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Cover Photo of Eastern Black Kingsnake, *Lampropeltis nigra*, by **Haley Oakley, TN Tech University**

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An Analysis of the Turtle Assemblage in the Freed-Hardeman University Research Ponds

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Abstract. — Studies of assemblages of common turtle species from small ponds are rare. Here we describe a turtle assemblage occupying two small ponds on a university campus in rural west Tennessee. We deployed two double-throated hoop nets and two wire catfish nets in each pond during each sampling session. We captured a total of 56 individuals representing five species between 2019 and 2021. The most abundant species was the Southern Painted Turtle (*Chrysemys dorsalis*) followed by the Eastern Musk Turtle (*Sternotherus odoratus*), the Pond Slider (*Trachemys scripta*), the Snapping Turtle (*Chelydra serpentina*), and the River Cooter (*Pseudemys concinna*). Survey area H' was 1.37, J' was 0.85, and S was five. Sex ratios did not significantly differ from 1:1 within species. Morphometric variables only significantly differed between sexes of the Southern Painted Turtle. Species richness in each pond was four. H' between the two ponds was not significantly different. The Eastern Musk Turtle was the only species that used one pond significantly more than the other. This information serves as a baseline to identify future changes in the assemblage. Identifying negative impacts on populations and their potential causes is a reasonable first step in the goal of keeping common species common.

Key Words. — Community, Lentic, Species Diversity, Species Richness, Small Ponds, Tennessee

Originally presumed stable and flourishing in their geographic ranges, native populations of many turtles are imperiled globally (Gibbons et al. 2000). Of the 357 turtle species identified worldwide, the International Union for Conservation of Nature (IUCN) has classified 171 of those as threatened, signifying that 47.9% of the total turtle species are Critically Endangered, Endangered, or Vulnerable (Turtle Taxonomy Working Group [TTWG] 2021). A turtle species placed on the IUCN Red List under such a categorization is facing threats to either its population stability, geographic range, or both, which can be traced to anthropogenic origins, including habitat destruction and modification, disturbance, and exploitation for food and the pet trade (Burger and Garber 1995; TCF 2002; IUCN 2012).

Turtles offer many unique services to the ecosystem in which they inhabit (Agha et al. 2018). Specifically, turtles are major contributors to the overall biomass of an ecosystem, which indicates the availability of energy within a system. As ectotherms, turtles incorporate acquired energy more efficiently into their biomass than their endotherm counterparts (Iverson 1982; Agha et al. 2018). Consequently, predators of turtles will have access to more bioavailable energy. Acting as both prey and predators, turtles can exhibit both top-down and bottom-up cascades. Through top-down effects as predators, turtles can have profound effects on the landscape of an ecosystem (Davenport et al. 2020). As prey, turtles influence the available food for predators at higher trophic levels (Colbert et al. 2010). Furthermore, by digging burrows for shelter and concealment of eggs, turtles offer safe refuge for other species from predators (Agha et al. 2018; Goodman et al. 2018; Madden et al. 2008). Some turtles, such as the Eastern Musk Turtle (*Sternotherus odoratus*), use the burrows of other turtles for egg deposition. (Niemiller et al. 2013). Therefore, turtles play a critical role in the functioning of healthy ecosystems.

While there are many studies on the role of turtles in their ecosystems, much less appears to be known about the community structure of turtles inhabiting small ponds, especially when compared with lotic environments (House et al. 2011). The few studies on small ponds concentrate their efforts on analyzing abundance, biomass, richness, and population density (Stone et al. 2005; House et al. 2011). One study conducted on small ponds surveyed differences in species diversity, richness, abundance, and sex ratios between ponds located on agricultural and golf course lands (Dorcas et al. 2007).

Figure 1. Freed-Hardeman University North Pond (A) and Middle Pond (B) within FHU Wetland, Henderson, Tennessee, USA.

Much like small pond systems, common species are routinely overlooked and understudied (Gaston 2010). Despite the lack of interest and concern for abundant species, these species and their habitats are often exploited (Gaston 2010). For example, the Snapping Turtle (*Chelydra serpentina*) is harvested in the United States and exported to Asian countries for consumption (TCF 2002). Thus, it is imperative to understand the community structure of common turtles before they become less common. Such work can provide baseline knowledge which may demonstrate the root causes of population declines in the future.

The purpose of our study was to describe the turtle community, made up of species considered common in Freed-Hardeman University's (FHU) small pond system. Specifically, we assessed species richness, species diversity, and relative abundance, sex ratios, and morphology for turtles within the system between 2019 and 2021. We also compared species richness, species diversity, and species usage between the two ponds to explore possible differences in habitat use.

Methods and Materials

Study Site. — Our survey area was two small ponds (North Pond and Middle Pond) (Fig. 1) within the FHU Wetland, Henderson, TN, USA (Fig. 2). The FHU Wetland is a 2 ha. restored wetland that formally was an agricultural field that lies on the edge of the South Fork Forked Deer River floodplain. FHU Wetland is bordered on the north by a field containing a cross country course, on the south by TN Highway 100, on the east by South Mifflin Avenue, and on the west by a field containing a cross country course and disc golf course. Municipal sewage lagoons and a forested flood plain including Sugar Creek lie south of TN Highway 100. North Pond lies east to west and has a length of 68 m and a width of 19 m. South Pond has a north south orientation and has a length of 89 m and a width of 19 m. The southern edge of North Pond is approximately 30 m from the northern edge of Middle Pond. Both ponds are approximately 1.5 m deep full pool. The banks of both ponds are surrounded by trees dominated by Oak (*Quercus* sp.). More than 30% of the surface of each pond is covered with Water Lilies (*Nymphaea* sp.).

