DEVELOPING A PREDICTIVE GEOSPATIAL HABITAT MODEL FOR A RARE SPECIES OF SALAMANDER IN TENNESSEE: A CASE STUDY FOR THE GREEN SALAMANDER (ANEIDES AENEUS)

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ABSTRACT

Green Salamanders (*Aneides aeneus*; Cope and Packard 1881) are a secretive and cryptic species of salamander that can be relatively difficult to detect, due to their occupation of arboreal habitats. The incorporation of geospatial tools is critical in developing models that can be used to predict undocumented locations in Tennessee and elsewhere. Locating the species in Tennessee was accomplished through a total of 18 localities and with that, several habitat characteristics were able to be identified including, but not limited to: appropriately shaded and structured rocks, typical fauna co-occurring within the area, and general topography of the area. Of the 91.8% of sites that were deemed to have suitable habitat, 40% of those had positive presence, the remaining 60% is indeterminate for presence at this time as more field visits should be conducted in the future.

DEDICATION

I would like to dedicate this work to the memory of my dear friend Ryan Bartel and to all those who through their encouragement, made this research possible.

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I want to thank all of those who have supported and encouraged me through this process. I begin by thanking my advisor, Dr. Thomas Wilson for his guidance, encouragement, and his overall trust in my abilities to take on such an ambitious project. He has shown the utmost care and utter investment not only in my project but as my overall person. I also thank Nyssa Hunt, who from the beginning realized my capabilities to grow in the GIS field. She has kept me grounded, caffeinated, and fed. Nyssa has been my rock through this process and has been nothing less than supportive. I am truly grateful for her mentorship and knowledge. I would also like to thank Dr Qin for his time, insight, and expertise as a member of my committee.

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LIST OF ABBREVIATIONS

AIC, Akaike Information Criterion

AUC, Area Under the Curve

Bsal, Batrachochytrium salamandrivorans

Esri, Environmental Systems Research Institute

HW, Head Width

JB, Jarque-Bera p-value

K(BP), Koenker (BP) Statistic p-value

MaxEnt, Maximum Entropy

MB, Millibars

OLS, Ordinary Least Squares

SA, Global Moran's I p-value

SDM, Species Distribution Model

SVL, Snout-Vent Length

TBL, Total Body Length

TL, Tail Length

VIF, Max Variance Inflation Factor

CHAPTER I

INTRODUCTION

Effects on Amphibian Populations

Amphibians have been facing an overall global decline over the past several decades. In 1989, a meeting of the First World Congress of Herpetology brought forth awareness of these global declines; because of this, an influx of studies on the declines were conducted (Corser 2001). There are a variety of factors which contribute to the decline: Land-use changes, commercial exploitation, introduction of alien species, and disease (Mendelson et al. 2006). Because of amphibians' close association with both water and land, they experience the environmental stressors that are present in both these systems (Blaustein and Kiesecker 2002); therefore, increasing their likelihood of exposure to anthropogenic affairs, as degradation and loss of habitat spanning from human activities creates a negative association to the area and amphibian richness (Cordier et al. 2021). These activities not only contribute to harmful chemical releases into the environment, but also to a loss of biodiversity overall.

Dodd's 2009 Amphibian Ecology and Conservation A Handbook of Techniques includes a refined list of factors which explain why the loss of amphibians should be of concern to the everyday individual: Economics, ecosystem functions, aesthetics, and ethics.

Beginning with the economic functions of amphibians, they are considered to many as a food source, kept as pets, and used in the scientific field for medical research and teaching (Dodd 2009). To further discuss amphibians as pets, exploitation of amphibians for the pet trade has

been an ongoing issue within the last century, with their trade exponentially increasing over the last few decades, and more and more species continuing to be added to the activity (Carpenter et al. 2014). Issues surrounding this activity are three-fold: contributing to the decline of natural populations, the introduction of alien species, and spread of diseases (Measey et al. 2019). Removing amphibians from their natural populations contributes not only to amphibian population declines (Mitchell et al. 1999), but also to the disruption of trophic systems due to synergistic effects.

Amphibians play a vital role in how our ecosystem functions, by contributing to nutrient cycling and a food source for many birds and mammals (Dodd 2009). The loss of amphibians has and will continue to cause instability in the natural order of our ecosystems. Aesthetically speaking, artists have used amphibians as a source of inspiration for their creative and artistic endeavors; for many, the sight of migrating frogs and salamanders is a pivotal indication of spring for many, with the calling of male frogs adding to the ambiance of a crisp spring night. Ethically, amphibians have the right to exist and have inherent value to the ecosystem (Dodd 2009). No single animal could be used to study every element of our ecosystem; however, understanding amphibian roles in the ecosystem is crucial (Hopkins 2007).

Salamander Decline

Species included within the order Caudata, are not excluded from this downward spiral. The family, Plethodontidae, is a family of salamanders known commonly as "woodland salamanders", members of this family are lungless and often terrestrial; with several species that exhibit direct development. Due to woodland salamanders' performance of gas exchange via cutaneous respiration, they are quite susceptible to changes of their microclimate (Spotila 1972).

Plethodontids are unique in that they are physiologically connected to both microclimates and successional processes that greatly impact forest dwelling plants and animals (Welsh and Droege 2001), making their presence and abundance in such ecosystems critical to the health and wellness of the area. Rucker et al. (2022) suggests fragmentation of forests in high elevation Appalachia could be affecting the microclimates of adjacent mature forests and therefore influencing salamander occupancy of these nearby areas. Highton (2005) observed major declines of plethodontid salamanders in the 1980s; subsequently, using his own historical records and revisiting 205 populations, it was found that the mean number of salamanders observed was 41.6% less than his original observations from the 30-year period.

Typical timber management practices often rotate from 80-100 years in Southern Appalachian forests, and the timber roads used to access these sites persist and as more forestry roads are added over time, the effects of forest fragmentation accumulate, compounding the loss of available habitat for woodland salamanders (Semlitsch et al. 2007). Regardless of the fact that a forest system may regenerate after timber harvest events, terrestrial salamander abundance may remain declined for several decades of time due to the altered environment (Petranka et al. 1993; Ash and Bruce 1994; Ash 1997; Herbeck and Larsen, 1999).

Immediate Concerns of Aneides aeneus

Aneides aeneus is noted as a species that suffers from both data deficiencies and population declines (Blackburn et al. 2015). There are a multitude of factors that a population's health may depend on: abundance, age structure, gene flow, disease, and environmental stressors (Hinkle et al. 2018). In addition to these ongoing factors, Wilson (2001) worried that collection

of *A. aeneus* from either research or hobbyist endeavors would contribute to a decline of their populations; particularly those occupying the Blue Ridge.

In the 1970s, a large population crash of *A. aeneus* was recorded throughout Georgia, South Carolina, and North Carolina (Snyder 1991). Between the 1970s and 1990s these areas saw a loss of 98% of the original *A. aeneus* populations. By 2001 evidence of recolonization was lacking, leading to the concern that immigration rates of the area may be too low to return the sites to their previous levels of occupation (Corser 2001).

A current concern for the species is the prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*). These fungi can occur in both soils and water, with potential to cause Chytridiomycosis in amphibians, which can lead to mass mortalities of amphibians (Weinstein 2009). Blackburn et al. (2015) conducted a study on Virginia populations of *A. aeneus* confirming that they can contract both chytrid and ranavirus, suggesting that these pathogens could cause a threat of population declines of the species in the future; especially when drought occurs, as ranavirus spreads more readily during these periods. Martel et al. (2014) estimated the threat of an infectious chytrid fungus that is currently emerging in the Western Hemisphere, known as *Batrachochytrium salamandrivorans* (*Bsal*), it has been concluded that this species of fungus has potential to rapidly infect plethodontids. Newman et al. (2019) swabbed 41 *A. aeneus* individuals over a two-year period in South Carolina to detect any pathogens that may be present and found a single individual with Chytrid but no detection of ranvirus or *Bsal* (which has yet to be detected in North America).

A. aeneus habitat locations have been frequently described as being similar to *Plethodon* glutinosus, taking refuge in rock crevices and shale banks (Bishop 1994); however, A. aeneus tends to select crevices that are higher than most other plethodons will extend to (Petranka et al.

