The experimental chamber housing the snakes was maintained at 23 °C with a 12 h light/dark cycle generated by white overhead lights. The daytime period began at 0800h and ended at 2000h each day. Snakes were housed in captivity to facilitate an experiment involving timeseries data. As part of this experiment, we observed snakes continuously (24 h/day) six days a week for a period of approximately three months.

During daytime hours, an observer was present in the experimental chamber who would continuously monitor snakes for signs of shedding behavior. Daytime observation did not involve active disruption of snake behavior as the provided hide boxes were translucent. This permitted observers to view individual snakes through the transparent walls of the enclosure without physical disruption or touching of the animal. During nighttime hours, an observer would enter the dark experimental chamber and observe each snake individually using a red headlamp positioned several centimeters from the side wall of the enclosure. Red light was chosen as it is generally considered to be less disruptive to sleeping or nocturnal animals than white light (Ouyang et al. 2017. Global Change Biology 23:4987–4994). We repeated this process once every thirty minutes for the extent of each nighttime period. Each nighttime observation period took between 5–10 minutes. During this time, each snake was individually inspected for between 30 seconds and 2 minutes without any physical disturbance. In the later part of the experiment, some snakes were exposed to intermittent red light via an automated lighting rig to mimic observation periods by human observers.

Over the course of this experiment (which lasted from 31 May 2019 to 21 August 2019), ten shedding events took place among the captive snake population. Prior observations of snakes maintained in captivity for long periods of time have not reported a bias for nocturnal or diurnal shedding behavior (Lamonica et al. 2007. International Journal of Morphology 25:103–108). However, we observed eight shedding events that occurred during daytime hours and two shedding events that occurred during nighttime hours. Previous work has shown that ambient temperatures effect overall rates of ecdysis in snakes, but little is known about the mechanisms that influence a snake already in a pre-shed state, to begin the shedding process (Semlitsch. 1979. Society for the Study of Amphibians and Reptiles 13:212–214).

Between the dates of 25 June 2019 and 15 July 2019, seven shedding events took place. All of these shedding events, except one, took place during the daytime observation window. The one nighttime shedding event which took place during this period occurred over the weekend of 4 July 2019 when nighttime observation was temporarily suspended, hence, this snake was not exposed to intermittent red light, via observer activity, on the night of the shedding event. It was determined that this animal had shed when the lights were off as routine animal wellness checks were still conducted that evening (2000h, during which no shed was found) and the following morning (0800h, during which the shed was found). This led us to hypothesize that nighttime observation activities, particularly exposure to light, may alter snake shedding behavior.

To investigate this, we constructed a lighting rig that utilized red lights (n=2 light bulbs, UTILITECH Model #YGA08A54-8W-RED) placed 15 cm away from the enclosures and interval timers to automatically expose snakes in a pre-shed state (development and subsequent loss of cloudy eyes) to red light throughout the night. The lights of this system came on for 30 minutes and turned off for 30 minutes in a repeating cycle throughout the nighttime period. We utilized this lighting rig to expose two snakes exhibiting a pre-shed state to red light without other observer activities. One animal was exposed to light using this system for one night before shedding during the day on 16 July 2019. Another animal was exposed to light using this system for two nights before shedding during the day on 17 July 2019. Thus, both snakes exposed to this automated lighting system shed during daytime observation hours.

The final shedding event recorded during this experiment occurred on the night of 20 August 2019. This was the last night before the experiment was concluded and, like the weekend of 4 July 2019, nighttime observation was suspended. As with the previous nighttime shedding events, health checks were conducted the evening before and the morning after the shedding event took place. This allowed us to determine that the shedding event had occurred when the overhead lights were off in the experimental chamber.

In our observations, every snake that shed during the day had been exposed to intermittent red light the night before the shedding event took place. Alternatively, the two snakes that shed at night had not been exposed to intermittent red light on the night that they shed. Recent research investigated the expression of three visual pigment genes across snakes and suggested that most snakes exhibit cone dichromacy resulting in the lack of an ability to perceive the color red (Simões et al. 2016. Molecular Biology and Evolution 33:2483–2495). However, Natricine snakes possess vision which is sensitive to wavelengths of light which correspond with the color red (Fenwick et al. 1992. Journal of Comparative Physiology A 170:701–707). Consequently, when exposed to a red light in an otherwise dark room, study snakes would perceive the illumination caused by the light source. From the perspective of the snake, it might appear as a yellow light source rather than red.

Our observations suggest that intermittent exposure to red light may have an effect on when snakes begin the shedding process. Northern Watersnakes are known to display crepuscular activity cycles during summer months in some parts of their range (Ernst et al. 2012. The Herpetological Bulletin 121:23–28). Other members of the genus *Nerodia* display varying degrees of nocturnality depending on season (Mushinsky & Hebrard 1977. Canadian Journal of Zoology 55:1545–1550). Additionally, snakes are generally considered to be more vulnerable to predation during the shedding process (Loughran et al. 2015. Northwestern Naturalist 96:156–160). Thus, study snakes may have preferentially shed at night. However, when nighttime disturbance was induced, via intermittent exposure to red light, study snakes may have switched to shedding during the daytime, when only passive observation occurred. Further study may elucidate if shedding behaviors can be modified via exposure to distinct lighting regiments.

Submitted by **ALEXANDER S. ROMER**, Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA; **JACKSON WEST**, Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA; **EMMA PHIPPS**, Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA; **KYLIE MOE**, Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA; **DONALD M. WALKER**, Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee 37132 USA (email Donald.Walker@mtsu.edu).

TABLE 1. Overview of snake shedding behavior with regard to lighting exposure.

Red Light Exposure	Shedding Window	Exposure Method	Snake ID	Date	Shed Start Time
Yes	Diurnal	Observer Flashlight	C4	25-Jun	1530h
			E5	28-Jun	1230h
			E7	28-Jun	1300h
			E6	4-Jul	1000h
			E10	9-Jul	0900h
			C7	15-Jul	0930h
Yes	Diurnal	Lighting Rig	C10	16-Jul	0900h
			C4	17-Jul	1200h
No	Nocturnal		E9	7-Jul	2000h–0800h
			E10	20-Aug	2000h–0800h

The 26th Annual Meeting of the Tennessee Herpetological Society was canceled due to COVID-19. Instead, we hope you will join us and participate in the virtual conference in February 2021. See the following website for registration and more information:

https://www.tnherpsociety.org/copy-of-2019-ths-meeting

Students – Consider applying for the Chadwick Lewis Memorial Grant to support your research! See this website: https://www.tnherpsociety.org/chadwick-lewis-memorial-grant