Data Collection. — From late-March to early-May and mid-August to early-October of 2019 through 2021, the FHU Turtle Team sampled the two ponds by deploying two types of nets. Two double-throated hoop nets (76 cm diameter, 193 cm length; Memphis Net and Twine, Memphis, Tennessee, USA) and two wire catfish nets (43 cm diameter, 122 cm length; Memphis Net and Twine, Memphis, Tennessee, USA) were set in each pond. Nets were set by submerging one-half of the net in the water, leaving the other half open to air to allow captured turtles to breathe. On days that heavy rain was expected, the nets were set higher to ensure they did not become completely submerged. Nets were secured by iron rods and baited with raw chicken, chicken nuggets, fish scraps or meat scraps based on availability. After a 24-hour interval, the nets were checked for turtles and rebaited. The same type of bait was used in all nets in both ponds during each trapping session.

Figure 2. Freed-Hardeman University Wetland and surrounding area. Henderson, Tennessee, USA. N = North Pond and M = Middle Pond.

Table 1. Comparison of turtle species composition in Freed-Hardeman University Research Ponds, Henderson, Tennessee, USA. Data presented as number of individuals (relative abundance). S = species richness, H' = Shannon Index, J' = Equitability

Common Name	Scientific Name	North Pond	Middle Pond	Survey Area
Snapping Turtle	*Chelydra serpentina*	5(0.19)	5(0.13)	7(0.13)
Southern Painted Turtle	*Chrysemys dorsalis*	13(0.48)	12(0.31)	23(0.44)
River Cooter	*Pseudemys concinna*	1(0.04)	0(0)	1(0.02)
Pond Slider	*Trachemys scripta*	8(0.3)	10(0.26)	13(0.23)
Eastern Musk Turtle	*Sternotherus odoratus*	0(0)	12(0.31)	12(0.21)
S		4	4	5
H'		1.15	1.48	1.37
J'		0.83	0.96	0.85

Captured turtles were identified to species and sexed based on secondary sexual characteristics, such as tail and claw length (Ernst and Lovich 2009). Turtles were measured with 40 cm tree calipers (Haglof Inc., Madison, Mississippi, USA) to obtain straight-line measurements of maximum carapace length (CL), maximum plastron length (PL), maximum carapace width (CW), and shell height (SH), recorded to the nearest mm. All turtles were weighed to the nearest g using Ohaus top loading digital scales (Ohaus, Parsippany, New Jersey, USA) or Pesola digital scales (Pesola, AG, Baar, Switzerland). The carapace of each turtle was marked with a unique notching pattern, a variation of the technique described by Cagle (1939). Each turtle was then examined for damage or injury. After physical examinations were completed, turtles were returned to the pond from which they were trapped.

Data Analysis. — We counted the number of species captured at each pond and collectively in the survey area to determine species richness (S) and calculated relative abundance (RA) of each species by determining the proportion of each species relative to the total number of individual turtles captured. We calculated a natural log Shannon Index (H') to describe diversity and Equitability (J') to estimate heterogeneity for each pond and the sampling area collectively.

We performed a chi-square test to determine if observed sex ratios for each species differed significantly from 1:1. We compared means of each morphometric variable between sexes within each species with t-tests. We used one-tailed t-tests for the Snapping Turtle, Southern Painted Turtle, and Pond Slider because male Snapping Turtles are known to be larger than females, and female Southern Painted Turtles and Pond Sliders are known to be larger than males. We used a two-tailed t-test to compare CL between sexes of the Eastern Musk Turtles because sizes are often similar in other populations (Niemiller et al. 2013).

We compared H' between North Pond and South Pond with a Hutchison t-test. We searched for differences in pond usage within species with two-tailed Wilcoxon-Signed Rank tests. For all tests, $\alpha = 0.05$.

RESULTS

We captured a total of 56 individuals representing five species. The most abundant species was the Southern Painted Turtle (*Chrysemys dorsalis*) followed by the Eastern Musk Turtle (*Sternotherus odoratus*), the Pond Slider (*Trachemys scripta*), the Snapping Turtle (*Chelydra serpentina*) and the River Cooter (*Pseudemys concinna*) (Table 1). Survey area H' was 1.37, J' was 0.85, and S was 5 (Table 1). Sex ratios did not significantly differ from 1:1 (Table 2) within species. Shell morphology did not differ between sex save that all morphometric variables significantly differed between sexes of the Southern Painted Turtle (Table 3).

Species richness in each pond was four (Table 1). H' between North and Middle ponds was not significantly different ($t = 1.48$, df = 39, $p = 0.15$). The Eastern Musk Turtle was not captured from North Pond and the River Cooter was not captured from Middle Pond. The Eastern Musk Turtle was the only species that used one pond significantly more than the other (Table 4). Individuals of three species moved between ponds. Three of 7 Snapping Turtles, 7 of 13 Pond Sliders and 2 of 23 Southern Painted Turtles were captured in both ponds, respectively.

Table 2. Sex ratios of turtles in the Freed-Hardeman University Research Ponds, Henderson, Tennessee, USA.

Common Name	Scientific Name	Male	Female	Sex Ratio	X^2	*p*
Snapping Turtle	*Chelydra serpentina*	3	4	0.75:1	0.14	0.71
Southern Painted Turtle	*Chrysemys dorsalis*	13	9	1.44:1	0.73	0.39
Pond Slider	*Trachemys scripta*	5	8	0.63:1	0.70	0.41
Eastern Musk Turtle	*Sternotherus odoratus*	8	2	4:1	3.60	0.06

Table 3. Mean comparisons of morphometric variables between sexes within turtle species in the Freed-Hardeman University Research Ponds, Henderson, Tennessee, USA. Values given ± one standard error. Asterisks (*) indicate significance at alpha 0.05.

