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Cover Photo: Four-toed salamander (*Hemidactylium scutatum*), **Bryce S. Wade**

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Distribution, genetic structure, and wetland characteristics of four-toed salamanders (*Hemidactylium scutatum*) in Catoosa Wildlife Management Area

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Abstract. — The four-toed salamander (*Hemidactylium scutatum*) is a small, wetland-breeding amphibian that is patchily distributed and listed as a species of conservation concern across much of its range. Despite ranging throughout Tennessee, little is known about its ecology or distribution in the state. We conducted four-toed salamander surveys across 45 wetlands in Catoosa Wildlife Management Area (WMA) on the Cumberland Plateau to assess distribution, genetic structure, and habitat use. Four-toed salamanders were detected at 19 wetlands (42.2%) across the eastern two-thirds of Catoosa WMA and Lone Mountain State Forest. Population genetic analyses revealed spatial genetic structure and were largely congruent to previous genetic clustering analyses within the WMA. Using high-resolution geospatial data, we compared characteristics between wetlands where we detected four-toed salamanders and wetlands where we did not and found that wetlands where we detected four-toed salamanders had significantly greater within-wetland variation in slope and lower average solar radiance. This indicates that topographic complexity and natural shade could promote microhabitats that are suitable for nesting. These findings demonstrate that four-toed salamanders are more widespread within Catoosa WMA than previously documented and promoting future surveys and protecting small, topographically variable wetlands will be critical for conserving this species in Tennessee.

Key Words. — Cumberland Plateau, microhabitat, nesting, Plethodontidae, principal component analysis, rivers, solar radiance

The four-toed salamander (*Hemidactylium scutatum;* Fig. 1) is a wetland-breeding salamander that is native to the eastern United States and Canada (Petranka 1998). Despite its large geographic range, this species is patchily distributed, poorly understood, and often assumed to have low abundance when present. It thus carries special conservation listing status in 18 US States and 3 Canadian Provinces. Our understanding of four-toed salamander ecology and distribution is somewhat limited in the southeastern United States where relatively few studies have been conducted (but see: King and Richter 2022; Carter et al. 2025; Wade et al. 2025). Although, the southeast may harbor unique evolutionary lineages (Herman and Bouzat 2016). Four-toed salamanders are found across the state of Tennessee but are known mostly from sporadic records (Niemiller and Reynolds 2011). For this reason and their reliance on sensitive wetland habitat, the species is listed as In Need of Management by the Tennessee Wildlife Resources Agency and was considered in the 2025 State Wildlife Action Plan to be one of Tennessee's species of greatest conservation need (TNSWAP 2025).

Four-toed salamanders represent a monotypic genus that is deeply diverged (~80 MYA) from any other species of extant salamander (Stewart and Wiens 2025). These salamanders also have a reproductive life history that is unique among North American wetland-breeding amphibians. In the early spring, females will lay clutches of 30–80 eggs in moss (or other organic material) that overhangs or is adjacent to the wetland rather than in the pond basin as is typical in most wetland breeding amphibians (Blanchard 1923; Wood 1953, 1955). The female will remain with the eggs for multiple weeks while protecting them against microbial pathogens (Banning et al. 2008; Lauer et al. 2008). When suitable nesting substrate is limited, four-toed salamanders will form communal nests, and nests containing several hundred eggs have been observed (Wood 1953). Female four-toed salamanders have high site fidelity and individuals have been observed returning to the same patch of moss in multiple years (Harris and Ludwig 2004; Hamed 2014), which may contribute to fine-scale population structure (Wade et al. 2025).

Four-toed salamanders will nest in a wide variety of wetland types including ponds, bogs, seeps, and slow-moving streams (Chalmers and Loftin 2006; Wade et al. 2023; Ferguson and Hamed 2024). Because they have a short larval period compared to most temperate wetland-breeding amphibians (as short as 3 weeks; Vagila et al. 1997), four-toed salamanders can utilize wetlands that are exceptionally ephemeral. These small, ephemeral wetlands likely serve as critical habitat, as four-toed salamander larvae are small (often less than 1 cm; Wood 1953) and vulnerable to predation by fish or larger pond breeding amphibians like eastern newts (*Notophthalmus viridescens*) and mole salamander larvae (genus *Ambystoma*). Because four-toed salamanders have specific requirements for nesting, they are more dependent on within-wetland habitat features (e.g., areas of high slope, shaded areas with stable moisture, sensitive vegetation; Chalmers and Loftin 2006, Wahl III et al. 2008, King and Richter 2022) compared to other wetland-breeding salamanders. Therefore, variation in topography (i.e., structural heterogeneity) within a wetland (e.g., stumps, mounds, and small islands) or in the adjacent landscape (e.g., shade-providing cliffs or hills) that facilitates these wetland features may be especially important in shaping suitable breeding habitat.

Four-toed salamanders' reliance on small, cryptic wetlands and a general lack of detectability outside of the breeding season can make systematic surveys challenging and has potentially contributed to their seemingly patchy distribution in Tennessee (Chalmers and Loftin 2006; Niemiller and Reynolds 2011; Wade et al. 2023). Therefore, we performed dedicated four-toed salamander surveys in Catoosa Wildlife Management Area (Catoosa WMA) on the Cumberland Plateau of Tennessee. We also utilize next-generation sequencing data to assess spatial genetic structure in Catoosa WMA and make comparisons to previously published analyses for the site. Lastly, we aim to characterize wetlands using high-resolution spatial data to compare wetlands where we detected four-toed salamanders and wetlands where we did not. We predicted that four-toed salamander wetlands would tend to be smaller, more shaded (by forest cover and surrounding topography), and have higher topographic variation within the wetland. This topographic variability may be indicative of habitat features like stumps, mounds, or channels that support successful nesting.

Fig. 1. An adult female four-toed salamander (*Hemidactylium scutatum*) from Tennessee.

Materials and methods

Study Site—. We conducted surveys in Cumberland and Morgan Counties on the Cumberland Plateau in Tennessee. Most surveys were in Catoosa WMA, but we also surveyed 2 wetlands in the adjacent Lone Mountain State Forest. These surveys were primarily conducted to collect samples for a landscape genomic analysis (results reported elsewhere; Wade et al. 2025). Catoosa WMA is over 300 km^2 of primarily deciduous forest within the Emory River drainage and contains many large waterbodies such as Daddy's Creek, Otter Creek, and the Obed River. These riverine systems constitute an entrenched drainage network within the dissected Cumberland Plateau, marked by pronounced incision, steep sandstone bluffs, and narrow V-shaped valleys. They sustain a diverse assemblage of aquatic predators and display flashy flow regimes, with seasonally high discharges driven by intense precipitation, which likely present considerable barriers to salamander movements across the broader landscape. The site ranges from 360–610 m in elevation. Ongoing prescribed burns are currently being applied to recover rare Cumberland Plateau savannah habitat at the site (Bowers et al. 2016; Vander Yacht et al. 2017). Because of its extensive area, variation in habitat, and unique species assemblage, Catoosa WMA represents an important area for herpetological conservation in Tennessee. However, despite the large size of the WMA and abundance of aquatic habitat, four-toed salamanders were previously recorded from only a single wetland (Herman and Bouzat 2016) and were undetected during extensive herpetological inventories of the WMA (Strebler et al. 2025).

*Survey methods—.*We primarily located wetlands in Catoosa WMA by visually assessing leaf-off aerial imagery. However, because many four-toed salamander breeding sites are small and shallow, we also used hydrologic modeling to search for wetlands that are difficult to see based on satellite imagery. We generated a topographic wetness index (Beven and Kirkby 1979) in ArcGIS Pro (v3.3.0, Esri) using a 1-m digital elevation model (DEM) obtained from United States Geologic Survey 3D Elevation Program (USGS 2024). To identify areas of the landscape with a high probability of wetland presence, we then visually assessed the model by identifying patches of the landscape with high topographic wetness that were roughly the same size and shape of a wetland but did not correspond to known water bodies. While these methods of wetland identification are certainly imperfect and a more rigorous remote-sensing framework could have allowed us to more comprehensively identify wetlands (Guo et al. 2017), they allowed us to identify a large number of sampling sites in a relatively small geographic area. We located a total of 45 wetlands, 43 of which were in the east and central portions of Catoosa WMA and 2 across the Emory River in the adjacent Lone Mountain State Forest property.

