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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

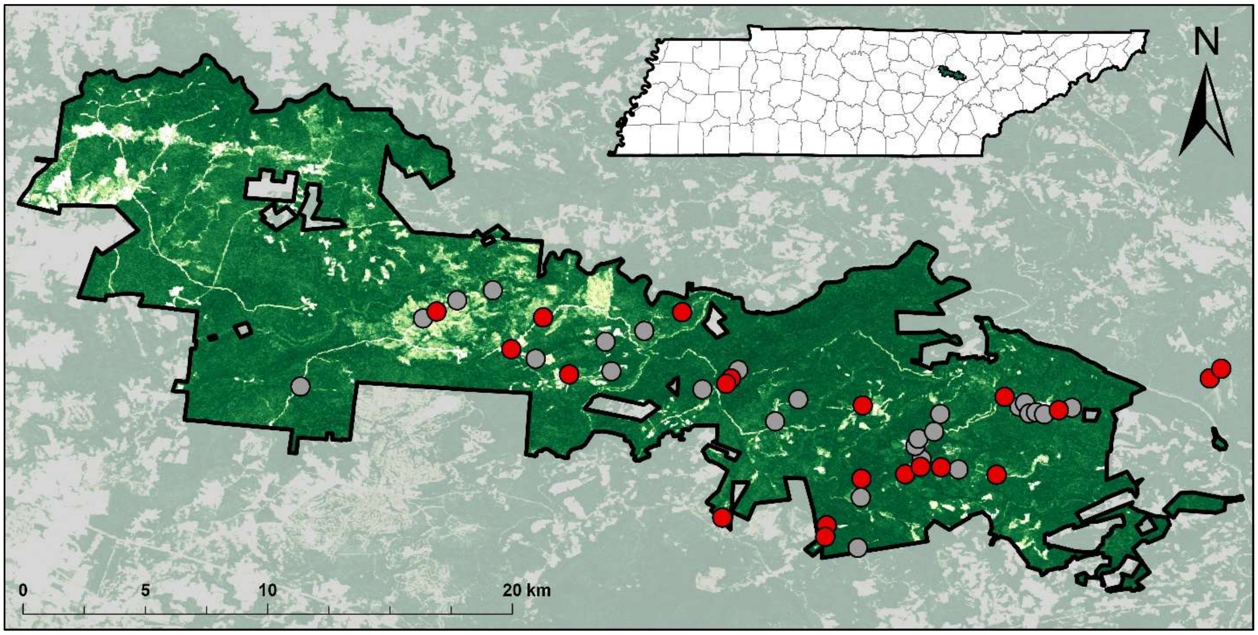

Fig. 2. A map of wetlands surveyed for four-toed salamanders (*Hemidactylium scutatum*) in Catoosa Wildlife Management Area with its position within Tennessee displayed in an inset map. Red points represent wetlands where four-toed salamanders were detected, and gray points represent wetlands where they 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).

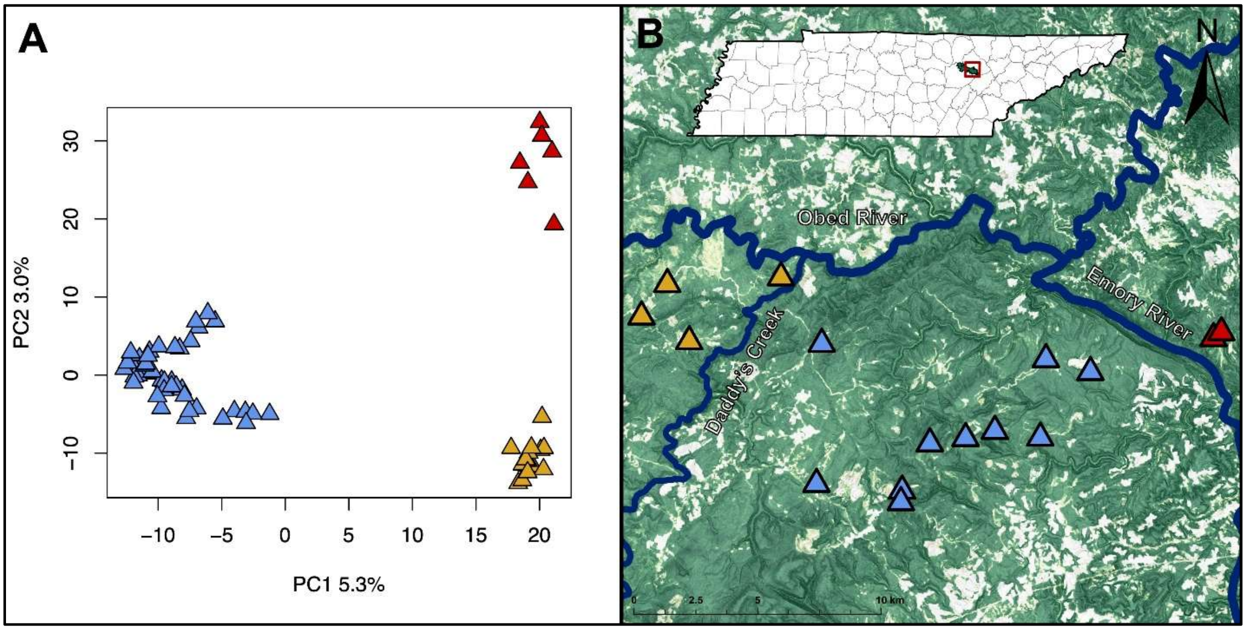

Fig. 3. The results of a principal component analysis (PCA) of single nucleotide polymorphism data for four-toed salamanders (*Hemidactylium scutatum*) showing PC1 vs PC2. Visual clusters are denoted by color (A), and their geographic positions are displayed on a map (B) in which major rivers and creeks 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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