Variable	Male	Female	*t*	*P*
Chelydra serpentina	n = 3	n = 4		
Carapace Length	324.3±17.7	293.5±24.4	0.95[a]	0.19
Carapace Width	285.0±18.0	233.8±23.0	1.65[a]	0.08
Plastron Length	227.3±13.2	197.3±14.8	1.45[a]	0.10
Shell Height	128.5±17.5[c]	113.3±8.7	0.90[a]	0.21
Mass	7590±1183	4770±1363	1.50[a]	0.10
Chrysemys dorsalis	n = 13	n = 9		
Carapace Length	96.9±6.0	124.7±9.7	2.57[a]	0.01*
Carapace Width	73.8±4.9	95.2±5.9	2.80[a]	0.01*
Plastron Length	88.9±5.7	118.8±8.7	2.97[a]	0.004*
Shell Height	34.2±2.0	47.3±3.4	3.49[a]	0.001*
Mass	135.7±12.5[d]	295.9±51.1	3.46[a]	0.001*
Pseudemys concinna	n = 1	n = 0		
Carapace Length	256			
Carapace Width	176			
Plastron Length	221			
Shell Height	86			
Mass	1530			
Trachemys scripta	n = 5	n = 8		
Carapace Length	205.0±7.9	214.8±11.4	0.61[a]	0.28
Carapace Width	155.2±5.7	164.1±9.1	0.71[a]	0.25
Plastron Length	187.0±7.7	197.5±9.9	0.75[a]	0.23
Shell Height	77.2±4.1	85.5±6.3	0.95[a]	0.18
Mass	1105.8±135.5	1464.6±243.2	1.10[a]	0.15
Sternotherus odoratus	n = 8	n = 2		
Carapace Length	89.6±3.8	82.5±0.5	0.90[b]	0.39
Carapace Width	60.4±2.4	57.5±2.5	0.57[b]	0.59
Plastron Length	63.4±2.4	55.0±1.0	1.64[b]	0.14
Shell Height	36.4±1.2	33.5±0.5	1.13[b]	0.29
Mass	112.8±15.8	100.0±10.1	0.39[b]	0.71

[a] = one-tailed test; [b]= two-tailed test; [c] Sample size was n = 2; [d] Sample size was n = 12

Discussion

We documented the presence of every lentic turtle species expected to be in West Tennessee, except for the Eastern Mud Turtle (*Kinosternon subrubrum*), from our two ponds within the FHU Wetland (Niemiller et al. 2013). The most common of these turtle species captured included the Southern Painted Turtle, Eastern Musk Turtle, and Pond Slider, followed by the Snapping Turtle. All four lentic species are reported to be common members of the communities in which they inhabit in Tennessee (Niemiller et al. 2013).

Of these four common species, the Southern Painted Turtle represents one of the most abundant turtle species native to North America along with the closely related Painted Turtle (*Chrysemys picta*) (Niemiller et al. 2013). Similar studies on small pond systems have also documented painted turtles (*Chrysemys*) as the most abundant members of their communities with Pond Slider nearing the same levels of abundance (Stone et al. 2005; House et al. 2011; Niemiller et al. 2013). Still, other studies describe Pond Sliders as the dominant members of their community whereas Southern Painted Turtles are unable to attain the same densities (Dreslik et al. 2005; Niemiller et al. 2013). Although neither species is monitored as a species of concern, both are vulnerable to the impacts of harvesting and landscape alteration (Niemiller et al. 2013).

The other two lentic species captured in this study, the Eastern Musk Turtle and the Snapping Turtle are considered stable species of least concern globally on the IUCN Red List, and in Tennessee, both are reported to be common (Niemiller et al. 2013; van Dijk 2012; van Dijk 2015). Such reports on the Eastern Musk Turtle align with the findings of our study as the Eastern Musk Turtle represented 21% of the turtles recorded, approaching similar numbers to the Pond Slider. Though not as bountiful, the Snapping Turtle exhibited abundance levels comparable to House et al. (2011). Despite being evaluated as common species, both encounter threats to their stable populations. The Eastern Musk Turtle suffers

Table 4. Results of Wilcoxon-Signed Rank tests comparing turtle usage between North Pond and Middle Pond of the Freed-Hardeman University Research Ponds, Henderson, Tennessee, USA.

Common Name	Scientific Name	W	*P*
Snapping Turtle	*Chelydra serpentina*	14	>0.05
Southern Painted Turtle	*Chrysemys dorsalis*	128.5	>0.05
Pond Slider	*Trachemys scripta*	23.5	>0.05
Eastern Musk Turtle	*Sternotherus odoratus*	0	<0.05*

*significance at an alpha of 0.05.

more from habitat destruction and less from travel-related deaths from crossing roads as this species does not journey far from their residence unlike the Snapping Turtle (Niemiller et al. 2013). However, Snapping Turtles uniquely are at risk by potential overharvesting as they are considered a game species in Tennessee as well as many other states (Congdon et al. 1994; Colteaux 2017; Niemiller et al. 2013). Additionally, because populations of turtles in Asia are rapidly declining from overharvesting, demand for Snapping Turtle meat has dramatically increased (Colteaux 2017; TCF 2002).

The River Cooter was the least common species captured. Similar studies on small pond systems have documented the low occurrence of the River Cooter (Stone et al. 2005, House et al. 2011). House et al. (2011) attributes this to the habitat preference of the River Cooter, which is a species that selects lotic environments such as rivers as opposed to still water ponds. We suspect the lone River Cooter to be a transient that wandered to North Pond from the South Fork Forked Deer River floodplain.

We did not capture the Eastern Mud Turtle during our study. However, the turtle is known from the FHU Wetland (Butterfield et al. 2014) and FHU students previously captured individuals in turtle nets from both North Pond and Middle Pond between 1998 and 2007 (unpublished data). We suspect that development of the adjacent areas including the construction of athletic fields and paved roads have negatively impacted the Eastern Mud Turtle by hindering its access to the FHU Wetland. However, we do not know if our failure to detect the Eastern Mud Turtle during the time period of this study reflects a decline of the species within the area adjacent to the FHU Wetland.

Morphometric variables between sexes for all species did not differ significantly except for the Southern Painted Turtle. For the Southern Painted Turtle, Pond Slider, and River Cooter, sexual dimorphism is well documented, with females typically attaining larger sizes than males (Rowe 1997; Niemiller et al. 2013). This difference in size allows females more space for eggs (Rowe 1997). Yet, in the Snapping Turtle, males attain larger sizes than females, while the Eastern Musk Turtle does not display size dimorphism between the sexes (Niemiller et al. 2013). However, we found that Southern Painted Turtle males grew to larger sizes than adult females, which may be a consequence of our small sample size. Our failure to detect sexual size dimorphism among the other species may also be a consequence of small sample sizes. However, our morphometric variables between sexes of the Snapping Turtle and Pond Slider did trend in the predicted directions. That is, morphometric values for male Snapping Turtles were greater than females, and those for female Pond Sliders were greater than those for males.