We visited all 45 wetlands between February and April in 2024 and 2025 when four-toed salamanders are most detectable. Each wetland was searched a single time. During each survey we flipped available cover objects surrounding the wetland to search for male, juvenile, or non-nesting female four-toed salamanders. We also searched for nests by gently parting moss where it overhung the water. This is the preferred method for surveying for four-toed salamanders, and it tends to have high detection rates, especially compared to surveys for larvae or off-nest adults (Denton and Richter 2013; Drayer and Richter 2016; Roberts et al. 2024). For example, in a resurvey of historically occupied wetlands in Minnesota, Roberts (2024) detected four-toed salamanders at 83.3% of wetlands using nest surveys compared to only 38.5% using larval surveys. During the spring of 2024, nonlethal genetic samples (tail tips) were collected from all adult or juvenile individuals found at a wetland and individuals were immediately returned to their point of capture (no genetic samples were collected in 2025). We also opportunistically collected eggs from failed nests (depredated nests, nests that had fallen into the pond, etc.). These represent the same genetic samples analyzed in Wade et al. (2025). Because nest surveys may be disruptive to attending females, we attempted to reduce the amount of disturbed habitat when possible. In the 2024 field season, surveys ended after 7 genetic samples were collected and this threshold was associated with the landscape genomic analyses for which these surveys primarily occurred (Wade et al. 2025). In the 2025 field season, surveys ceased after one four-toed salamander nest was detected in a wetland. Otherwise, we attempted to exhaustively search all available nesting substrate in the wetland. To do this, we would locate areas with dense, suitable moss and systematically part moss moving only 2–4 cm at a time. This method was time consuming, and large wetlands with extensive moss coverage regularly took over an hour.

*Assessment of population genetic structure—.*We used next-generation sequencing to assess spatial genetic structure among our 67 samples (see Wade et al. 2025 for details regarding DNA extraction, library preparation, and bioinformatic processing). We filtered data in our assembly using the analysis toolkit in ipyrad v0.9.84 (Eaton and Overcast 2020) to: (1) include only loci present in at least 80% of samples with a minor allele frequency of 0.05 (Paris et al. 2017); (2) impute missing data from other individuals from the same area (i.e., using the "kmeans" method in ipyrad, which imputes genotypes based on allele frequencies in k populations); and (3) include only one SNP per locus to ensure SNPs are unlinked. We defined k in kmeans as 4 based on STRUCTURE (Pritchard et al. 2000) results from Wade et al. (2025) in Catoosa WMA. Filtering yielded 21,307 SNPs from 744,685 total sites. Wade et al. (2025) conducted population genetic and Bayesian clustering (STRUCTURE) analyses for the same samples we present in this study. However, as this was a multi-site study answering broad ecological questions, a detailed discussion of genetic structure in Catoosa WMA is absent from Wade et al. (2025). Here, we present a more detailed assessment of genetic structure within Catoosa WMA which can provide a deeper understanding of the impact of site-specific geography. We conducted a principal component analysis (PCA) using the ipyrad analysis toolkit to visually assess population genetic structure as a complement and comparison to the previously published clustering analyses.

*Assessment of wetland characteristics—.*We assessed the difference between wetlands where four-toed salamanders were detected and undetected using remote-sensed geospatial data. We first generated polygons for each wetland using leaf-off aerial imagery. We calculated the area of each polygon as a measure of wetland size. Because four-toed salamanders require shaded nesting substrate, we assessed the average canopy cover using a 30-m resolution dataset from the United States Forest Service within a 100 m buffer of each wetland as a measure of wetland canopy closure. Because four-toed salamanders require complex wetland habitat and surfaces with high slope to lay nests, wetlands with high variation in slope may be more likely to support breeding populations. Therefore, we also assessed the variability of topographic slope (standard

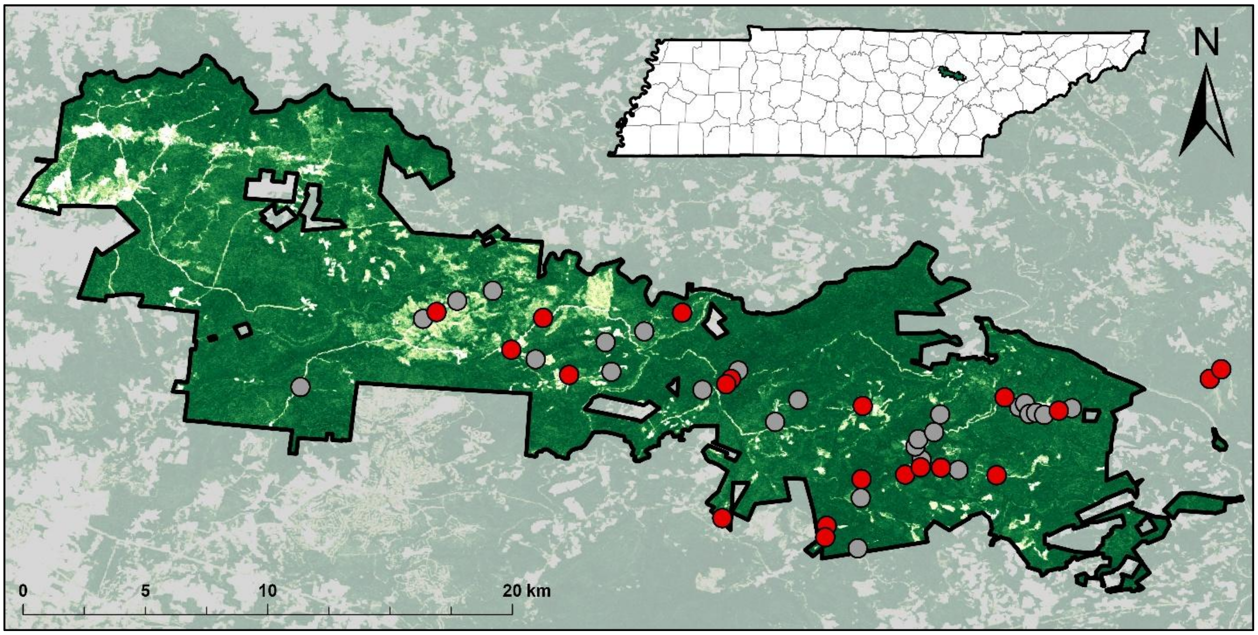

Fig. 2. A map of wetlands surveyed for four-toed salamanders (*Hemidactylium scutatum*) in Catoosa Wildlife Management Area with its position within Tennessee displayed in an inset map. Red points represent wetlands where four-toed salamanders were detected, and gray points represent wetlands where they were not detected. Background coloration represents percent canopy cover, with darker green indicating higher canopy cover.

deviation of cells within a wetland) in each wetland at 1-m resolution using a 1-m DEM and the "Slope" tool in ArcGIS Pro. Additionally, because four-toed salamander nests are sensitive to overheating and desiccation (Wahl III et al. 2008), the amount of sunlight a wetland receives based on broader topography may influence the availability of nesting habitat. Therefore, we calculated the mean amount of solar radiation each raster cell in a wetland receives during the nesting season (February, March, and April) using the "Raster Solar Radiation" tool and a 1-m DEM in ArcGIS Pro. One wetland was removed prior to all analyses that relied on a DEM as it displayed odd behavior (many pyramidal-shaped mounds in the wetland basin) that may be attributable to error in the DEM. We then compared each of these habitat characteristics between wetlands in which four-toed salamanders were detected and undetected using Welch's two sample t-tests.