1998). Wyatt (2010) observed several plethodon species occupying the same outcropping as *A. aeneus*, although there were no observations of any species cohabiting crevices. *Plethodon glutinosus* are considered to have similar life histories to that of *A. aeneus* (Petranka 1998) and occasionally occupy the same rock faces, they may be a high risk for transferring the disease to *A. aeneus* (Armstrong 2010).

Why Green Salamanders?

Aneides aeneus, commonly known as the Green Salamander (Cope and Packard 1881), or less so as the Bronzed Salamander, is a Plethodon species of salamander native to areas surrounding the Appalachian Mountains, and of particular interest to this study, the eastern portion of the state of Tennessee. A. aeneus is globally considered a vulnerable species, with a rank of G3 (NatureServe Explorer 2021). In the state of Tennessee, they are ranked at S3S4, considering them to be secure to imperiled with a high risk of extirpation.

Snyder (1991) advocated for *A. aeneus* to be considered a target species that aids to "track the overall health of an ecosystem". He gives several rationale to this idea: (1) their inherent risk to desiccation; (2) their unique life history as a terrestrial species; (3) their somewhat "extensive range" may allow resilience against local crises, although they are not unaffected by large environmental degradation; (4) due to their limited contact with the ground, they may be more exposed to acid precipitation than other amphibians; (5) bioconcentration of toxicants may be possible due to their carnivorality; and, (6) standardizing a census effort on this species may be possible due to their microhabitat preferences and the ability to check the same crevices over multiple visits. To summarize Snyder's (1991) points, *A. aeneus* are an ecologically significant species.

Species Distribution Modeling

Most species occurrence data is limited and as a result, information on their distributions is insufficient (Elith et al. 2006). Understanding a species distribution is an important feat, giving insight to their complex ecological and evolutionary history (Brown et al. 1996; Gaston 2003; Barve et al. 2011). To supplement data gaps, Species Distribution Models (SDMs) have become a popular method to predict spatial patterns of various biota (Pradervand et al. 2014). These model types function by utilizing a species occurrence in conjunction with a variety of environmental variables, considering statistical processes that yield varying results depending on the program chosen and settings by the user. Some techniques are even used to predict future distributions of species via climatic factors. SDMs are often loosely used to describe similar methodologies or objectives: bioclimatic models, climatic envelopes, ecological niche models, habitat models, resource collection functions, range maps, correlative or spatial models are all techniques that fall under the SDM umbrella (Elith and Leathwick 2009). In a simplistic definition these model types fit under this umbrella, having a target species occurrence as the independent variable and physical attributes as the dependent variables; however, the focus of this research lies on the more traditional definition of habitat modeling.

Geospatial Predictive Software

MaxEnt is a species distribution modeling technique that is widely available due to it being an open-source geospatial type software. The function is to find the probability distribution of maximum entropy, which is the best probability of distribution when several distributions are possible. Features or variables from known distributions of a species are analyzed for areas of similar aspects to predict potential distributions for that target species (Phillips et. al 2006). The

software can be used when there are limited amounts of distribution data of a species and only requires data on the species occurrence to predict habitat suitability, making it advantageous compared to other algorithms (Byeon et. al 2018). Due to its use of an organism's presence as a function to habitat variables, MaxEnt's versatility and availability creates an optimum distribution research application. Hardman (2014) conducted a study on *A. aeneus* occurrences using MaxEnt and successfully located a previously unknown population, though the study was restricted to the Blue Ridge Escarpment. More Recently, Thames et al. (2021) built a similar model via MaxEnt for *A. aeneus* in Tennessee and Niemiller et al. (2022) utilized the model to determine the species geographic extent and identify present and future stressors to the species.

Objectives

If integral habitat variables of this species life history can be identified, then utilization of a predictive model will have the innate ability to identify available habitat for the species. This study aims to address the question: can MaxEnt accurately predict the occurrence of *Aneides* aeneus within areas containing their appropriate habitat? The primary objectives of this research are to investigate the following factors:

- (1) Analyze whether Maxent is an appropriate tool for creating a predictive habitat model.
- (2) Locate potential populations of *A. aeneus* in Tennessee, to better understand and recognize the landscape metrics for the species to be present.
- (3) To evaluate suitable habitats that may facilitate occupancy and to gain insight for future studies on their distribution.

The approach appointed to these obstacles was to focus on what is known about *Aneides* aeneus life history and their habitat associations. Variables such as climatic, terrain and land use

metrics, in combination with occurrence data were chosen to develop the model. Creating a predictive species distribution model may give us better insight on how to locate this species, to aid land managers and conservationists in Tennessee in decision making for future projects and assessing the species status.

CHAPTER II

MATERIALS AND METHODS

Species Distribution Modeling

MaxEnt is an open-source modeling program that requires downloading from the internet and needs only be extracted and installed on a computer. MaxEnt was determined to be the best software available to utilize for this specific study. Merow et al. (2013) notes that the MaxEnt is software package is highly favored for modeling of species distribution and environmental niche modeling, due to two probable rationales: first, that it typically will outperform in predictive accuracy compared to other methodologies; and second, MaxEnt is uniquely uncomplicated to execute. MaxEnt possesses the accessibility, utility, and reliability that was necessary for this research. MaxEnt's function is to evaluate the probability distribution of maximum entropy, that is, a distribution that is either broadened or uniform (Phillips et al. 2006).

To use this program, a data file containing the species observations must be used and the environmental variables must be indexed as either categorical or continuous type data.

Continuous variables contain numeric values derived from measurements whilst categorical variables have a limited number of possible values (Phillips and Dudik 2008). Examples of the differences between such data types would be elevation and soil type.

Habitat

Aneides aeneus encompasses a range that extends from Pennsylvania, Ohio, and Indiana down through Maryland, Virginia, West Virginia, Kentucky, Tennessee, Georgia, Alabama, and

Mississippi. There are disjunct populations which occur in the Blue Ridge Escarpment of North Carolina and South Carolina. In total, they occur in 13 states of the Eastern United States (Figure 1.1).

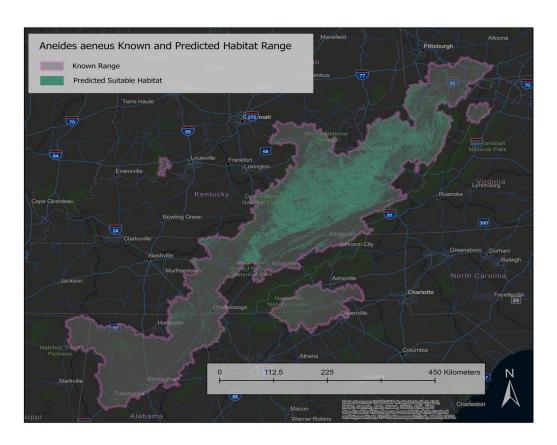


Figure 2.1 The known range and predicted suitable habitat for *Aneides aeneus*. Data acquired through the U.S. Geological Survey's Gap Analysis Project

The Southwestern Appalachians include the Cumberland Plateau region that resides within Tennessee. This region encompasses low hills, upland, valleys, and ridges. These ridges are about 300m higher in elevation than that of the ecoregions to its west; therefore, it receives cooler temperatures and more precipitation than the surrounding areas. The geology includes Pennsylvanian-aged conglomerate, sandstone, siltstone, coals, and shale; all of which have acidic

and well drained soils laid upon them. *A. aeneus* are known to occupy this ecoregion within the rocky outcroppings (Niemiller and Reynolds 2011).

As Tennessee has a vast array of topography and habitats within its borders, narrowing the study area for the species involved analyzing where they have been historically located and which surrounding areas had the potential for their presence in the present day. Topographically speaking, *A. aeneus* range is visibly located on the western edges of the Appalachian Mountains, including the Ridge and valley as well as portions of the Appalachian plateau; in Tennessee, this includes the Cumberland Mountains also known as the Southwestern Appalachians. The exception to this is the disjunct populations located within the Blue Ridge Escarpment in North and South Carolina. It is often cited that Weller (1931) located a population within the Great Smoky Mountain National Park; however, no records of such population have been made since (Petranka et al. 1998). Further west, the landscape begins to drop from the eastern highland rim into the Nashville basin and drops further as you continue to approach the Mississippi River, making suitable habitat and topography lessen across the state's landscape.