We found no difference in diversity between the two ponds. We also documented moment between ponds for three species. This was not surprising because the ponds are near each other and have near identical habitat. The Southern Painted Turtle, Pond Slider, and Snapping Turtle were found in both ponds whereas, we found the Eastern Musk Turtle exclusively in Middle Pond. We currently have no convenient explanation why the Eastern Musk Turtle was not found in North Pond. The northern bank of North Pond is bordered by a cross country track which causes more human foot traffic near the pond. Further investigation is needed to assess a possible link between turtle behavior and human traffic in our study area, and with the recent installation of a disc golf course near the ponds, the turtles may be experiencing more disturbance than ever before.

Assemblages of common turtles in small ponds are largely understudied. Here we provide descriptive data from a turtle assemblage in small ponds on a university campus in rural Tennessee. This information serves as a baseline to identify future positive or negative changes in the assemblage. Identifying negative changes and potential causes of these changes to turtle assemblages is a reasonable first step in the goal of keeping common species common (Stone et al. 2014).

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Natural History Notes

***EURYCEA LONGICAUDA* (Long-tailed Salamander)**. USA: TENNESSEE: Sullivan Co.: Steele Creek Park (36.5684°N, 82.2359°W; datum WGS 84). 29 June 2023. Meredith J. Hart and Jean Van Olst. Verified by Jessica Grady. David H. Snyder Museum of Zoology, Austin Peay State University (APSU 20738; photo voucher). There is one Sullivan County record from 1934 (UMMZ 76329) from the University of Michigan Museum of Zoology (Redmond and Scott 1996. Atlas of Amphibians in Tennessee. The Center for Field Biology, Austin Peay State University, Clarksville, Tennessee. Internet version, available at https://www.apsubiology.org/tnamphibiansatlas/ [updated 18 November 2019]; accessed 11 July 2023). That record is located within the Keenburg, TN topographic quadrangle in the town of Bluff City with exact locality data on file at the David H. Snyder Museum of Zoology, Austin Peay State University (J. Grady, pers. comm.). Our record, 10.53 km away, is the second confirmed record as well as a new locality in Sullivan County. This record adds to the previously reported list of amphibians and reptiles in Steele Creek Park (Jessee et al. 2022. Southeast. Nat. 21:63–73.). One individual was observed underneath a rock in an intermittent stream within forested shale knobs.

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Abstracts of the 29th Annual Meeting of the TN Herpetological Society, Union City, TN

Student Oral Presentations

The Importance of Early Successional Habitat for Conservation of Herpetofaunal Assemblages in Tennessee

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Increasing anthropogenic influence has exacerbated climate change and ecosystem degradation, leading to global biodiversity loss, specifically within herpetofauna. However, snake population status is exceedingly difficult to determine due to cryptic and elusive behaviors, brief and patchy movement patterns, and use of inaccessible habitats. The Western Pygmy Rattlesnake (*Sistrurus miliarius streckeri*) is classified as Threatened within Tennessee (TN) and primarily occupies floodplain habitat adjacent to early successional habitats (ESH) along the western portion of the Interior Plateau ecoregion. ESH support unique flora and fauna but have declined over the last few decades mostly as a result of urban encroachment and disturbance suppression. We used a combination of drift fence and box trap arrays, road cruising, and radio telemetry to evaluate the importance of ESH for herpetofaunal conservation, with an emphasis on Pygmy Rattlesnakes. Preliminarily, we observed robust recaptures of common herpetofauna species, more frequently occurring at forest edges nearest ESH. We found box trapping to be an effective method for passive sampling of highly cryptic Pygmy Rattlesnakes, with all individuals captured within 150 meters of field edges. Evidence from this work suggests importance of forested edge habitat for herpetofaunal conservation.

Livin' la vida local: philopatry results in consistent patterns of annual space use in a long-lived lizard

Jocelyn B. Stalker, Jason L. Jones, C. M. Gienger
Austin Peay State University, 601 College St, Clarksville, TN 37044

Some reptiles can live decades in the wild and experience extreme environmental variation that influences patterns of habitat use. Individuals may modify their use of space over time, reducing the utility of single-year home range estimates. VHF telemetry data were collected for Gila monsters at three Mojave Desert sites in Clark County, Nevada and home ranges were calculated using an autocorrelated KDE. Home range size was consistent within individuals and populations and did not vary across years. To measure home range fidelity, we calculated Bhattacharyya's Coefficient (BC) for each combination of years in which an individual was tracked and averaged estimates across individuals and populations. Average BC was 0.86 (scale from 0-1) and did not vary among populations. Analysis of the frequency of movement by individuals, average distance traveled per movement, and cumulative distance traveled per active season revealed that movement patterns vary considerably by year. Heterogeneity of space use among populations and individuals suggests that individual and local environmental variation, rather than annual variation in resource availability, may drive home range size and movement patterns of Gila monsters in southern Nevada. Annual variability in movement patterns did not translate to variability in home range size or location, and the species exhibits extremely high philopatry, using the same areas for periods of at least 3-5 years.

Incubation temperature has population-specific effects on the Streamside Salamander (*Ambystoma barbouri*)

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Climate change will impact all animals but especially developing embryos of vertebrate ectotherms which rely on environmental temperature for physiological activities and receive little parental care. The effects of a warming climate on non-avian reptile development have been studied extensively; however, there is comparatively less known about impacts on amphibians, particularly salamanders. The Streamside Salamander (*Ambystoma barbouri*), a Tennessee state endangered species, has a disjunct range across relatively wide latitudes which may have resulted in population-specific responses to environmental temperature. To determine how thermally sensitive traits vary across populations and, thus, how populations may respond to climate change, we collected eggs from across the Streamside Salamander's range and incubated them at various temperatures. At each location, we deployed temperature loggers to characterize the thermal environment of nests. We measured developmental rates and recorded resultant survival and morphology of larvae and metamorphs in the laboratory. We report nest temperatures and the effects of temperature during embryonic development, highlighting trends between disjunct populations. These results will aid in targeted conservation efforts as they will demonstrate if and how endangered Tennessee populations differ phenotypically from more robust populations in the northern portion of the range.