Results

*Survey results—.*We detected four-toed salamanders at 19 out of our 45 sampled wetlands (Fig. 2). We also detected numerous other species of wetland breeding amphibians during our surveys including spotted salamanders (*Ambystoma maculatum*), marbled salamanders, (*Ambystoma opacum*), American toads (*Anaxyrus americanus*), eastern newts (*Notophthalmus viridescens*), mountain chorus frogs (*Pseudacris brachyphona*), spring peepers (*Pseudacris crucifer*), green frogs (*Rana clamitans*), and wood frogs (*Rana sylvatica*). We located four-toed salamander nests in a variety of species of moss, but nests were generally located in American tree moss (*Climacium americanum*), sphagnum moss. (*Sphagnum spp.*), and delicate fern moss (*Thuidium delicatulum*). We lack the data to quantify the proportion of four-toed salamander nests in each moss species, but other studies consistently find nests in these mosses, and they are considered important for four-toed salamander nesting (e.g., King and Richter 2022; Carter et al. 2025; Hilt et al. 2025).

*Population genetic structure—.*After filtering, our PCA was conducted with 21,307 SNPs and 67 individual salamanders from 14 wetlands in Catoosa and 2 in Lone Mountain State Forest (no genetic samples for wetlands located in 2025 were collected). Principal components 1 and 2 explained 5.3% and 3.0% of the variation in our data, respectively. Based on a visual assessment of our PCA, we found support for three clear genetic groups within our sampling area that corresponded well to major streams and rivers within the site (Daddy's Creek and the Emory River; Fig. 3).

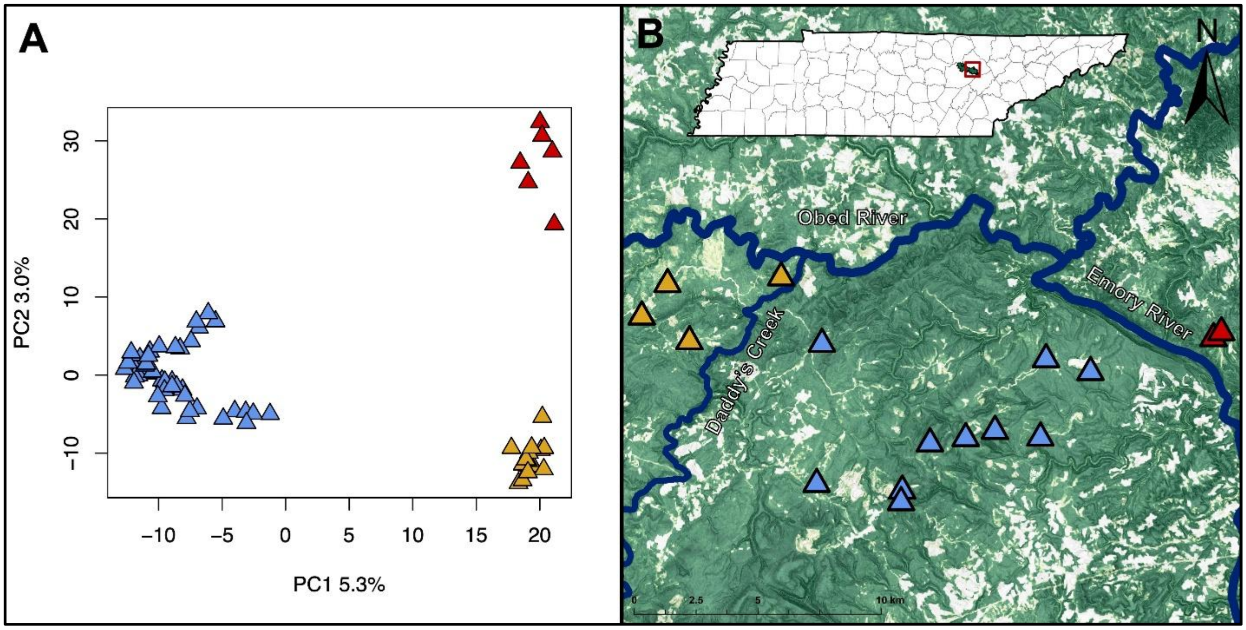

Fig. 3. The results of a principal component analysis (PCA) of single nucleotide polymorphism data for four-toed salamanders (*Hemidactylium scutatum*) showing PC1 vs PC2. Visual clusters are denoted by color (A), and their geographic positions are displayed on a map (B) in which major rivers and creeks are labeled. Each triangle in the PCA represents an individual salamander while each triangle on the map represents a sampled wetland. The area's position within Tennessee and Catoosa Wildlife Management Area is denoted by a red square in an inset map.

*Assessment of wetland characteristics—.*The area of surveyed wetlands ranged from 24–5762 m^2. The mean area for sites where four-toed salamanders were detected was 812 (± 753.67) m^2, and the mean area for sites where four-toed salamanders were undetected was 1252.08 (± 1397.11) m^2, though this difference was not significant based on a Welch's two sample t-test ($P = 0.18$, df = 38.03; Table 1). The mean percent canopy cover within 100 m of each wetland varied between 27.38% and 91.67%. The mean percent canopy cover for sites where we detected four-toed salamanders (75.28% ± 14.61%) did not differ significantly from that of sites in which four-toed salamanders were undetected (77.22% ± 15.6; $P = 0.67$, df = 41.39; Table 1). The variation in slope within each wetland ranged from 0.22–6.96. The mean variation in slope was 3.34 (± 1.71) for sites where we detected four-toed salamanders and 2.25 (± 1.11) for sites where salamanders were undetected. This difference was statistically significant ($P = 0.02$, df = 31.76; Table 1). The mean solar radiance was 114.49 (± 1.91) kWh/m^2 for sites where we detected four-toed salamanders and 115.66 (± 1.26) kWh/m^2 for sites where salamanders were undetected. This difference was statistically significant ($P = 0.02$, df = 33.30; Table 1).

Discussion

Despite the lack of detection of four-toed salamanders during previous surveys of Catoosa WMA and their cryptic nature, we found four-toed salamanders at 42.2% (19/45) of wetlands surveyed. It is important to note that this study only included single visits to each wetland and thus results represent naïve occupancy rates. Without multiple visits and an understanding of imperfect detection (MacKenzie et al. 2002), it may be difficult to estimate true occupancy rates and 42.2% is likely a conservative estimate. However, as four-toed salamander nests are static throughout the breeding season (compared to adult salamanders which are mobile), the probability of detection may be quite high for this survey method if conducted in the breeding season. For example, in 5 years of systematic surveys and resurveys of historically occupied wetlands in the Great Smoky Mountains National Park, Corser and Dodd (2004) detected four-toed salamander nests in 92.7% of surveys. This indicates that the detection probability of four-toed salamander nests can be quite high, even when population densities are low. Therefore, as long as surveys occur after nests are laid and wetlands are searched thoroughly, naïve occupancy rates may be good approximations of true occupancy rates in this system.

The high detection rate in this study is similar to that presented by Chalmers and Loftin (2006) in Maine (43%) but is higher than other surveys in the southeast (Miller et al. 2005; Drayer and Richter 2016; Strebler et al. 2025). It is also important to note that we did not survey the western third of Catoosa WMA, and thus the number of occupied wetlands within the WMA property may far exceed the 19 in which we detected four-toed salamanders. The large number of wetlands in which we

TABLE 1. Comparisons of the means (± SD) for variables in wetlands in which four-toed salamanders (*Hemidactylium scutatum*) were detected and undetected. P-values are for the difference between groups using a Welch's t-test.