A. aeneus are known to inhabit rock faces consisting of limestone, sandstone, granite, or schist (Waldron and Humphries 2005). They have an affinity to the naturally occurring crevices that are present within these geologic structures and prefer deeper crevices further from the opening, than shallow ones; it is possible that they prefer this as a mechanism to avoid predation (Gordon and Smith 1949; Armstrong 2010; Smith et al. 2016). Occupation of crevices offers several advantages to this species. One of which is the more consistent temperatures (Armstrong 2010) that are likely due to the insulative properties of the rock structures. In this case, humidity levels would also have higher retention. This is an important aspect when considering the permeability of amphibians' skin and their enhanced vulnerability to desiccation. There have

been accounts of *A. aeneus* occupying woody habitats (Pope 1928; Welter and Barbour 1940); however, there is little evidence suggesting that they firmly occupy these areas. An explanation may be that they may move to these areas occasionally, but do not strictly occupy them for long periods of time. Gordon (1952) postulated that *A. aeneus* began occupying rock structures as a secondary habitat preference as competition with other species increased, as even their closest western relatives are considered "more arboreal" than *A. aeneus*.

Multiple variables contribute to adequate habitat for the species; Hinkle et al. (2018) recognized that rock outcrops differed through the vegetation and crevice types present, higher rock walls have lower degrees of canopy cover as the trees occur at greater distances.

Because of their strict habitat requirements, *A. aeneus* distribution across its range is patchy (Petranka et al. 1998). This species is known for its cryptic nature and can be relatively difficult to detect, even when suitable habitat is plentiful. When *A. aeneus* are observed, it is unusual to see them in abundance (Newman et al. 2018). This restricts the capabilities of collecting adequate sample sizes for statistical analyses as there is potential for populations to be much greater than what has been perceived by the researcher.

Several authors have noted the correlation between the distribution of *A. aeneus* that occurred during the tertiary period, at which time deciduous forest types spanned the continent, and the association this species has with mixed-mesophytic forest types of today, which strictly occur in the Appalachian region (Lowe 1950; Gordon 1952; Wake 1966; Bruce 1968; Waldron and Humphries 2005). Due to this historical distribution in relation to their current distribution and habitat associations, *A. aeneus* can be considered a historical marker of these ancient forest systems and habitat structures.

Species Overview

Aneides Aeneus, can be described as having a black dorsum with irregular green to yellow markings that are "lichen-like" and the ventrum is a lighter yellow (Bishop 1928). They are more simply described by Zim and Smith (1956) as being "dark with greenish blotches"; in addition, A. Aeneus have unique morphological adaptations for climbing, as they have squared-toe tips and a prehensile tail (Patton et al. 2019) which are optimal attributes for climbing in semi-arboreal environments. This species is particularly agile, having the ability to jump many times its length and the ability to obtain inverted positions on surfaces (Bishop 1928). Gordon (1952) denotes them as the most terrestrial of the plethodontid species occurring in the eastern United States, whilst Dunn (1926) describes them to be the most primitive member of their genus due to Plethodontidae originating from the Appalachian Highlands. A. aeneus was thought to be the only species semi-suited to arboreal habitats in the eastern United States for over a century, until recently. The Hickory Nut Gorge Salamander Aneides caryaensis was specified as a distinct species from A. aeneus due to DNA sequencing and morphological differences. This species is located in the Hickory Nut Gorge of western North Carolina (Patton et al. 2019).

During their active season, *A. aeneus* are thought to move out from the rock crevices that they normally occupy to forage and search for mates at night (John et al. 2019). Their active period generally ranges from late April to November, with four main periods of their annual cycle (Cupp 1991). Breeding can be said to take place around May through late September, from September to November the species aggregate and disperse, from November to late April they hibernate, and in late April into May they aggregate and disperse again (Gordon 1952). It is key to keep in mind that various studies suggest slight differences in the timing of the four periods, likely due to the location of the study and its seasonality.

Geospatial Data Acquisition

Data was acquired through an assortment of sources that were available to the general public. Variables were chosen based on the target species life history (Thames et al 2021; Hardman 2014; Niemiller and Reynolds 2011; Waldron and Humphries 2005; Petranka 1998; Cupp 1991; Gordon 1952; Bishop 1928) through a literature review process (Table 1.1). Selecting the appropriate covariates is crucial as selecting features that are not inherently related to the species occurrences could result in low AIC scores or biases with skewed probabilities.

Table 2.1 Variables used for the development of the MaxEnt predictive habitat model of *Aneides aeneus*; processed in ArcGIS Pro 3.x.x

Variable	Data Type	Source
Tennessee <i>A. aeneus</i> Observations	Occupancy Variable	Tennessee Wildlife Resources Agency (Personal Contact)
Elevation	Continuous	U.S. Geological Survey
Aspect	Continuous	*Derived from Elevation
Slope	Continuous	*Derived from Elevation
National Land Cover Database (NLCD 2019)	Categorical	Multi-Resolution Land Characteristics Consortium- U.S. Geological Survey
NLCD 2016 USFS Tree Canopy Cover (CONUS)	Continuous	Multi-Resolution Land Characteristics Consortium-U.S. Geological Survey
Geology Type	Categorical	U.S. Geological Survey
1-19 Bioclim	Continuous	WorldClim
Soil Types Soil Survey Geographic Database (SSURGO)	Categorical	Natural Resources Conservation Service- U.S. Department of Agriculture
Resilience	Categorical	Conservation Gateway- The Nature Conservancy

Data was processed using ArcGIS Pro 3.x.x software and transformed to the same projection (NAD 1984) and layers that were available as vectors were converted to rasters. All of the rasters were processed in 30M resolution. For a few layers, appending and merging of data was necessary due to the format of the source data. Each variable data layer was processed to the same extent using a clipped Tennessee counties layer. Because Tennessee encompasses a broad range of topography and therefore habitats, the extent of the study region was determined by using the historical range and locations of *A. aeneus* observations. All of the variables being utilized for the model were then converted from rasters to ascii files, as MaxEnt requires this format for running the program (Hunt 2018). A subsample run type was used, meaning that 75% of the observation points used to build the model train it and the remaining 25% of points are used to test the model.

Once MaxEnt completed the output, the resulting ascii files were opened in ArcGIS Pro. Files were reverted to rasters, polygons, and finally to points. The result raster points were reclassified as a percentage: 0.0-1.0 (Figure 1.2), where the values represent the probability of suitable habitat for the target species within that area. Due to the intensive nature that it takes to survey for this species, points that occurred in areas with an 80% or above suitable probability were selected for further analysis.

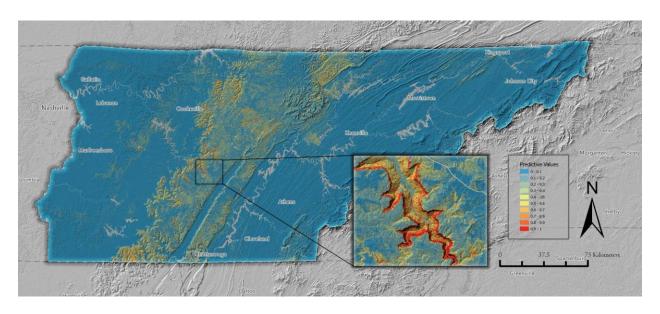


Figure 2.2 The MaxEnt output, where raster pixels were reclassified to a probability scale of 0.0-0.1 to represent possible *Aneides aeneus* habitat

Constricting points was a necessary step due to the accessibility and time constraints of the project. Overall, 22,246 pixels within the study region were ranked as 80% probability or above (Figure 1.3). The PAD US 2.2 layer (hosted by the Gap Analysis Project under The U.S. Geological Survey) was used with the "select by attributes" function to isolate points that occurred within public and natural areas that were owned by the state (Figure 1.4) and therefore negating the need to gain accessibility to privately owned lands (Table 1.2). This process trimmed the possible areas to 7,773 pixels remained; from that set, pixels were further isolated using the Tennessee trails layer (Figure 1.4) and a buffer of 3000M, this distance was selected as it was to be the maximum distance traveled off trail by the researchers. Ultimately, 3,782 pixels remained with 80% probability of suitable habitat, while meeting the proximity criteria for sampling. The "Create Random Points" tool was then utilized, to reduce bias of chosen survey points, using a maximum of 500 possible points and a parameter of 100M between them. The maximum points input was set to 500 to ensure that an adequate number of field locations would

be considered and the parameter of 100M was set to ensure randomized points would be spatially balanced and not occurring within the same raster pixels, allowing for optimal observations of an area.