Professional Oral Presentations

Stream Restoration Design Considerations for the Streamside Salamander (*Ambystoma barbouri*) at a Sumner County, Tennessee Mitigation Site

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The state endangered streamside salamander (*Ambystoma barbouri*) is known from a single reach of a stream & wetland

mitigation site in Sumner County, Tennessee. Permitting & design work associated with the project was conducted from 2018-2023. Existing conditions within the reach occupied by *A. barbouri* found high bank erosion and poor floodplain connectivity. Hydrology was intermittent with flow only during the winter & spring. The existing riparian buffer was absent and/or largely composed of invasive species. Proposed stream restoration followed Natural Channel Design (NCD) methodologies which catered to the ecology of *A. barbouri*. The design incorporated a Rosgen "C" stream type with riffle pool geomorphology consistent with reference sites occupied by *A. barbouri*. A pool depth ratio >2.5 and % pool of >50% provided developmental areas for larvae in the spring & early summer. Constructed riffles will be top-dressed with native slab rock currently used by *A. barbouri* for breeding activities at this location. Invasive species will be cleared from the restoration reach. The project conservation easement will be re-planted with native tree and shrub species to establish an ecologically appropriate riparian buffer. Construction activities started in July 2023 and work on the occupied reach is planned to continue through the fall of 2023.

Early-life Manipulations of the Gut Microbiota in a Vertebrate Ectotherm Affect their Heat Tolerance

Jason W. Dallas, Anna Kazarina, Sonny Lee, Robin Warne
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The gut microbiota is known to influence and have regulatory effects in diverse physiological functions of host animals, but only recently has the relationship between host thermal biology and gut microbiota been explored. Here, we examined how early-life manipulations of the gut microbiota in larval amphibians influenced their critical thermal maximum (CTmax) at different acclimation temperatures. We removed the resident microbiome on the outside of wild-caught wood frog egg masses via an antibiotic wash, and then either maintained eggs without a microbiota or inoculated eggs with pond water or the intestinal microbiota of another species, green frogs, that have a wider thermal tolerance. We predicted that this cross-species transplant would improve the CTmax of the recipient wood frog larvae relative to the other treatments. In line with this prediction, green frog-recipient larvae had the highest CTmax while those with no inoculum had the lowest CTmax. Both the microbiome treatment and acclimation temperature significantly influenced the larval gut microbiota communities and alpha diversity indices. Our results are the first to show that cross-species gut microbiota transplants alter heat tolerance in a predictive manner. This finding has repercussions for the conservation of species that are threatened by climate change and demonstrates a need to further explore the mechanisms by which the gut microbiota modulates host thermal tolerance.

There and Back Again; A Herpetologist's Tale

Michael C. Fulbright
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My professional research began in Tennessee as an undergraduate student, but graduate school took me south to Cajun country. Luckily, I gained the opportunity to return as a new faculty member at Cumberland University. I would like to share some of my past research experiences related to the physiological ecology and functional morphology of reptiles, as well as talk about my future Tennessee herp research plans. At the University of Louisiana at Lafayette I worked on a variety of research projects related to the functional morphology of reptiles. I collaborated on several projects that measured the striking kinematics of ratsnakes, cottonmouths, and western diamond-backed rattlesnakes. My dissertation research focused on the feeding mechanics and digestive physiology of microcephalic and megacephalic map turtles (Graptemys spp.). I measured voluntary bite forces, and compared those with stimulated maximal bite forces, and theoretical models of maximum bite force derived from the craniofacial muscle anatomy of adult map turtles. To develop those theoretical models, I took a classical anatomy approach to dissect and describe the muscle fiber arrangements of each portion of the jaw adductors in map turtles. I also measured the standard metabolic rate and the differences in the energetic costs of feeding on different prey bases by map turtles with differing trophic morphologies. Now I am back in Tennessee at a small, teaching institution. I want to work to share my expertise and establish new partnerships and research collaborations.

Status and Update of Amphibian and Reptile Atlases of Tennessee

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Center of Excellence for Field Biology and Department of Biology, Austin Peay State University, Clarksville, TN 37040

The David H. Snyder Museum of Zoology at APSU houses the largest amphibian and reptile collection in Tennessee. The specimens in the collections were the basis for the biogeographic, ecological, and taxonomic information used in the publication of the Atlas of Amphibians in Tennessee (1996) and the Atlas of Reptiles in Tennessee (2008; Redmond and Scott). Data from the atlases are important for conservation planning, including the Tennessee State Wildlife Action Plans. For 23 years online versions of the atlases were updated quarterly (Redmond and Scott), but updating the static content of those legacy atlases is no longer possible. Here we present recent changes to the Tennessee Herp Atlas system including migrating all content to a database-driven webpage and a more contemporary digital framework drawing species occurrence information from multiple online data sources. A live demo of the new atlases will be given.

Natural History and Status of the Pygmy Rattlesnake (*Sistrurus miliarius*) in Tennessee

William B. Sutton, Carlin Frost, Jesse Eaker, Shawn Snyder, Rob Colvin, and Brian Flock
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Tennessee is home to 35 snakes, with 4 of these species belonging to the family Viperidae. One species within this family, the Pygmy Rattlesnake (*Sistrurus miliarius*), has a limited range in Tennessee, with historical detections occurring in habitats associated with the Tennessee River in the Western Highland Rim and Southeastern Plains regions. Historically, this species has been confirmed in seven Tennessee counties, with questionable reports from an additional six counties. Since 2016, the Tennessee State University Wildlife Ecology Lab and

Tennessee Wildlife Resources Agency have collaborated to better understand the natural history, ecology, and distribution of this species in Tennessee. We report data from 67 detections (59 new captures and 7 recaptures) of Pygmy Rattlesnakes in Tennessee, including body size, sex ratios, and activity patterns. In addition to noting important behavioral observations (e.g., courtship), we have also documented the presence of neonate snakes in one population. Our work in combination with citizen science, primarily the Tennessee Snake ID Page, has resulted in the documentation of the species in three previously unconfirmed counties. The Pygmy Rattlesnake appears to be patchily distributed throughout the known range in Tennessee; however, reliable detection of the species at suspected localities remains a difficult issue.