Variable	Mean (detected)	Mean (undetected)	P-Value
Area (m^2)	812 ± 753.67	1252.08 ± 1397.11	0.18
Forest Cover (%)	75.28 ± 14.61	77.22 ± 15.6	0.67
Slope Variability	3.34 ± 1.71	2.25 ± 1.11	0.02
Solar Radiance (kWh/m^2)	114.49 ± 1.81	115.66 ± 1.26	0.02

detected four-toed salamanders suggests that Catoosa WMA may serve as important core habitat for the species in Tennessee, and the protection of wetland habitat should be prioritized during events such as general maintenance, road construction, or logging. Additionally, as Catoosa WMA is a focal area for prescribed burns, care must be put into the timing and location of burns, so they do not interfere with four-toed salamander nesting and migration. While fire can sometimes improve wetland quality in the southeastern United States (McWilliams et al. 2007; Flores et al. 2011), the timing of fire in four-toed salamander wetlands should be considered carefully as prescribed burns can harm nests or alter sensitive moss communities that these salamanders rely on (Noble et al. 2018; Davies et al. 2023).

We found evidence of spatial genetic structure in our samples despite the relatively small sampling area. We found that both Daddy's Creek and the Emory River appear to structure four-toed salamander populations in our study area leading to three visually distinct genetic clusters in our PCA. Wade et al. (2025) also identified these three clusters using Bayesian clustering analyses, along with a fourth cluster located between Daddy's Creek and the Emory River (the blue cluster in Fig. 3). This fourth cluster is somewhat visible in our PCA (separation within the blue cluster; Fig. 3) and may represent genetic differentiation caused by isolation by distance as this cluster covers the largest geographic area in our study (Fig 3). Wade et al. (2025) found a clear signal of isolation by distance in four-toed salamanders at each landscape assessed. However, it is important to note that geographically distant samples unseparated by rivers are still closer to one another in PCA space than nearby wetlands that are separated by rivers. Genetic isolation caused by areas of deep water and high topographic variation (i.e., a river gorge) are congruent with the results of landscape genetic analyses with four-toed salamanders (Wade et al. 2025) and many amphibians (e.g., Richardson 2012, Homola et al. 2019, Waraniak et al. 2022). Though four-toed salamanders are capable swimmers (author pers. obs.), they may either refuse to cross or fail to cross larger bodies of flowing water. Additionally, they may avoid steep areas like river gorges due to the energetic cost associated with crossing them. On larger scales, major river systems (e.g., the Tennessee River) may structure four-toed salamanders at the species level (Herman and Bouzat 2016).

Wetlands where four-toed salamanders were detected were, on average, smaller than those where they were not detected, though this difference was not statistically significant. Despite their general association with small, boggy wetlands (Chalmers and Loftin 2006; Vitale 2013; Wade et al. 2023) we found that four-toed salamanders will occasionally use large wetlands that contain fish predators (e.g., sunfish [*Lepomis spp.*]). However, because fish presence directly influences spatiotemporal extirpation dynamics of wetland amphibians, these populations may be particularly unstable over time (Cosentino et al. 2011). We did not find any difference between the average forest cover of wetlands with or without four-toed salamanders. It is important to note that we found four-toed salamanders using wetlands with low levels of forest cover surrounding them (see the western-most site where we observed four-toed salamanders in Fig. 2). However, these wetlands in our study were primarily located in restored Cumberland Plateau savannah habitat and should not be directly compared to areas experiencing anthropogenic development or timber harvest.

We found that wetlands where we detected four-toed salamanders had higher variation in slope and lower solar radiance than areas salamanders were not detected. These differences likely capture the importance of suitable within-wetland microhabitat conditions for successful nesting in this species. The importance of microhabitat conditions created by broader scale landscape complexity is well established for small, terrestrial salamanders (e.g., Jaeger 1980; Peterman and Semlitsch 2014; Cosentino and Brubaker 2018). However, in addition to these complex microhabitat requirements, four-toed salamanders are unique in that they also require a different set of fine-scale microhabitat conditions for nesting. Because four-toed salamander nests are laid terrestrially they are particularly prone to desiccation when exposed to direct sunlight (Wahl III et al. 2008) and thus many small mounds with north facing slopes may provide abundant nesting substrate compared to a single north facing wetland bank. Additionally, wetlands that are generally shaded by the larger landscape (e.g., hills protecting part

Fig. 4. Examples of wetlands where we detected four-toed salamanders (A; *Hemidactylium scutatum*) and wetlands where we did not (B) in Catoosa Wildlife Management Area. Note the small, mossy mounds in the wetland where salamanders were observed.

of the wetland from afternoon sunlight) may have a higher abundance of cool, moist areas where nests can be laid.

Wetlands that contain topographic heterogeneity within the wetland basin (e.g., mounds, tree stumps, channels) will contain more suitable breeding habitat than flat wetlands of a similar size (i.e., nesting habitat availability does not scale with perimeter in these wetlands) (Fig. 4). This reflects a well-established ecological principle that increased habitat structural complexity increases the availability of different microhabitats (MacArthur and MacArthur 1961; Semlitsch 2000; Tews et al. 2004). This differs from other species of North American wetland-breeding salamanders (i.e., Ambystomatids) in which the size of breeding populations tends to scale with the surface area of the wetland, not habitat complexity (e.g., Wang et al. 2011, McCartney-Melstad et al. 2018, Wendt et al. 2021). The reliance of four-toed salamanders on specialized microhabitats in the terrestrial landscape and within breeding wetlands make them a unique spatial conservation challenge in which managers must jointly consider both distinct habitat requirements.

As four-toed salamanders represent a species of greatest conservation need in Tennessee, it is important that continued surveys are conducted to identify critical habitat for this sensitive species. We provide evidence that four-toed salamanders may inhabit more wetlands than originally assumed across large protected areas like Catoosa WMA. Future surveys, especially those that target nests during early spring, could be useful to evaluate if this trend holds across other areas of the state. Surveys may be guided by geospatial analyses, especially those that predict the presence of abundant cool, moist nesting habitat. However, we note that wetlands that are not typically associated with four-toed salamander presence (e.g., wetlands with fish, wetlands with low forest cover) should not be completely ignored. Future work should attempt to further quantify the importance of different habitat characteristics within four-toed salamander breeding wetlands. Specifically, an assessment of how habitat characteristics influence four-toed salamander nest abundance would be appropriate as we were unable to explore this concept given our study design.

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Data Availability.— Raw sequence reads associated with this work are publicly available in the SRA (BioProject PRJNA1229940). Data used in analyses or spatial files can be obtained from the authors upon request.

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Natural History Notes

ANEIDES AENEUS **(Green Salamander)**. Ground-dwelling behavior. Green salamanders (*Aneides aeneus*) are well-known as crevice-dwelling salamanders that primarily inhabit exposed rock faces (and on rare occasions are observed arboreally) in the southeastern United States (Gordon 1952. Am. Midl. Nat. 47: 666–701). These salamanders, with their black and green mottled pattern, tend to blend in well with mossy rock faces and utilize these specialized habitats for nesting and brumation (Niemiller et al. 2022. Herpetol. Conserv. Biol. 17:249–265). Green salamanders are known to inhabit the Walden's Ridge geologic feature on the eastern edge of the Cumberland Plateau, including Prentice Cooper State Forest in east Tennessee (PCSF; Niemiller et al. 2022. Herpetol. Conserv. Biol. 17:249–265).

On 17 February 2024 we recorded two green salamanders engaging in abnormal ground-dwelling behavior at PCSF. During surveys for the wetland-breeding four-toed salamander (*Hemidactylium scutatum*), we flipped two individual green salamanders (one at 1149 h and the other at 1558 h) under logs directly adjacent to two different ephemeral wetlands at PCSF (exact localities withheld for conservation concerns; Fig. 1). One individual was found under the same cover object as a single adult southern zigzag salamander (Fig. 1; *Plethodon ventralis*). Neither wetland was in the vicinity of any emergent rock formations that are considered typical habitat for this species, but both were found in forested habitat. We believe that this behavior may have been triggered by abnormally rapid drop in temperature from a high of 11.1 ºC on 16 February 2024 to –2.2 ºC on the morning of 17 February 2024. These salamanders may have both been occupying arboreal habitat and then forced to descend and shelter under substantial cover objects when temperatures rapidly dropped below freezing. These occurrences may suggest that this species may be willing to use a variety of habitats that are not considered suitable (i.e., habitat other than rock crevices and trees).