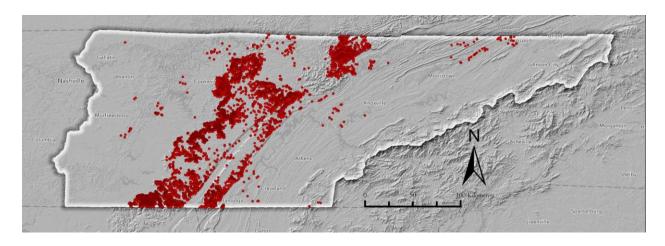


Figure 2.3 Pixels that ranked as 80% probability of suitable habitat or above within the study region were converted to polygons and then to points, giving 22,246 points of possible suitable habitat

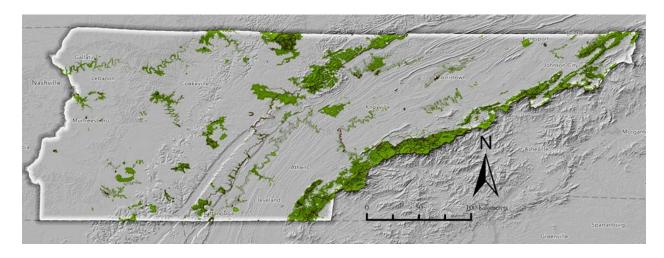


Figure 2.4 The PAD US 2.2 (hosted by the Gap Analysis Project under The U.S. Geological Survey) and Tennessee trails layers used to restrict points for suitable accessibility

Table 2.2 Vector layers used with the "Extract by Mask" tool to isolate study areas and potential field points

Additional Vector Layers	Source
Tennessee Counties	Info GIS Map (https://www.igismap.com/)
Protected Areas Database of the United States (PAD-US) 2.1	U.S. Geological Survey (https://www.usgs.gov/)
Tennessee Trails	U.S. Geological Survey (https://www.usgs.gov/)

The layer was then transformed to WGS 84, the appropriate coordinate system for the GPS system (Garmin GPSMAP® 64st). Garmin BaseCamp® (Version 4.7.4) is a free downloadable software that allows for the user to upload and download geographic points or routes between a computer and a GPS unit. The program was used to import the point layer into the GPS system. For route planning and traveling simplification, Google MyMaps was used by uploading the points for the purpose of mapping potential routes of travel. This also aided in the ability to share my travel location with other colleagues and plan appropriately.

Habitat Data Acquisition

Field locations were visited from June 11th, 2022 – October 17th, 2022 (Figure 1.5). Due to the extraneous effort that visiting these sites required and considering the heightened temperatures that occur in the afternoons, typically 1-3 sites that were within hiking distance of each other were visited during a single field day. When visiting the target sites, if exceptional habitat was spotted along the route, it was also searched for the target species. In instances when these untargeted areas produced a sighting, the same habitat measurements were collected.

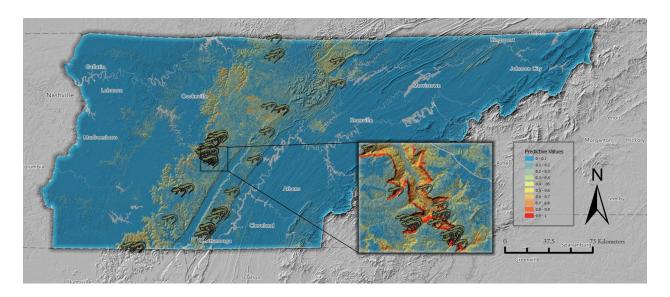


Figure 2.5 Generated randomized points used for field surveys

The target location was approached as close as practical, due to the steep cliff sides and overall terrain, it was not always possible to get to the exact location. A new coordinate point, the time, temperature, relative humidity, dew point, barometric pressure, elevation, aspect, surface temperature, dominant foliage, geology types, and the canopy density were collected for each cardinal direction and overall, at each predetermined site.

A. aeneus has made more frequent debuts in scientific literature in more recent years, there are still elements of their habitat or landscape selection that are unknown. Due to this somewhat limited availability of knowledge on A. aeneus, microhabitat characteristics and additional measurements were sought. Upon observation, if a species of salamander was located within the vicinity of the target site, another suite of data were collected. The surface temperature of the location they were found, the nearest neighbor foliage type and distance, largest overstory tree contributing to the area, if A. aeneus were found within a crevice, the depth, width, and height were recorded. If the specimen was able to be captured, the weight, snout-vent length

(SVL), tail length (TL), head width (HW), and total body length (TBL) measurements were taken.

Exploratory Regression and Ordinary Least Squares

To further understand the spatial trends and distributions of *A. aeneus*, tools known as Exploratory Regression and Ordinary Least Squares (OLS) were utilized within the ArcGIS Pro 3.x.x software. These programs require explanatory variables in conjunction with a dependent variable to process regression analyses (Hunt 2018). The Exploratory Regression analysis purpose is to assess all possible combinations of explanatory variables in order to identify potential the model best suited for Ordinary Least Squares analysis (Esri 2019). Ordinary Least Squares (OLS) assume fixed relationships by applying a single equation to all features and producing a predictive model of the dependent variable (Esri 2019). OLS accounts for the percentage of variations in the dependent variable based upon the chosen explanatory variables, if the relationship between variables is consistent (Ortiz-Yusty et al. 2013).

The occupancy variable consisted of *A. aeneus* observations from both the fieldwork conducted in 2022 and the TWRA observation data. Points from the TWRA were restricted within the same buffer areas as the randomized field points and observations that occurred prior to 2004 were excluded. Both data were included to ensure there were enough points for the program to properly execute the analysis. In total, 51 observation points were used, each having a total count of *A. aeneus* spotted within the area. To complement the observation points, 51 pseudo-absence points were then generated within the same buffer areas and were merged with the observational data to create a singular vector layer for analysis. The Elevation, Slope, Aspect, and Canopy Cover rasters that were processed for the MaxEnt analysis were employed as the

explanatory variables. This required extracting the raster values at all 102 points, by using the Extract by Mask tool. These values were then merged into the observation vector attribute table.

CHAPTER III

RESULTS

Model Summary and Field Results

The area under the curve (AUC) score generated from the MaxEnt program was 0.93 with a standard deviation of 0.013. The top five contributing variables all contributed above 8%, with the major variable being soil type at 33.5% contribution to the overall model (Table 1.3). The Jackknife of regularized training gain gives further insight to the contribution of each variable to the model and further highlights the contribution that soil type made to the model, denoted as "SSURGO_Ras" (Figure 1.6).

Table 3.1 The top 5 contributing environmental variables to the MaxEnt model, with their associated contribution values

Variable	Percent Contribution
Soil Type	33.5
Aspect	11.9
Mean Diurnal Range	10.9
Canopy Cover	10.1
Geology Type	8.3

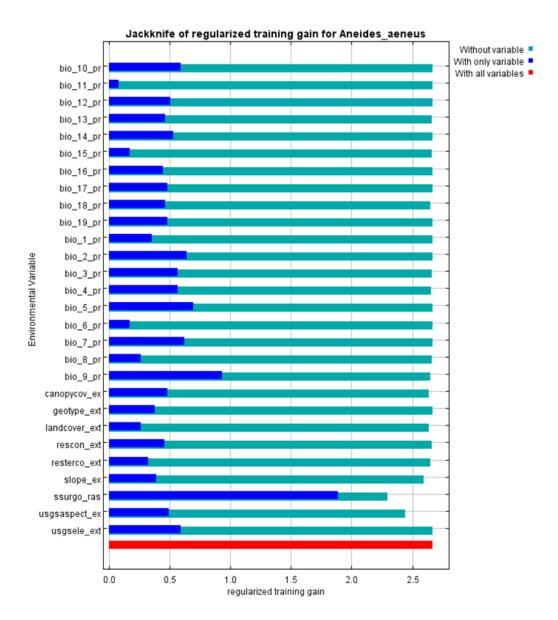


Figure 3.1 Jackknife of regularized training gain for *Aneides aeneus*, created by the MaxEnt program. Teal bars indicate the overall model's strength without the inclusion of that specific variable. Dark blue bars indicate the model's overall strength when the variable is used independently

Over the course of the single field season, a total of 49 field locations out of the possible 77 (63.6%) were visited; of those, 18 were found to have positive presence of *A. aeneus* (36.7%). Thirteen of those sites were previously undocumented by the Tennessee Wildlife Resources Agency (72.2%). Forty-five of my 49 sites had suitable habitat (91.8%), with four being deemed

as having inadequate habitat (8.2%). One of those sites was in the parking lot of a trailhead and the other was in a powerline clearing adjacent to a dammed lake. Each of the sites were surveyed a single time across a single field season.