Poster Presentations

Fecundity Selection and Sexual Dimorphism in the Gila Monster (*Heloderma suspectum*)

Alexandra Anderson, Christopher M. Gienger
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The Gila monster (*Heloderma suspectum*) is a large-bodied venomous lizard that inhabits deserts across the Southwestern United States and Northern Mexico. Previous studies have observed sexual dimorphism in this species; males have larger heads than females after accounting for body size. This trait is thought to be under sexual selection due to male-male combat in their mating system. Sexual dimorphism that could facilitate higher fecundity in females, like abdomen size, has not been previously tested. Female Gila monsters lay a clutch every 2-4 years, with each clutch being relatively large, suggesting they could benefit from having larger trunk sizes (relative to males) to accommodate large clutches and more offspring. Using morphometric data from preserved zoological museum specimens, analyses were conducted to determine if trunk size could be a target of selection and if a difference existed between males and females. Our results indicate that after accounting for body size, there is no significant difference between trunk sizes of males and females and that males are larger than females on a multivariate basis. Trunk size for both sexes had a significant effect on the amount of reproductive tissue (testes or eggs) in the specimens we measured, indicating that a larger abdomen could overall hold more eggs.

The search for a rare salamander: the use of eDNA in detection of *Eurycea junaluska* in the Great Smoky Mountains National Park

Ben F. Brammell, Sara A. Brewer, Elizabeth K. Strasko, Jarrett R. Johnson
Ben F. Brammell and Sara A. Brewer - Department of Science and Health, Shaw School of Sciences, Asbury University, Wilmore, KY 40390, USA; Elizabeth K. Strasko and Jarrett R. Johnson - Department of Biology, Western Kentucky University, Bowling Green, KY 42101, USA

Eurycea junaluska is a small, semiaquatic plethodontid salamander with a very restricted range, much of which is encompassed within the Great Smoky Mountains National Park (GSMNP). *Eurycea junaluska* was first described in 1976 and was initially only known from three creeks in Graham County, North Carolina. Currently, *E. junaluska* is known to exist in a total of five counties, all within TN and NC. The objectives of this study were to A) develop and validate a species-specific assay which detects *E. junaluska* and B) utilize this assay to reassess the distribution of *E. junaluska* within GSMNP boundaries. We obtained *E. junaluska* DNA (Swain Co., NC) and amplified and sequenced a 650 BP segment of cytochrome b (cytb). We aligned this sequence with the only *E. junaluska* cytb sequence published in public databases and designed an assay (primers and probe) utilizing conserved regions of these two sequences (95% similar). This assay was tested with sympatric species in silico (22 species) and in vitro (7 species) to confirm specificity. Fifty water samples were collected in July 2023 throughout the GSMNP; samples were collected in every major drainage, on both the TN and NC sides, and spaced as evenly as possible throughout the park. Samples were filtered in the field using sterile disposable filter funnels and extracted in the lab. qPCR analysis is currently in progress. These data should expand our understanding of exactly how rare *E. Junaluska* is and facilitate conservation efforts.

An improved assay for eDNA detection of four-toed salamanders (*Hemidactylium scutatum*): significance of mitochondrial genome region in primer development

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Environmental DNA (eDNA) is an emerging tool that promises to greatly increase the ease, efficacy, and scope of ecological studies. Recent works have highlighted the need for carefully tested assays for use in species-specific marker studies, and the value of thoroughly vetting eDNA primers using as many local sequences as available. We developed a species-specific assay (primers and probe) for use in qPCR eDNA detection of *Hemidactylium scutatum* and tested these primers in silico (22 species) and in vitro (7 species) against sympatric species to ensure specificity. We field tested our assay at 15 field sites in central and eastern Kentucky and detected *H. scutatum* DNA at 10/15 sites in a manner mostly consistent with field observations. Additionally, we collected *H. scutatum* tissue from specimens from four locations in central and eastern KY and obtained DNA from a New York collected *H. scutatum* specimen; our assay was successful in amplifying *H. scutatum* DNA from each collection locality. A previously published *H. scutatum* eDNA assay was used to detect specimens from NY, but failed to detect specimens from KY; this assay targets the intergenic spacer region (IGS), while our assay targets cytochrome b. We completed sequencing of the cytb and the IGS region from both a KY and NY *H. scutatum* specimen and confirmed conservation of cytb but significant sequence differences within the IGS region of the two specimens. These results are consistent with our understanding of mitochondrial evolution and highlight the importance of locus selection in assay development.

A Novel Application of Membrane Inlet Mass Spectrometry to Study Larval Physiology of the Streamside Salamander (*Ambystoma barbouri*)

Kaitlyn F. Darnell, Justin Murdock, Trevor Crawford, Julia Thulander, Joshua M. Hall
Tennessee Tech University, 1100 North Dixie Avenue, Cookeville, TN 38505

Urbanization often causes environmental temperatures to increase due to the replacement of vegetation with heat-absorbing substrates. Thus, the physiology of ectotherms inhabiting urbanized areas may be detrimentally affected. The Streamside Salamander (*Ambystoma barbouri*) in Tennessee is primarily threatened by urbanization, which, in addition to habitat destruction, causes temperature increases in breeding streams around the Nashville metro area. During early development, salamanders are particularly sensitive to temperature increase due to their limited thermoregulatory capacity. To determine effects of rising temperatures on larval physiology, we applied an experimental application of a Membrane Inlet Mass Spectrometer (MIMS) to examine oxygen consumption of larval *A. barbouri* at two distinct temperatures (10 and 20 °C). These temperatures span nearly the entire range of environmental temperatures experienced in the wild. Statistical analysis revealed a Q10 of 2.6 based on oxygen levels measured from individual larvae at the two thermal treatments. A Q10 of 2.6 is within the range expected for biological reactions and that observed in other species. Therefore, these data support the hypothesis that larvae function normally within this range of temperatures and warmer or cooler temperatures would be required to induce thermal stress. The results of this study offer crucial insights into the physiological adaptations of *A. barbouri* and provide a foundation for the use of MIMS to study larval physiology in the future.