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Fig. 1. (A) An adult green salamander (*Aneides aeneus*) and an adult southern zigzag salamander (*Plethodon ventralis*) flipped under a small log at Prentice Cooper State Forest. (B) The wetland habitat directly adjacent to the log. Photos by B.S.W. and A.M.M.

Geographic Records

CARPHOPHIS AMONENUS **(Eastern Wormsnake)**. USA: TENNESSEE: Madison Co.: (35.547250°N, 88.715278°W; datum WGS84). 17 Aug 2025. Lee J. Barton, Brian P. Butterfield, and Jonathan Graves. Verified by Jessica Grady. David H. Snyder Museum of Zoology, Austin Peay State University (APSU 20795, color photo). We searched VertNet and yielded no unpublished records in Madison County. (https://www.vertnet.org/occurrence/search?taxonKey=2457092&stateProvince=Tennessee [accessed on 2025-09-11]). This species was first documented from Madison County in 1939 (Endsley, J. R. 1954. An annotated listing of a herpetological collection mainly from Tennessee. Journal of the Tennessee Academy of Science 29:36-41; 35.500700°N, 88.716900°W; datum WGS84). This report represents the first vouchered specimen for Madison County and was found ca. 5.17 kilometers north of the individual reported by Endsley (1954, op. cit.). Our specimen was found dead on road between two residential areas, one maintained lawn on the north side of the road, and a dwelling with numerous hickory and oak trees on the south side of the road.

LEE J. BARTON (lbarton@fhu.edu), **BRIAN P. BUTTERFIELD** (bbutterfield@fhu.edu), and **JONATHAN GRAVES** (jonathan.graves@students.fhu.edu), Freed-Hardeman University, 158 E. Main Street, Henderson, Tennessee 38340, USA.

2025 Bob Hatcher Award: Dr. Danny Bryan

Danny started his academic career at Motlow State Community College, where he earned an Associates of Science Degree before moving to Knoxville and earning a B.S. in Plant and Soil Science at the University of Tennessee. He received a graduate teaching assistantship at Middle Tennessee State University which allowed him to complete his master's thesis on the growth rates and mortalities of dogwood trees relative to orientation with the magnetohydrodynamic coal-fired facility in Tullahoma. After his MS work, he took an instructor position at Martin Methodist College in Pulaski which allowed him to teach zoology and microbiology courses. He then moved to Cumberland University where he worked his way up the academic ranks as a biology professor. While teaching at Cumberland, he completed his Ph.D. in Environmental Science at Tennessee Tech which focused on the survival and natural history of the timber rattlesnake at Center Hill Lake in Dekalb County, TN.

In his 29 years at Cumberland University Danny taught an incredible breadth of courses on a number of topics in biology from flowering botany to vertebrate biology. He helped foster internship and directed research opportunities for a number of undergraduates. Danny was a cornerstone of the Science Department at Cumberland. He worked to maintain accreditation for biology courses and led the department as program director for nearly a decade. His dedication to the sciences and his work to help grow enrollment at Cumberland led to his appointment as Emeritus Professor of Biology in 2023. Aside from his academic duties at Cumberland University, Danny was the head wresting coach. In 2024 he was inducted into the Cumberland University Sports Hall of Fame.

Danny is perhaps the most vocal advocate for timber rattlesnakes in the state of Tennessee. He has been working to understand the distribution, habitat preferences, ecology, and population health of Tennessee timbers for decades. In his work at Center Hill Lake, he was the first researcher to detect *Chrysosporium* in Tennessee which was later reclassified as the fungal pathogen *Ophidiomyces ophiodiicola*. Danny consulted with the Tennessee Wildlife Resources Agency about the presence and effects of the disease and later authored a technical report for monitoring the disease. Additionally, Danny co-authored the Tennessee chapter in The Timber Rattlesnake: Life History, Distribution, Status, and Conservation Action Plan. This tome was a major effort sponsored the Partners in Amphibian and Reptile Conservation group in 2021.

Danny expanded his research on timber rattlesnakes by tracking and monitoring individuals at the Bridgestone Reserve at Chestnut Mountain which is managed by the Nature Conservancy. It was during this research that one of the most impactful events in Danny's career occurred, he was envenomated by one of his research animals. In the true form of a pure educator, he used his bite as a teaching opportunity. Danny delivered many presentations, news media and podcast interviews about his envenomation and recovery. Danny helped raise snakebite awareness, while also promoting personal responsibility and stressing the importance of rattlesnake conservation.

Danny was a founding member of the Tennessee Herpetological Society and since the beginning he has been an active member of the group that has helped to guide and solidify the trajectory of the society. His service to the THS includes election to the board as Middle TN rep, vice president, and member at large, membership on the Chadwick Lewis Scholarship Committee, service on the conservation committee, and a position on the editorial board for the Tennessee Journal of Herpetology. He also served as host for the Annual Meeting in 2005 and 2011.

It is difficult to overstate how vital Danny Bryan has been to the Tennessee herpetological community. Danny's involvement as an educator, researcher, and advocate for Tennessee herpetofauna has cemented his legacy as a dedicated herpetologist. Because of this, we feel that he is an ideal candidate for the Bob Hatcher Conservation Award. Danny is the Tennessee "Rattlesnake Man" and mention of his name is synonymous with advocacy and conservation of timber rattlesnakes in the state. Over Danny's vast career he has worked to promote the Tennessee Herpetological Society's mission of educating the public and working hard to study and conserve reptiles and amphibians.

Michael Fulbright, Cumberland University; **Lisa Powers**, Froghaven Farm

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Abstracts of the 31st Annual Meeting of the TN Herpetological Society, Lebanon, TN

Student Oral Presentations

Urbanization and Tennessee's Streamside Salamander: Updates and Initial Findings After Two Years

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The Streamside Salamander (*Ambystoma barbouri*) is a unique Ambystomatid that reproduces in ephemeral streams, specifically those with limestone substrates. This species was likely historically abundant in Tennessee's Central Basin but is now listed as Endangered in the state due to the rapid urbanization of the Nashville metro area. We have completed two years of a four-year project using multiple research methods to understand the impact of urbanization on Streamside Salamanders in Tennessee. As part of an occupancy and abundance model framework, we have conducted repeat surveys at occupied sites across the range of development. At each site visit, we recorded the number of eggs, larvae, and adults, measured water quality metrics, and assessed stream morphology. In an effort to better understand the species' distribution in the state, we created the first species distribution model for the Streamside Salamander in Tennessee. We also conducted a radiotelemetry study, tracking salamanders at several sites to gain further insight into the species' movement ecology and habitat use. I will discuss the observed differences in abundance and occupancy across sites, as well as variations in breeding phenology found in the repeated surveys. Additionally, I will elaborate on the species' distribution model and initial findings about the species' movement ecology from radiotelemetry efforts. I will also discuss future analyses and research goals and highlight how this work will support the conservation of the species in Tennessee.

