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

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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*)

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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.

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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

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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
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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
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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.