Incubation moisture and temperature influence embryo physiology but not hatchling morphology in the Eastern Fence Lizard (*Sceloporus undulatus*), a model vertebrate for global change research

Lydia Dudley, Haley Oakley, Joshua M. Hall
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There is great need to understand the physiological limits of organisms to predict responses to climate change. Researchers often incorporate organismal thermal limits into predictive models, but we know comparatively less about how factors like water availability interact with temperature to mitigate or exacerbate heat stress. Embryos of vertebrate ectotherms (e.g. lizards) are particularly vulnerable to fluctuations in temperature and moisture because eggs are left to develop under prevailing conditions. Therefore, to understand the independent and interactive effects of moisture and temperature on development, we incubated eggs of the Eastern Fence Lizard (*Sceloporus undulatus*) in a factorial experiment of two moisture and two temperature treatments. One temperature regime subjected eggs to stressful temperatures so we could understand how moisture influences embryo responses to heat stress. Effects of moisture and temperature were expected: dry conditions reduced water absorption and survival, high temperatures increased embryo heart rate, developmental rate, and lowered hatching success. We observed no statistically clear interaction of moisture and temperature on any phenotype; however, egg survival was greatest under cool, moist conditions and lowest under hot, dry conditions indicating that high incubation moisture mitigates adverse effects of extreme temperatures. We highlight the need for additional work combining realistic temperature and moisture treatments to understand the relationship between multiple abiotic factors and global change.

Environmental Influences on Gila Monster Spatial Ecology

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The home range concept is a valuable tool for understanding how individuals use available habitat in relation to resource abundance and individual behavioral decisions. As resource abundance is tied to environmental conditions (precipitation, temperature, productivity), organisms may adjust their home range during periods of resource scarcity and retract range size in times of resource abundance. These patterns may be particularly apparent in organisms that live in highly seasonal environments with a wide range of suitable habitat types. We compiled telemetry data for Gila Monsters at eight sites throughout the states of Arizona, Nevada, and Utah. Annual home range utilization distributions were calculated using a weighted autocorrelated kernel density estimator. Annual environmental variables quantifying different aspects of precipitation, temperature, and habitat productivity were extracted and calculated for each individual in each year it was tracked. Annual home range size was positively correlated with annual heat moisture index and thermal seasonality, and negatively correlated with annual precipitation during the current year and annual precipitation from the year prior to each annual home range. These findings suggest that temperature and precipitation are the primary environmental variables influencing resource availability, and thereby home range size, of Gila Monsters.

Transcriptome assembly, annotation, and comparative analysis for characterizing adaptive potential of the Streamside Salamander (*Ambystoma barbouri*)

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Transcriptome sequencing and annotation use next-generation technology to sequence transcribed regions of the genome (i.e. mRNA) and assign them biological significance using a reference genome. Transcriptome sequences and gene expression levels result from existing genetic variation and its responses to environmental conditions. Thus, populations may vary in adaptive potential due to their existing genetic variation which reflects long-term historical population size and historical selection pressures like climatic history. Therefore, comparing transcriptomes across latitudinally separated populations can produce insights into how populations have adapted to past climates which may indicate how species will respond to future

climate change. In this study, we use Streamside Salamanders (*Ambystoma barbouri*) in a common garden experiment combined with genomic technology to obtain RNA-sequencing data from 80 individuals representing 6 populations across the latitudinal gradient of the species' range and two thermal treatments (10 & 20 °C). This sequencing data will be used in differential gene expression analysis to quantify population-level variation in response to thermal conditions as well as transcriptome assembly and annotation. Our data will be used to create genomic resources for conservation purposes as well as bolster our general understanding of the underlying genetic architecture of the species which may help predict population responses to future climate conditions.

Effects of Food Availability on Energy Allocation in *Sceloporus undulatus*

Haley Oakley, Lydia Dudley, Joshua M Hall
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Individuals have a limited amount of energy they can spend on reproductive effort, growth, and maintenance. The amount of energy that is beneficial for an individual to invest in each of these is dependent on the amount of food available in the environment, however, food availability is subject to change seasonally and between years. My objective is to better understand energy investment in maintenance, growth, and reproduction for *Sceloporus undulatus*, the Eastern Fence Lizard, under differing food availability conditions. I collected pairs of *S. undulatus* from two populations, provided them a standard diet until they laid their first clutch and then, if they reproduced, placed them under one of two feeding treatments to simulate a good quality or poor quality environment. This design specifically considers energy allocation in the late season when reproduction is particularly costly. I measured clutch and egg size, egg composition, hatchling morphology, and adult growth and body composition via necropsy at the end of the experiment. My statistical analyses focus on achieving two major objectives: determining what factors influence early-season reproductive traits in *S. undulatus* and understanding how females invest in maintenance, growth, or reproduction based on food availability across the season. This study enhances our general understanding of life-history theory while bolstering our knowledge related to *S. undulatus*, a model species for understanding life-history trade-offs.

Monitoring herpetofauna and *Ophidiomyces ophiodiicola* in response to pine restoration in Bankhead National Forest.