Survival of Head Started Hellbenders in Big Swan Creek

Taina McLeod, Sherri Doro-Reinsh, Dale McGinnity, Pia Sandonato, Rebecca Hardman, Brian Flock, and William B. Sutton
Tennessee State University, 3500 John A Merritt Blvd, Nashville, TN 37209

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large-bodied, fully aquatic salamander that occupies streams and rivers throughout the eastern United States. However, due to habitat fragmentation, sedimentation, increased water temperatures, and other factors, there has been a drastic decline and extirpation of some populations throughout their range. To supplement the current population of eastern hellbenders in central Tennessee, zoo-raised individuals have been released and are being radio-tracked to assess movement, habitat use, and survival once translocated. A total of 72 animals were released across three seasons (y1=24, y2=27, y3=21) across five sites in Big Swan Creek, Tennessee. Survival proportions varied greatly between cohorts due to stochastic weather events (hot/dry conditions, high water events, etc.). Overall, it was found that mass at release is the most significant factor that affects hellbender survival, where larger animals face a greater risk of mortality. Assessing the post-release survival of eastern Hellbenders will give managers an idea of the proportion of animals that will survive after a larger-scale translocation event, as well as potential management practices that can help increase long term survival.

The long-term effects of incubation temperature on morphology and performance in the Streamside Salamander (*Ambystoma barbouri*)

Cindy D. Scruggs, Julia Thulander, Joshua M. Hall
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Embryos of vertebrate ectotherms are particularly vulnerable to changes in temperature because they have little ability to thermoregulate. Although much research demonstrates embryonic temperature can alter fitness-relevant phenotypes via developmental plasticity, little research has considered the long-term effects of developmental temperature on ectotherms. The streamside salamander (*Ambystoma barbouri*) is an endangered species that oviposits in shallow, ephemeral streams which are subject to thermal variation over space and time. The purpose of this study is to determine the long-term effects of developmental temperature during embryogenesis on fitness-relevant traits of adults. Eggs were collected from natural nests, incubated at ecologically-relevant temperatures (5°C, 10°C, and 20°C), and resultant metamorphs have been raised to adulthood to assess temperature effects on morphology (body size, head size, body mass), performance (speed and endurance), and physiology (growth rate). We present preliminary morphology results for salamanders 12 months and 20 months post metamorphosis and preliminary endurance results at 20 months. We aim to assess the potential for incubation temperature to influence fitness of the streamside salamander via lasting impacts on traits critical to reproduction and survival.

Professional Oral Presentations

Tennessee Streamside Salamanders differ from core range populations in life history traits and developmental physiology

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The largest continuous portion of the Streamside Salamander (*Ambystoma barbouri*) range extends from northern Kentucky into southern Ohio and Indiana. While populations in this core range appear robust, disjunct populations in West Virginia, western Kentucky, and central Tennessee are isolated and may face extirpation. Due to reproductive isolation and possible local adaptation, these populations may be genetically and phenotypically distinct, potentially qualifying as Distinct Population Segments under the Endangered Species Act. To assess differences, we collected eggs from across the core range and Tennessee populations, incubated them at various temperatures, and reared larvae, metamorphs, and juveniles in a laboratory common garden. We measured population-specific temperature responses and general phenotypic variation. Tennessee populations differed from core populations in morphology, life history traits, and embryo physiology. Combined with genetic evidence of distinctiveness, these results strengthen the case for recognizing Tennessee Streamside Salamanders as Distinct Population Segments.

The Timber Rattlesnake (*Crotalus horridus*) at Chestnut Mountain.

Danny L. Bryan
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The timber rattlesnake (*Crotalus horridus*) is facing serious threats throughout the United States, with population studies indicating a decreasing trend throughout its range. In Tennessee, it is listed as a species of Greatest Conservation Need and protected from take (Tennessee Code Annotated 70-8-104 and 70-4-403). This field study was conducted at The Bridgestone Nature Reserve at Chestnut Mountain. Chestnut Mountain is a 5,763-acre mountain forest located in Sparta, Tennessee. Protection and augmentation of habitat are common tools of resource agencies to maintain or enhance populations of game and non-game species. Monitoring population trends and movement patterns and identifying habitat components are needed to manage timber rattlesnake populations in Tennessee. In this study, six hibernacula and three gestation sites were identified, this is by no means all-inclusive and further investigations will be required to identify other locations. A vegetation analysis study consisting of eight 11.3-m radius plots was conducted in the area surrounding two gestation sites. Simpson's Diversity Index was calculated to determine the biodiversity in this ecological community. The area received a score of 0.782 (range = 0-1), with 13 different species documented indicating low biodiversity. The red maple has the highest importance value in each of the plots with an overall value of 84.12, indicating that it is the dominant species in the area.

Exploring the stability and resilience of the Cumberland Dusky Salamander in Tennessee

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Described in 2003, the Cumberland Dusky Salamander has largely been presumed to fill the role of a "mountain dusky" salamander within the southern Cumberland Plateau alongside other widespread dusky salamanders. However, investigations in the past decade revealed that their occurrence and distribution were far more limited and their ecology and phylogenetic origins differ from that of a "mountain dusky". We performed a series of surveys to investigate the status, vulnerability, and population structure of the Cumberland Dusky Salamander. The status of this species remains largely unchanged over 9 years. They are still isolated to waterfalls and bedrock cascades and prefer mature forests where they exist in very small populations. However, a new experiment revealed small but consistent negative effects of stream dewatering as is becoming more common. Furthermore, genetic analyses revealed a robust northern clade centered on dense populations found in the northern portion of their range whereas the remainder of their range generally hosts smaller populations with lower genetic diversity. The high habitat specificity of this species is likely responsible for its relatively high resilience to human activities on the Cumberland Plateau. However, shifting climate regimes may serve as press disturbances that could send southern populations toward extirpation.

Cave-associated amphibians of Tennessee

Matthew L. Niemiller, K. Denise Kendall Niemiller, E. River Niemiller, Amata Hinkle, Eric C. Maxwell, C. Lael Anderson, Bjorn V. Peterson, Jared P. Higgs, Brendan Cramphorn, Madison Richards, Brain T. Miller
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Caves and associated subterranean environments in karst regions are important habitats for many amphibian species that occur in Tennessee. Many species use cave habitats on a semi-regular to regular basis for several aspects of their life histories, including reproduction, refuge from harsh environmental conditions on the surface, and hibernation. Here we review salamander and frog diversity in and use of caves and other subterranean karst habitats in Tennessee. We compiled cave occurrence records for salamanders and frogs from scientific literature, museum accessions, and online repositories (GBIF, iNaturalist). In addition, we included occurrence data from 545 biosurveys of 298 caves in the state from our research over the past 20 years. Thirty-two species of amphibians (14 anurans and 18 salamanders) are known from caves and other subterranean habitats in Tennessee. Two salamanders in the region are considered troglobionts (obligate cave-dwellers: *Gyrinophilus gulolineatus* and *G. palleucus*. Although the occurrence of several species can be categorized as accidental, several non-troglobiotic salamanders breed in caves, such as *Eurycea cirrigera*, *E. longicauda*, *E. lucifuga*, *Gyrinophilus porphyriticus*, *Plethodon glutinosus*, *P. dorsalis/ventralis*, and *Pseudotriton ruber*. A growing body of evidence indicates that in addition to the surrounding surface habitats, caves are critical habitats for many species, and, therefore, should be protected for proper amphibian conservation and management.

Species Richness and Encounter Rates of Salamanders at Horse Creek Wildlife Sanctuary, Savannah, Tennessee

Lee J. Barton, Brian P. Butterfield and Mary K. Brackin
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Amphibians have faced marked declines globally over the past several decades for a variety of reasons including habitat loss. In Tennessee, habitat loss is a major threat to the state's amphibian fauna. The Horse Creek Wildlife Sanctuary (HCWS) in Savannah, Tennessee offers habitat that is suitable for a variety of amphibians, especially salamanders. This site is ideal for determining salamander species richness in a protected and relatively undisturbed site within the transitional hills of Tennessee. Our sampling period ranged from October 2023 to April 2024. We utilized time constrained area searches to survey salamander species. We recorded the number of individuals during each sampling period to calculate species richness (S) and encounter rates. Species richness was nine. This study provides baseline data on salamander richness from a protected site in Tennessee. This data should be useful for researchers and managers who will assess salamander diversities in more disturbed sites within the region.