The highest air temperature when presence was detected was 28.5 °C, while the lowest was 10.7 °C. Surface temperature of the rock structures would parallel the air temperatures, with the highest surface temperature occurring at the same location on the same day and time as the highest air temperature 29.17 °C and the lowest temperature being 10 °C. The highest humidity level was at 92.5% and the lowest humidity was measured at 44.5%. The highest Dew point measurement was 76.6% and the low was 33.6%. The highest barometric pressure was 1038.8 MB and the low was 965.32 MB. The maximums and minimums of each of the above measurements did not necessarily occur on the same field day, meaning that they occurred over the course of the field season (Table 1.4).

Table 3.2 Field recorded climatic measurements, taken at the time of arrival to target location

		Air Temperature °C		Barometric Pressure MB	Dew Point %
	23.5	27.22	80.5	1021	NULL
	22.6	22.22	82.5	1020.7	NULL
	22.1	25.67	79.5	1017.5	NULL
	21.3	20.78	64	1023.3	NULL
	26	24.5	89.4	NULL	72.9
	28.5	29.17	78	NULL	76.6
	24.7	24.22	92.5	1019.4	73.2
	21.5	22.5	84.2	1018.1	67.4
	21.3	22.28	88.2	1021.3	68.6
	23.6	21.22	90.4	1032	67.3
	24.8	23.39	90.3	1038.8	71.1
	19.7	21.61	89	1017.3	68.5
	20	21.44	74.8	1015	64.3
	12	10	77	1013.6	44.5
	10.7	13.5	60.2	1026.1	43.4
	12.4	12.94	44.5	107.2	33.6
	15.2	19.83	47.8	1027	33.7
	11.7	13.44	62.5	1026.8	47.3
Mean	20.09	20.89	76.41	965.32	59.46
Median	21.4	21.915	80	1020.85	67.35

A few studies have focused upon crevice size and selection by *A. aeneus* (Smith et al. 2017; Armstrong 2010; Rossell et al. 2009; Gordon 1952), so the collection of such data was executed during the field surveys when positive *A. aeneus* presence was observed (Figure 1.7). In several cases the structure would exceed 150 mm, which is the maximum length acquired by the calipers used. Five width measurements that exceed 150mm were taken by utilizing the calipers twice within the space; this technique was not used again as in several cases it was not possible due to the narrow nature of the structures. Typically, occupied crevices were horizontally orientated (86.8%); that is, they were wider than they were tall, which agrees with Gordon's (1952) observations of crevice structure in the Blue Ridge Escarpment and Smith (2017) in Virginia.

Crevice Structure

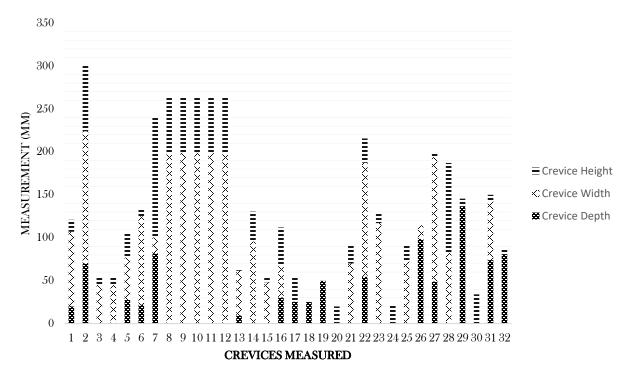


Figure 3.2 Measurements taken of crevices that were occupied by *Aneides aeneus*.

Measurements were taken in Millimeters and in many cases measurements in a singular direction would exceed 150 mm

Morphological measurements of *A. aeneus* were taken when possible, to aid in the lack of such data available (Table 1.5). All individuals measured were adults, in instances where extraction was deemed to cause stress on the animal, efforts to extract ceased. In a singular circumstance of a female guarding her eggs only a photograph was captured so as to avoid causing her unneeded stress.

Table 3.3 Morphological Measurements of *Aneides aeneus*

	SVL	TL	HW	TBL	Weight
	(mm)	(mm)	(mm)	(mm)	(gr)
	45.4	60	6.2	107.65	29.66
	51.16	66.2	8.66	120.16	34
	28.66	Null	5.83	Null	1.26
	35.66	46.83	6.16	83.33	1.46
	50.3	48.3	6.6	101.1	2.8
	31.5	41	5	71.5	1
	39.6	27.5	6.5	71.3	1.6
	39	59.6	7	99.6	2.6
	28.6	33.1	3.8	61.5	0.7
	50.16	61.66	6.66	100.16	3.56
	33.83	44.5	6.66	79.16	1.5
	31.66	28.33	5.5	62.83	1.2
	33	38.67	6.33	66.67	0.43
	36.17	46.83	6.67	84	1.77
	41.33	54.67	7.83	101.67	2.3
	36.17	48.5	5.17	87	1.8
	37.5	49	6	91.5	2.6
	33.3	37.3	5.16	72.56	2
	39.25	50.75	7	85.5	2.1
	35	43.6	5.5	78.6	1.3
Mean	37.8625	46.64947	6.2115	85.56789	4.782
Median	36.17	46.83	6.265	84	1.785
Mode	36.17	46.83	7		2.6

To further assess the data collected, a series of graphs were created utilizing the processed rasters from the development of the MaxEnt model. It was of interest to the author to further investigate possible trends of the available *A. aeneus* data. The TWRA data was combined with the field measurement point taken in the summer of 2022 to do this. Detection of *A. aeneus* was assessed and as expected, higher observations occurred through May and June, and then had a slight spike in September through October (Figure 1.8). These periods are the known active season of the species.

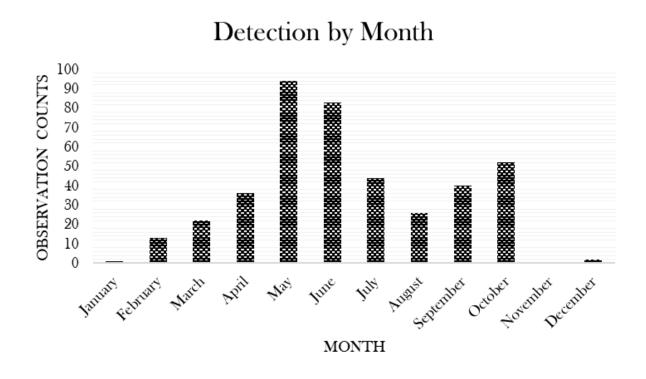


Figure 3.3 *Aneides aeneus* observation count by month observed. Data from field observations and Tennessee Wildlife Resources Agency

The aspect of inhabited rock structures was also of interest. There has been a mixed consensus on whether they primarily occur on certain facing slopes or not. The TWRA and study

observations were combined first (Figure 1.9) and then the Field surveys on their own (Figure 1.10). Lastly, the actual aspect which was recorded in the field was graphed to see if there was great difference between the raster values and the field observations (Figure 1.11). Between the two graphs that were generated with the raster values, they both show western facing structures to be the most common with *A. aeneus* observations. This goes against any literature that was considered in the literature review process. Interestingly, the graph created from the raster values and the one created from field observations do not agree with one another. Field observations of aspect seem to favor North and Southeastern facing slopes, which disagrees with observations made in other studies, where the majority were south facing (Newman et al. 2018 and Bruce et al. 1968). This differentiation is most likely due to spatial errors coinciding with the raster pixels as they cover a 30 m area and give the average of that overall area, whereas the field measurements are taken at the direct location of the rock structure where *A. aeneus* was observed.