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The US Forest Service is working to restore open-canopied pine forest across the southern range of William B. Bankhead National Forest through the strategic thinning/replanting of select stands and reintroduction of fire. To monitor how herpetofauna are responding to these efforts, we deployed 16 drift fence arrays with funnel traps and pitfalls across four different stand types: control, early, late, and mature. Control stands are unburned and unthinned, resulting in dense canopies and hardwood encroachment. Early replanting stands have high sunlight penetration and pine trees <10 years old. Late replanting stands have pine trees ranging from 20–30 years old with relatively less sunlight penetration. Mature stands are characterized by open canopies and mixed age pine trees ranging from seedlings to mature trees. Early, late, and mature stands are burned on a 2-3 year rotation. Preliminary results have shown that uncommon reptiles such as *Pituophis melanoleucus*, *Plestiodon inexpectatus*, and *Ophisaurus attenuatus* are more often found in mature and early replanting stands with open canopies. Conversely, amphibians such as *Pseudotriton ruber*, *Plethodon mississippi*, and *Pseudacris brachyphona* are more common in control and late replanting stands. In addition, skin swabs were taken from each snake captured to monitor for *Ophidiomyces ophiodiicola*, a pathogenic fungus known to cause Ophidiomycosis (Snake Fungal Disease). Early results indicate a prevalence rate of approximately 9% across all stands with mature stands having the highest prevalence (12%) among all treatment types.

Population Surveys of *Ambystoma barbouri* in Middle Tennessee

Jared T. Thompson, David Withers
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Streamside salamanders (*Ambystoma barbouri*) inhabit small first and second order streams in Tennessee, Kentucky, Ohio, Indiana, and West Virginia. In Tennessee, these streams often are misidentified as wet weather conveyances and may have little to no protection against degradation from regulated activities. Anthropogenic development poses a recognized threat to *A. barbouri* as human populations within its range continue to rise. We surveyed 47 populations of *A. barbouri* from December 2022 to April 2023 to assess the current status of *A. barbouri* within Tennessee. We discovered previously unknown populations that extended the established range of *A. barbouri* in the state. The known distribution now includes additional sites in tributaries of the Duck River in Marshall County, unnamed tributaries to West Fork Drakes Creek (Barren River watershed), Sumner County, and an unnamed tributary to Cumberland River/Cheatham Lake in Ashland City, Cheatham County. Several sites were also noted from the Western Highland Rim. Populations of *A. barbouri* were observed to occupy habitats at various states of development. We noted that some populations at disturbed sites exhibited reproductive strategy previously unreported for *A. barbouri* in Tennessee. In the absence of slab rock on bedrock habitat, females were observed utilizing unconventional substrates including grass, coarse woody debris, earthen bottomed channels, and human-made refuse. Results from this survey will be used to prioritize populations for conservation and management efforts.

Assessing the interaction of stress physiology and Bd infection in Arizona tiger salamanders (*Ambystoma mavortium nebulosum*)

Megan Zerger
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Amphibian biodiversity has greatly diminished in recent years due to panzootic pathogenic fungi *Batrachochytrium dendrobatidis* (Bd), the cause of the deadly disease chytridiomycosis (chytrid). The pathogenesis of chytrid is still

unclear, as certain species and individuals within a species are differentially affected. Susceptibility and mortality of Bd are influenced by prolonged corticosterone activity, which deleteriously affects many of the same physiological processes as Bd infections. Thus, the objective of our study is to assess the relationship of corticosterone variation and Bd spore load in Arizona tiger salamanders (ATS; *Ambystoma mavortium nebulosum*) of Colorado. In 2022 and 2023, we used a dermal swabbing method to collect baseline (resting) corticosterone from paedomorph (aquatic morph) and metamorph (terrestrial morph) ATS within three minutes of capture. Bd samples were then collected via skin swabbing. These samples will allow us to consider corticosterone and Bd spore load variation by morph, sex, location, and body condition. This study will provide a greater understanding of the pathogenesis of Bd and the interacting effects of glucocorticoid production and polyphenic life history on disease resistance. These results will develop the use of corticosterone as a predictor of Bd susceptibility and severity.

29th Annual Meeting of the Tennessee Herpetological Society
28-29 September 2023
Discovery Park of America, Union City, TN

Business Meeting Notes
Recorded by Julia Thulander

Award Recipients
Congratulations to the 2023 recipient of the Chad Lewis Memorial Grant: **Kalin Ferguson** of Virginia Tech for work on *The Influence of Seasonal Precipitation on Nest Site Fidelity and Annual Population Fluctuations of Four-toed Salamanders (Hemidactylium scutatum).*

Two students received the Niemiller Travel Award this year: **Jared (JT) Thompson** of Tennessee Tech and **Sara Brewer** of Asbury University in Kentucky.

The award for best student poster was given to **Sara Brewer** of Asbury University for presenting her work of *An improved assay for eDNA detection of four-toed salamanders (Hemidactylium scutatum): significance of mitochondrial genome region in primer development.*

The award for best student oral presentation was given to **Jocelyn B. Stalker** of Austin Peay State University for presenting her work entitled: *Livin' la vida local: philopatry results in consistent patterns of annual space use in a long-lived lizard.*

Awards Committee Report
Danny Bryan informed the society of his plans to leave the Chadwick Lewis Memorial Grant Committee. Vice President, Stephen Nelson, suggests that grant notifications be sent out earlier to allow for awardees attendance to be determined sooner.

Publication Committee
Herpetological Society Journal received few submissions in 2023, continuing an ongoing trend. Joshua Hall suggests it continues to be published as it serves as a society record. Both Joshua Hall and Matt Grisnik agree to continue serving as co-editors.

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 $9,421.54 and the investment balance was $26,264.88.

New Business
Michael Fullbright was nominated and approved as the new academic representative on the Chadwick Lewis Memorial Grant Committee.

The Middle TN Representative position was filled with a special 1-year term. Michael Fullbright was nominated and approved as the new Middle TN Representative.

Elections
President: Lee Barton
Secretary: Julia Thulander
West TN Representative: Bryan Butterfield
Sargent at Arms: Darrel England

Next Annual Meeting
2024 will see the 30th anniversary of the Annual Meeting of the Tennessee Herpetological Society. Look to the society website for updates concerning the dates and location of the next meeting.