Lightning Talks

Pokes and prods affect an aquatic frog's locomotor behavior during an escape response

Javier Montero and Chase Kinsey
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The use of touch to elicit an escape response in the aquatic frog, *Xenopus laevis*, is a primary mode of stimulus in previous studies. However, touch may not be the best method for eliciting escape response across developmental stages as morphology changes drastically. Here we assess locomotor behavior and performance with the use of varying stimuli across developmental stage (NF52, NF60, NF66) and lateral line ablation in wildtype and albino frogs. We hypothesized that escape response would vary across development stage, and lateral line ablation will change escape behavior. Frogs randomly received a prod and water pulse stimulus from a pipette to both their rostral and caudal side. The lateral line from a subset of frogs was then ablated using CoCl2 and frogs were randomly tested again. Escape responses were filmed with high-speed video and digitized in MATLAB. There was no significant effect of ablation or stimulus type on velocity between wild and albino frogs, though older frogs in both groups were faster than their younger conspecifics. However, escape latency and fleeing time did differ significantly across stage, ablation, and type of frog. Our data suggests that while maximal performance does not change across treatment groups, escape behavior does. Future studies on frog locomotion should consider the stage and type of stimulus used when interested in specific locomotor behaviors.

Historic disturbance explains contemporary patterns of genetic diversity in a red-cheeked salamanders

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Anthropogenic activities like habitat destruction can cause declines in the genetic diversity of wildlife populations. Loss of genetic diversity can exacerbate ongoing biodiversity declines as affected populations can suffer from a higher probability of extirpation and populations with low diversity may not have the adaptive potential to respond to future environmental change. The protection and enhancement of genetic variation is especially important for the conservation of high-elevation endemic organisms as they may need to adapt to warming conditions as habitat for range shifts is bounded by the upper elevation of mountains within their range. Here, we assessed spatial patterns of genetic diversity in a narrow-range endemic salamander, the red-cheeked salamander (*Plethodon jordani*) within the Great Smoky Mountains National Park (GSMNP). Using genetic samples from 535 *P. jordani* we found that historic logging within the GSMNP explains modern patterns of genetic diversity better than elevation alone. Specifically, we found that areas that were previously logged have lower genetic diversity, but these losses may be buffered at high elevations. Historic logging may have caused genetic bottlenecks and altered forest characteristics (e.g., understory density) leading to lower modern levels of genetic diversity in affected areas. It's important to understand the spatial distribution of genetic diversity in endemic species like *P. jordani* and understand the impact that anthropogenic activities may have on the genetic health of wildlife populations for many generations.

Poster Presentations

Effects of Tributyltin Chloride Exposure on Swimming in *Xenopus laevis*

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Neuromuscular development in amphibians is strongly regulated by thyroid hormones, yet little work has examined how endocrine disruption alters whole-animal performance. Because tadpoles absorb chemicals directly from their environment, they provide a useful model for testing swimming behavior under thyroid disruption. This study tested the effects of tributyltin chloride (TBTCl), a known thyroid disruptor, on *Xenopus laevis* tadpoles over a 15-day exposure period. We hypothesized that TBTCl exposure would cause significant alterations in swimming performance. Tadpoles were divided into three groups: untreated controls, DMSO-only controls, and TBTCl in DMSO. They were housed individually, with solutions refreshed every 48 hours, and swimming behavior was recorded daily on video for systematic review. Tadpoles exposed to TBTCl displayed hyperactivity, erratic trajectories, and frequent collisions with container walls, consistent with disrupted neuromuscular control. In contrast, DMSO-only tadpoles showed sluggish swimming, reduced velocity, and decreased survival, suggesting solvent toxicity. Untreated controls exhibited smooth, coordinated swimming and normal survival. These findings indicate that while DMSO alone can impair locomotion, TBTCl exposure amplifies disruption and produces distinct behavioral abnormalities. Overall, behavioral assays of swimming consistency and velocity may serve as sensitive early markers of endocrine disruption in amphibians, providing a simple and non-invasive tool for toxicological research.

Gill length correlates with gill surface area in the Streamside Salamander (*Ambystoma barbouri*)

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Gill branch length is a common and accessible measurement for respiratory potential. However, gill surface area is thought to be a more reliable measurement but is harder to obtain. Little studied is how the length of one gill branch (e.g. anterior gill) relates to other gill features, such as other gill lengths and areas. Correlations would indicate that measures of branch length are useful to understand physiology. We randomly selected salamander larvae from a previously conducted egg incubation study across 4 temperature treatments, and photos of each larva were taken across key time periods in development. Image measuring software ImageJ was then used to calculate gill branch lengths and one gill's area. We used linear mixed effects models in R to evaluate correlations among gill measures. The anterior gill length correlated with the other 2 gill lengths and the sum of all 3 gill lengths. Gills 2 & 3 correlated with each other, and gill area correlated with gill length. In general, the strength of the correlations increased over time, being strongest at 60 days. Strong correlation between gill length and area indicates a strong positive correlation between the length of a gill branch and its respiratory potential. In many cases, simple measures of gill branch length will indicate among individual, or among-group differences in gill area (respiratory function). The first gill's length variation seems to most strongly predict the other two gills' lengths in the latest growth period, implying that early gill length variation may be more dependent on other factors.

Current operations of the APSU Museum and the TN Herp Atlas

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The David H. Snyder Museum of Vertebrate Zoology and the Reptile and Amphibian Atlases of Tennessee have been serving the state since 1962 and 1996, respectively. Primarily this service has been verifying and cataloging important occurrence records and physical vouchers of reptiles and amphibians in Tennessee and adjacent states. However, other crucial functions include research data requests, help with verifying new county records, specimen loans, data georeferencing and digitization, and modernizing the atlases for open and sustainable use. The atlases are also used to aid in the proper training of new biologists and in public education/outreach events. Recently there has been uncertainty in the continued funding to support work in the collections and upgrades to the online atlases. The museum and staff are operating at a reduced capacity, but operations have not ceased. Museum staff continue to maintain physical specimens, occurrence records, and data requests, and publishing help is still being offered. However, these functions are being done at a much reduced rate and largely on a volunteer and student basis. It is our hope to continue to serve the herpetological community as best as possible until we are able to find funding to drive all our normal operations. Any direction or support from the THS community is greatly appreciated.

Effects of Incubation Temperature on Juvenile Morphology of the Endangered Streamside Salamander (*Ambystoma barbouri*)

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Urbanization alters ecosystems through habitat loss and thermal stress, often intensified by the urban heat island effect, where cities remain warmer than surrounding areas due to heat-retaining surfaces. Such warming is especially impactful for the endangered Streamside Salamander (*Ambystoma barbouri*) in Tennessee, which depends on intermittent streams that can be further warmed by urbanization. To assess how temperature influences this species, we examined the long-term effects of incubation temperature on body size at six months after metamorphosis. Salamanders were incubated at 5°C, 10°C, or 20°C, and we measured appendage length, total length, and body mass. Warmer incubation temperatures did not produce lasting effects on morphology; however, salamanders incubated at 5°C had poorer body condition, driven by reduced body mass. Individuals incubated at 10°C and 20°C did not show this decline. These findings suggest that while embryos and larvae can tolerate elevated incubation temperatures, prolonged exposure to cold conditions, such as 5°C, can impair long-term body condition in this endangered ectotherm.