Aspect of Positive Presence Points

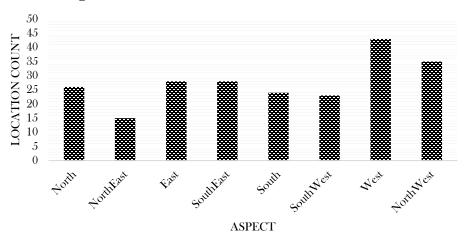
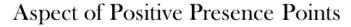


Figure 3.4 The aspect of the combined field and Tennessee Wildlife Resources Agency data. This data was derived using raster data



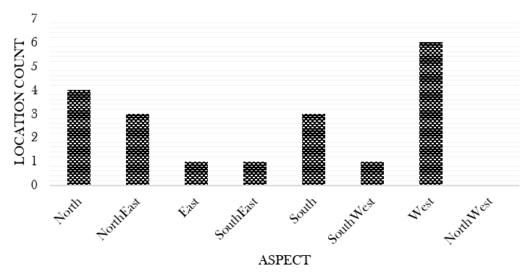


Figure 3.5 The aspect of the field collected positive presence points. This data was derived using raster data

Aspect Taken in the Field

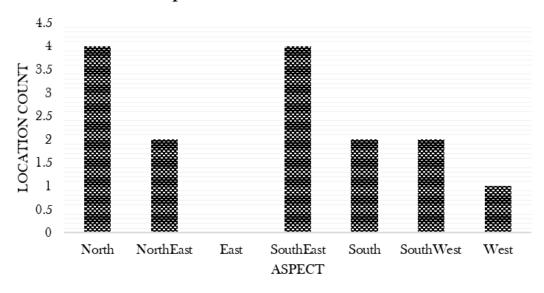


Figure 3.6 Aspect manually measured at each positive presence point

Finally, the predicted suitable habitat values generated via MaxEnt, were pulled for each locality with positive presence points, this was done to see if there was a difference between the predicted area and where presence was actually observed. For all observational data, the majority of points occur in the 60-80% range (Figure 1.12) and in the field observations the majority fall in the 40-70% range (Figure 1.13). Upon observation, many of the points coinciding with low predictability values are adjacent to pixels with much higher probability. The same spatial errors that are speculated for aspect are seen here as well, due to the size of the raster pixels in relation to the size of the rock structure.

Aniedes aeneus Probability Values According to MaxEnt

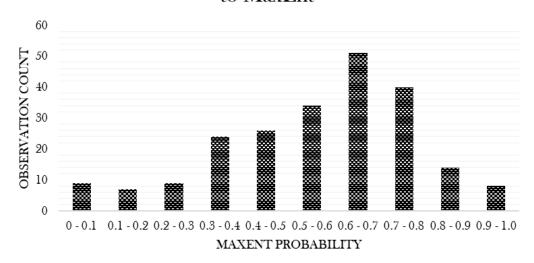


Figure 3.7 MaxEnt raster probability of suitable habitat at positive presence points of *Aneides aenus*. Derived from both Field collected data and the Tennessee Wildlife Resources Agency Data

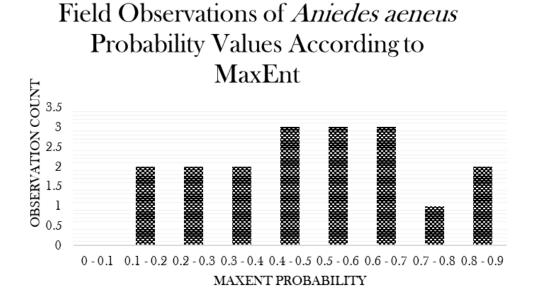


Figure 3.8 MaxEnt raster probability of suitable habitat positive presence points of *Aneides aenus*. Derived from field collected observational data

Exploratory Regression and OLS

The Exploratory regression results showed a model variable significance for slope of 0.01, this indicates strong positive significance of slope. Slope also contributed a positive significance to other variables, when modeled together (Table 1.6). Slope by itself had an adjusted R^2 of 0.05, this indicates that the variable is strongest when it is used solely.

Table 3.4 Results of the Exploratory Regression showing the Highest Adjusted R-squared Results. The asterisk (*) indicates model significance (* = 0.10; ** = 0.05; *** = 0.01)

Summary Choice out of 4	Model	Adjusted R ²	AICc	JB	K(BP)	VIF	SA
1	+Slope***	0.05	475.57	0.00	0.58	1.00	0.04
1	+Canopy Cover	0.00	481.14	0.00	0.48	1.00	0.04
1	+Elevation	-0.00	481.75	0.00	0.33	1.00	0.02
2	+Aspect +Slope**	0.04	477.56	0.00	0.70	1.00	0.03
2	+Elevation +Slope**	0.04	477.57	0.00	0.62	1.03	0.03
2	+Canopy Cover +Slope**	0.04	477.63	0.00	0.78	1.12	0.04
3	+Aspect +Elevation +Slope**	0.03	479.59	0.00	0.69	1.04	0.03
3	+Canopy Cover +Aspect +Slope**	0.03	479.68	0.00	0.84	1.13	0.03
3	+Canopy Cover +Elevation +Slope**	0.03	479.70	0.00	0.77	1.14	0.03
4	+Canopy Cover +Aspect +Elevation +Slope**	0.03	481.77	0.00	0.82	1.15	0.03

The OLS was executed by utilizing slope as the explanatory variable against *A. aeneus* localities as the dependent variable. Explanation of the following results was written with the aid of Esri's ArcGIS Online websites tool references (2023). The Robust t-value being a large number of 3.07 leads to the rejection of the model's assumed null hypothesis that slope is not influencing the model (Table 1.7). The Multiple R-squared and Adjusted R-squared are used to evaluate the model's performance; specifically, the adjusted R-squared value explains the

variation in the dependent variable (Esri 2019). For slope, R-squared =0.047987 (Table 1.8). Model significance is gauged according to the Joint F-statistic and the Joint Wald statistic, both of which are significant. The joint F-statistic is significant with a value of 0.035080, indicating that slope is an effective explanatory variable. The Koenker (BP) statistic represents the stationarity of the model; this test assumes that there is non-stationarity, which is rejected with a value of 0.583700. Lastly, the Jarquez-Bera statistic determines if there is model bias by assessing distribution, the model is not normally distributed with a significant value of 0; according to this and visually assessing the histogram, the model is biased (Figure 1.14).

Table 3.5 Ordinary Least Squares analysis result summary. The asterisk (*) indicates model significance (* = 0.10; ** = 0.05; *** = 0.01)

Variable	Coefficient	StdError	t-statistic	Probability	Robust_SE	Robust-t	Robust_Pr
Intercept	0.43	0.28	1.52	0.130308	0.22	1.92	0.056496
Slope	0.03	0.01	2.62	0.009792*	0.01	3.07	0.002641*

Table 3.6 Ordinary Least Squares diagnostics. The asterisk (*) indicates model significance (* = 0.10; ** = 0.05; *** = 0.01)

Number of Observation	118	AIC	475.567001
Multiple R-Squared	0.056124	Adjusted R-Squared	0.047987
Joint F-Statistic	6.897534	Prob(>F), (1,116) degrees of freedom	0.035080*
Joint Wald Statistic	9.447706	Prob(>chi-squared), (1) degrees of freedom	0.002114*
Koenker (BP) Statistic	0.300291	Prob(>chi-squared), (1) degrees of freedom	0.583700
Jarque-Bera Statistic	1249.370460	Prob(>chi-squared), (2) degrees of freedom	0.000000*

Histogram of Standardized Residuals

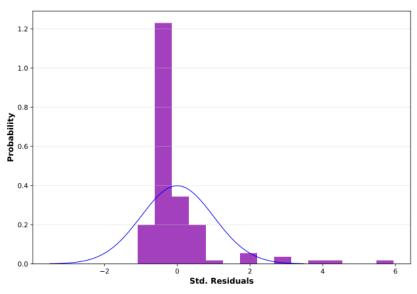


Figure 3.9 Histogram of Standardized Residuals. Bars represent the actual distribution; the thin blue line indicates where normal distribution would occur

CHAPTER IV

DISCUSSION

Data Relationships

Based on the successful locating of this cryptic species on a broad scale, MaxEnt was determined to be an appropriate tool for creating a predictive habitat model. Over 36.7% of sites visited were positive for *Aneides aeneus* presence and 72,2% of those were previously undocumented by the state. An AUC score of 93% with a standard deviation of 1.3% is an appropriate statistic for model fitness as it is neither over nor under fitting the model. Over or underfitting models can produce erroneous results which can lead to surveying areas with deficient habitat or over biased results which could lead to surveys of areas that do not quite fall into the zone of appropriate habitat, where slopes may be too steep or soil too thin for positive presence. Extracting the raster derived pixel values from the MaxEnt output revealed that the majority of my positive presence points coincided with areas that were estimated to have 40%-70% suitable habitat and overall with the TWRA observation data, areas were estimated to have 60%-80% suitable habitat.