Fuel vs. Fitness: Driving Forces Behind Locomotor Performance in Frogs

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Locomotor performance is driven in part by adequate nutrition and may be modulated by training effects. However, there is little empirical evidence under controlled laboratory settings to suggest a relationship. Here, we evaluate the relationship using the African Clawed Frog, *Xenopus laevis*, as a model system. 28 *X. laevis* frogs were split into four treatments: exercise with controlled food intake, non-exercise with controlled food intake, exercise with unlimited food, and non-exercise with unlimited food. Frogs were individually swam in a custom built flow tank at 2000 rpm 10 minutes daily for 30 days. After 30 days, frogs were filmed swimming in a 10-gallon tank and jumping on a custom built force plate using high speed cameras. Videos were digitized in MATLAB. Treatment groups with controlled food intake showed no difference in performance or mass between exercise and non-exercised groups. Future analysis will measure performance in the additional treatment groups. We expect the treatment group undergoing exercise with unlimited food availability to perform better than other treatment groups. The expected results, in conjunction with current results, might suggest that increased locomotor performance is driven primarily by an increase in nutrient consumption, not training effects in frogs. Additional data measuring variation in physiological cross-sectional area or myofibril length may provide additional insight into the relationship of locomotion, nutrition, and training.

Validating Dermal Corticosterone Swabs with Blood Assays in Green Frogs (*Lithobates clamitans*)

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Corticosterone (CORT) is released into the bloodstream of amphibians when the organisms encounter an acute stressor. While a short-term increase in CORT is necessary to increase the chances of survival in stressful events, long-term increases can indicate a population is experiencing chronic stress. Chronic stress can lead to increased susceptibility to disease, decreases in reproduction, and declines in individual health, making CORT a quantitative biomarker of population health. Currently, the preferred method of obtaining CORT data for amphibians is blood collection. Though accurate, it is highly invasive and can be impossible to do without lethality in many cases. The swabbing of skin secretions has been proposed as a new method of obtaining CORT data. This project aims to validate the dermal swabbing method as a non-invasive alternative.

Macroinvertebrate communities across an urbanization gradient in middle Tennessee

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The Nashville basin ecoregion is defined by its karst topography. This limestone dominated landscape provides the foundation for the intermittent streams relied upon by *Ambystoma barbouri* to reproduce and spend their larval stage. These streams, which typically flow from December to June, host a community of hardy benthic macroinvertebrates which must be able to aestivate for long periods of time, and must deal with the cold weather of their home stream's hydroperiod. Benthic macroinvertebrates are often noted for their role in breaking down organic matter, nutrient cycling, and as indicators of pollution. In a region that is experiencing urban and industrial growth, it is important to understand this macroinvertebrate community in the context of conservation of *A. barbouri*. It is also beneficial to explore this community as an indicator of the efficacy of current building regulations, and for its own unique contribution to the ecosystem. In the field, 20 sites were selected with historical presence of *A. barbouri*. I used two methods of invertebrate collection at each site, the Surber sampler and the Hester-Dendy (HD) multiplate sampler. The survey samples were collected at the time of the visit, whereas the HD samplers were collected after four weeks attached to the substrate to allow for colonization. Samples from developed sites will be compared with those from reference sites to assess the impact of development on the biotic community and possibly indicate changes in hydroperiod from anthropogenic effects. This research is ongoing.

Pathogen induced dysbiosis predictably restructures the snake skin microbiome in community enrichment experiments

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Snake Fungal Disease (SFD, ophidiomycosis) is an emerging disease that has been linked to declines in North American snake populations. *Ophidiomyces ophidiicola*, the causal agent of SFD, is an epidermal pathogen that causes dysbiosis to the skin microbiome of snakes across broad spatial and phylogenetic scales. Dysbiosis is defined as disruption to the typical composition and function of a microbiome, potentially resulting in adverse effects to host health. Limited understanding of the interactions between pathogenic fungi and skin microbial communities presents challenges for the effective management of fungal pathogen outbreaks in wildlife and human populations. This study will characterize the influence of *O. ophidiicola* on the structure of skin microbial communities in a simple and manipulatable laboratory system, to contribute to a greater understanding of the mechanisms dictating skin microbiome assembly. We have optimized an in vitro microbial community assembly system to test direct interactions between *O. ophidiicola* and the snake skin microbiome in synthetic minimal media that mimics skin chemistry. Using this experimental setup, we will measure shifts in community structure over time in response to pathogen induced dysbiosis of the microbiome. Observations will be paired with results spanning multiple experimental scales to cross validate observed ecological trends. Anticipated results will include the characterization of enriched snake skin microbiome structure as well as the influence of *O. ophidiicola* on snake skin microbiome α- and β-diversity.

Thermal Performance Curves of Tennessee Streamside Salamanders (*Ambystoma barbouri*)

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Urbanization increases environmental temperatures, posing risks for ectotherms whose body temperature and physiology depend on ambient conditions. While ectotherm performance increases with temperature, excessive heat causes enzyme conformational changes that slow or halt physiological functions. The streamside salamander (*Ambystoma barbouri*), a Tennessee state-endangered species, breeds in shallow, ephemeral streams around Nashville during winter. Because it is active in colder months, understanding warming effects from urbanization is critical. We tested swimming performance of 12 *A. barbouri* at four ecologically relevant temperatures (4, 15, 20, 25 °C). Individuals were randomly assigned temperatures, acclimated and fasted for 48 h, and swam in a 120 cm lane within a shallow (3 cm) aquarium. Three 30 cm sections were timed using Adobe Premiere Pro after swimming was induced by tail taps. Each salamander completed three trials per temperature with ≥10 min rest between trials. The fastest speed for each trial at each temperature was used to construct thermal performance curves. Speed increased linearly from 4 to 20 °C but plateaued thereafter, indicating that 20 °C may impose thermal stress. Temperature had a highly significant effect on performance, whereas body mass did not. These results suggest that projected warming in urban Nashville could reduce performance capacity in *A. barbouri*, with potential consequences for survival and reproduction.

31st Annual Meeting of the Tennessee Herpetological Society
25-26 September 2025
Cedars of Lebanon State Park, Lebanon, TN

Business Meeting Notes

Recorded by Joshua M. Hall

Award Recipients

Congratulations to the 2025 recipient of the Chad Lewis Memorial Grant: **Aiden Shaw** of The University of Tennessee at Knoxville. The funded project title is: *Reproductive isolation and patterns of co-occurrence in Black-bellied and Shovel-nosed Salamanders (Desmognathus)*

Cindy Scruggs of Tennessee Tech University received the Niemiller Travel Scholarship. And the inaugural Floyd Scott Memorial Award went to **Ross Rubin** of Middle Tennessee State University.

The award for best student poster was given to **Ross Rubin** of Middle Tennessee State university for presenting his work of *Pathogen induced dysbiosis predictably restructures the snake skin microbiome in community enrichment experiments*

The award for best student oral presentation was given to **Cindy Scruggs** of Tennessee Tech University for presenting her work entitled: *The long-term effects of incubation temperature on morphology and performance in the Streamside Salamander (Ambystoma barbouri)*

The Bob Hatcher Memorial Award was given to **Dr. Danny Bryan**, Emeritus Professor at Cumberland University

Publication Committee

Herpetological Society Journal received a more submissions in 2025 than 2024. We are still working to get the journal indexed so articles are more easily discovered.

Outreach and Social Media Committee

Please follow and interact with us on the Tennessee Herp Society accounts on Facebook, Instagram (@tnherpsociety), and Twitter (@TennesseeHerper).

Treasurer's Report

As of the meeting date, the balance in the checking account was $9,860.88 and the investment balance for the Chad Lewis Grant was $34,503.40. The previous year's conference (Steele Creek Park) income was $4,255 and conference expense was $3,406.64.

New Business

A new fund was created with a $1000 donation to offset the cost of conference attendance for retired and emeritus members.

Members voted to combine the membership and registration fee into a single payment. This allows all attendees to be members and vote on society business. The business meeting can now be interspersed throughout the meeting rather than put toward the end (and have low attendance).

Members voted for the society to provide a 100% match to the travel awards, which raises them to $200 awards.

Elections

President: Lee Barton
Secretary: Joshua M Hall
Sargent at Arms: Chase Kinsey
West, TN Rep: Brian Butterfield

Next Annual Meeting

2026 will bring a West Tennessee meeting. Updates will be on the society website.