These results also verify the objective to locate potential populations of *A. aeneus* in Tennessee. Identifying field variables which co-occurred in the vicinity of *A. aeneus*, such as, aspect, crevice structures, climatic data, and the best time of season to detect them aids in the understanding and recognition of landscape metrics for the species to be present. Individuals were found to be partially or fully emerged through a broad range of climatic conditions. Air

temperature varied broadly from 28.6 °C-10.7 °C and surface temperatures followed 29.17 °C-10 °C. Neither Relative Humidity (92.5%-44.5%) or Dew point (76.6%-33.6%) indicated that any of the areas were particularly dry across our field season and barometric pressure was never in any extreme, ranging from 1038.8mb-965.32mb., meaning the barometric pressure was never at a significant level.

The majority of occupied crevice structures observed were horizontally orientated (86.8%), which agreed with previous research (Smith 2017; Gordon 1952) and may aid future researchers as to where to search on rock structures. While morphological measurements were taken on selected individuals (n=20) out of the total number of *A. aeneus* actually observed (n=39), no analysis was conducted due to the sample size. Combining my field data with the data the TWRA offered a more robust data set to work with, which was able to provide further insight to trends occurring at positive presence localities. Detection by month showed that the most efficient time to survey may be May-June, knowledge which may assist future research if a population survey of an area was to be conducted. Looking at the aspect of the rock structure where *A. aeneus* occurs offered conflicting results as the raster derived data indicated western facing slopes to have the most prevalence, whereas my field collected data indicated North and Southeastern facing slopes to have more prevalence.

Evaluating sites with suitable habitat where presence was not observed, leaves one to wonder as to which habitat variables are inherently important for the species to be present? Or are climatic variables more fundamental to the observation of an individual, due to their ability to inhabit the deep crevasses of rock structures? Of my sites, 91.8% seemed to have adequate or above adequate habitat; however, presence was only observed at 36.7% of sites. Because these

were single field site visits, there is still potential for the species to be present in the remaining 63.3% of sites.

When analyzing habitat features such as slope, canopy cover, elevation, and aspect, the exploratory regression indicated that slope was a major contributing factor to A. aeneus presence (R^2 =0.05). To further test the model, an OLS was executed, the results corroborated the exploratory regressions; however, using the slope solely created an over biased model (Jarque-Bera Statistic X^2 =0.00), meaning that slope was overfitting as the model. This signifies that slope is a stationary variable, a consistent measure which correlates to A. aeneus presence. It is the recommendation of the author to include this variable in future models for A. aeneus.

Future Directions

Additional sites with predicted suitable habitat should be visited and habitat measurements should be taken at both new locations and sites where observations have historically occurred to gather more data on site selection preferences. Standardized methods for these surveys should be implemented to conduct population surveys of certain areas to get an idea of how well they are doing in certain natural areas, and the state overall. As Newman et al. (2018) states in her work with *A. aeneus*, single site field visits may have their own limitations when it comes to observation numbers due to uncontrollable circumstances, such as droughts.

Depending upon available data, similar models could be executed on finer scales to smaller regions or to the entirety of their range. A suggestion to any that may develop a model using similar methodology, would be to isolate points from a starting point using a buffer, such as a trailhead. Distance from the trail can cause sites to be selected several miles into an area which may be quite far from any access point. Utilizing LIDAR data in smaller regions has also

proven to be effective from Smith and Mullin's 2021 publication "Light Detection and Ranging (LiDAR)-Assisted Detection of Rock Outcrops in Appalachian Hardwood Forests". Using LIDAR post-MaxEnt processing could aid researchers in navigating the difficult terrain that the species occurs in. Allowing the selection of suitable sites to be more manageable. The main hardships to study this species is the overall terrain and areas that the species occurs in, secondly locating this species is particularly difficult as they seamlessly blend into their habitat due to their overall coloration and patterning.

Other habitat variables should be considered to identify if or how they relate to *A. aeneus* presence to an area. One such variable may be distance to a water source. *A. aeneus* have a reputation for being one of the "dryest salamander species" at least in the Southeastern United States; however, they are still amphibians and therefore do require some access to water. The majority of their water access may be from the humidity and dew accumulation that occurs on and within rock structures. As temperature and pressure changes, many rocks "swell" and release this moisture; particularly, those with clay minerals intermixed (Vermeulen 2011; Chugh and Missave 1981). Newman et al. (2022) indicated that these rock structures may also act as a buffer from major temperature and humidity shifts. Proximity to a water source may aid in the overall humidity of an area and therefore support the accumulation that occurs on and within the large structure.

Ray (1958) studied the responses of 8 salamander species to water loss, 3 of which were western occurring *Aneides* members. He concluded that ultimately, the salamander's tolerance to water loss directly correlates to the animal's environment; if they inhabit drier microclimates, they will have a greater tolerance to desiccation. Importantly, his results reflect what we know about *A. aeneus*'s life history, in that they are adapted to dryer conditions and may tolerate more

extremes than some of their sister taxa; nevertheless, they are still of the class amphibia and therefore will inevitably desiccate with prolonged inaccessibility to water. *Plethodon jordani melaventris*, now simply classified as *P. jordani*, was compared against *A. aeneus* by Gordon (1952) to determine the species moisture losses in a controlled environment. Overall, *A. aeneus* was able to withstand higher water losses and endure desiccation greater than *P. jordani*, leading the author to conclude that *A. aeneus* has the advantage of being able to withstand harsher conditions than that of its sister taxa and thus is able to evade competition of habitats.

Other aspects that should be considered are the overall biota and fauna that coinhabit these structures. If no organismal activity was detected on or within the rock structures, *A. aeneus* were never spotted. It may seem apparent; however, one must wonder why some of these otherwise seemingly suitable structures are desolate of any life to begin with? Camel Crickets (*Ceuthophilus sp.*) were more commonly than not found to be residing in the same rock structures as *A. aeneus*, along with slugs (*Philomycus sp.*) and millipedes (Diplopoda), all of which are noted as to co-occurring with *A. aeneus* (Cupp 2017; Gordan 1952). Interactions between *A. aeneus* and *Philmycus sp.* have rarely been noted in previous publications (Newman et al. 2022). Additionally, a variety of other organisms were frequently seen in or on the rock structures such as: Araneae species, gastropods, moss, and lichen. *A. aeneus* were located on the same rock structures as other plethodon species; however, they were never found to be cohabiting with one another, and never a plethodon of another genus. Possible interactions of other taxa with *A. aeneus* should be studied to determine if any type of relationships exist.

Possible Threats

Aneides aeneus is ranked as Near Threatened under criteria A3cde for The IUCN Red List of Threatened Species, where current populations are considered decreasing with the species being most recently assessed in 2021. Without proper population surveys, the A. aeneus status across its range in many cases is more speculative than certainty. Currently, there are two website results when you perform an internet search to order an A. aeneus individual (Indoor Ecosystems and Frogs Direct via The Frog Whisperer, LLC ®), which may indicate the public's imminent awareness of their existence. The source of these salamanders is not disclosed on either website so it cannot be said decisively if they were captively reared or not. Petranka et al. (1998) suggests that timber activities should refrain from approximately 100m from a rock outcropping, lack of canopy cover causes the crevices to dry and could cause a local population to extinction, as it inhibits their ability to perform necessary functions such as foraging and nesting. Newman et al. (2018) contradicts this stating that 14 of their survey sites held occupation regardless of the forest buffer only covering <20m of space between the rock outcrops and anthropogenically caused clearings. During my surveys, three A. aeneus individuals were observed approximately 20m from a major road at a historical observation site. Without more historical records, it cannot be determined if these clearings have any impact on A. aeneus populations; however, it is an important aspect to consider when selecting sites for surveys or land management practices.

Available Habitats in Tennessee

As stated by Gordon (1952), *A. aeneus* distribution refrains from the central Appalachian Mountains but rather coincide with the "Fenneman" physiographic regions, Appalachian Plateaus, the Ridge and Valley, the Blue Ridge, and the Piedmont physiographic sub provinces

(Ulack and Raitz 1981). Further, greater interfamilial relationships may have deterred A. aeneus from occupying the central Appalachian area (Newman et a. 2022; Cliburn and Porter 1987; Gordon 1952), as this region has the highest abundance and richness of salamander diversity on the globe (Petranka et al. 1993). The peaks of the central sections of the Appalachian Mountains are distinctly higher than those that occur towards the edges; many significantly exceeding elevations that are typical for A. aeneus occupation. Due to formation differences between the central Appalachians and the Fenneman regions there is a lack of exposed rock structures in many areas. Geologically, the Valley and Ridge region and central Appalachians differ and are separated by a fold-thrust belt (Anders et al. 2022). The valley and ridge formed as a result of the thrust fault (Woodward et al. 1988) meaning the older geologic structures beneath the surface were pushed upwards. These older stratifications are much of what we observe today. From personal observation, soil is thicker, and slopes have less than ideal rock structures, if any at all in the central Appalachians. The regions the species occurs in within Tennessee, westward from the Blue Ridge, are noted for their river gorge formations and with that, the enormity of suitable and available habitat for the species (Niemiller et al. 2022).

CHAPTER V

CONCLUSIONS

With one of the larger challenges of studying *A. aeneus* being the inability to properly survey their status, understanding how to accurately locate this species is crucial for land managers and researchers alike. Available data for this species is quite limited in several cases. Many authors reiterate and cite much of the same literature when it comes to habitat preferences, which are overall vague. Observational data tends to be just that, presence and absence of the species with a lack of habitat associations, morphological information, and often enough missing specific dates of the encounter. As geospatial methodologies improve and become more commonly incorporated to wildlife and conservation research, an influx of available spatial data has been slowly increasing as well (Reynard 2018). Publicly available datums play a crucial role in researcher's abilities to conduct more needed research on species and with finer and more accurate results.

Due to the availability of much of this data, the integral habitat variables identified for this species from the literature review process were able to be utilized for the creation of the predictive habitat model, which was able to identify suitable habitat for the species. It is key to bear in mind that MaxEnt cannot predict actual populations across the landscape; but rather, their prevalence is a consequence of a well fit habitat model. The first objective of this study was to determine if MaxEnt was and appropriate tool for creating a predictive habitat model, with 91.8% of the sites having suitable habitat for the species in addition to the 36.6% having positive

presence it was determined that MaxEnt was in fact an appropriate tool for the task. A future study comparing MaxEnt to other geospatial modeling programs may be beneficial. The second objective of locating the species in Tennessee was accomplished with a total of 18 localities and with that, several habitat characteristics were able to be identified including, but not limited to: appropriately shaded and structured rocks, typical fauna co-occurring within the area, and general topography of the area. Finally, for the third objective, of the 91.8% of sites that were deemed to have suitable habitat, 40% of those had positive presence, the remaining 60% is indeterminate for presence at this time as more field visits should be conducted in the future.

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APPENDIX A SUPPLEMENTARY GRAPHS

Dominant Foliage at Positive Presence Locations

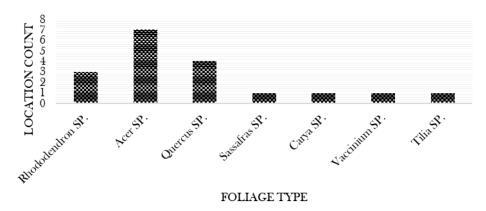


Figure 4.1 Dominant foliage types recorded in the field at positive presence localities of *Aneides Aeneus*

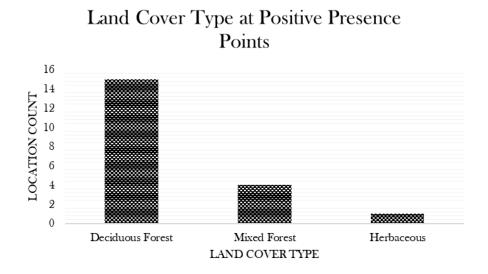


Figure 4.2 Land cover classifications from positive presence points in the field, data derived from raster values

Geology Type at Positive Presence Points

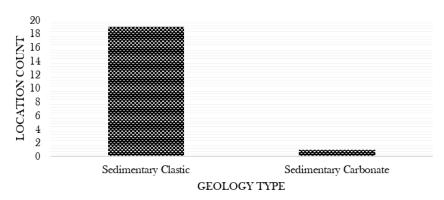


Figure 4.3 Geology types from positive presence points in the field, data derived from raster values

Canopy Cover at Positive Presence Locations

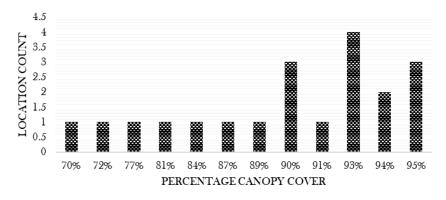


Figure 4.4 Canopy cover densities from positive presence points in the field, data derived from raster values

Soil Type at Positive Presence Points

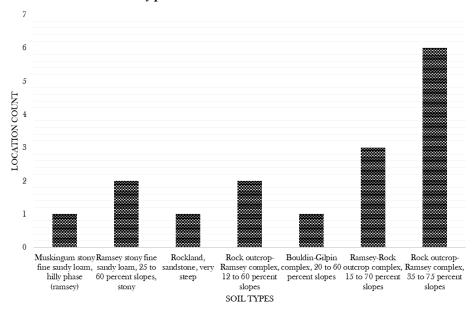


Figure 4.5 Soil types from positive presence points in the field, data derived from raster values

APPENDIX B

SUPPLEMENTARY PHOTOGRAPHS TAKEN DURING FIELD SURVEYS



Figure 5.1 Measuring an Aneides aeneus individual in the field (Pictured: Erin Gaylord)



Figure 5.2 an Aneides aeneus individual observed out in the open proceeding a rain event

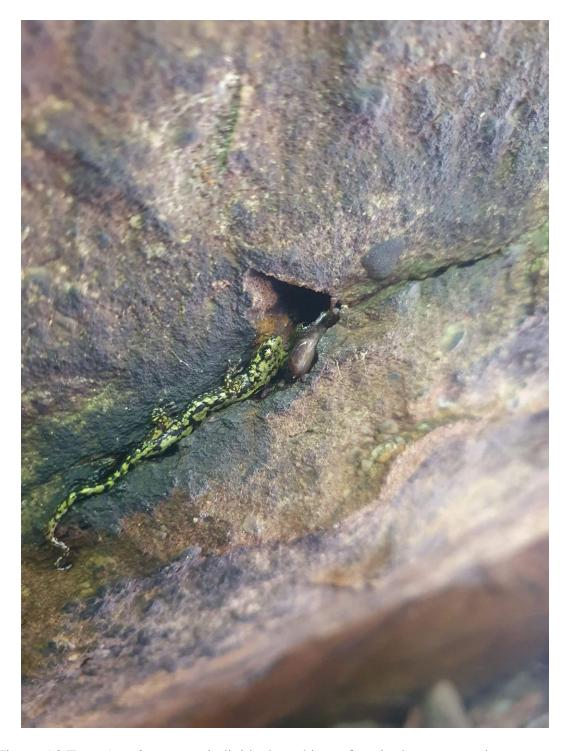


Figure 5.3 Two Aneides aeneus individuals seeking refuge in the same crevice



Figure 5.4 an Aneides aeneus individual guarding her clutch of eg

VITA

Erin Gaylord was born in Maryland and shortly moved to Northern Virginia after her birth. She spent much of her time looking for frogs, toads and salamanders at her grandparent's lake house in New York; this is where her love for amphibians grew. She graduated from Frostburg State University with her Bachelor of Science degree with a major in Wildlife and Fisheries Biology. Post-graduation she spent several summers taking on seasonal positions in various locations in the country. In August of 2021, she began her master's degree in environmental science at the University of Tennessee at Chattanooga. Erin was able to gain experience in the geospatial technologies field during her time in graduate school and found her niche in utilizing geospatial analysis for conservation. She hopes to continue efforts of applying geospatial analyses to conservation efforts focusing on amphibians.