Tennessee Herpetological Society

30th Annual Meeting

The Nature Center at Steele Creek Park, Bristol, TN

September 26-27, 2024

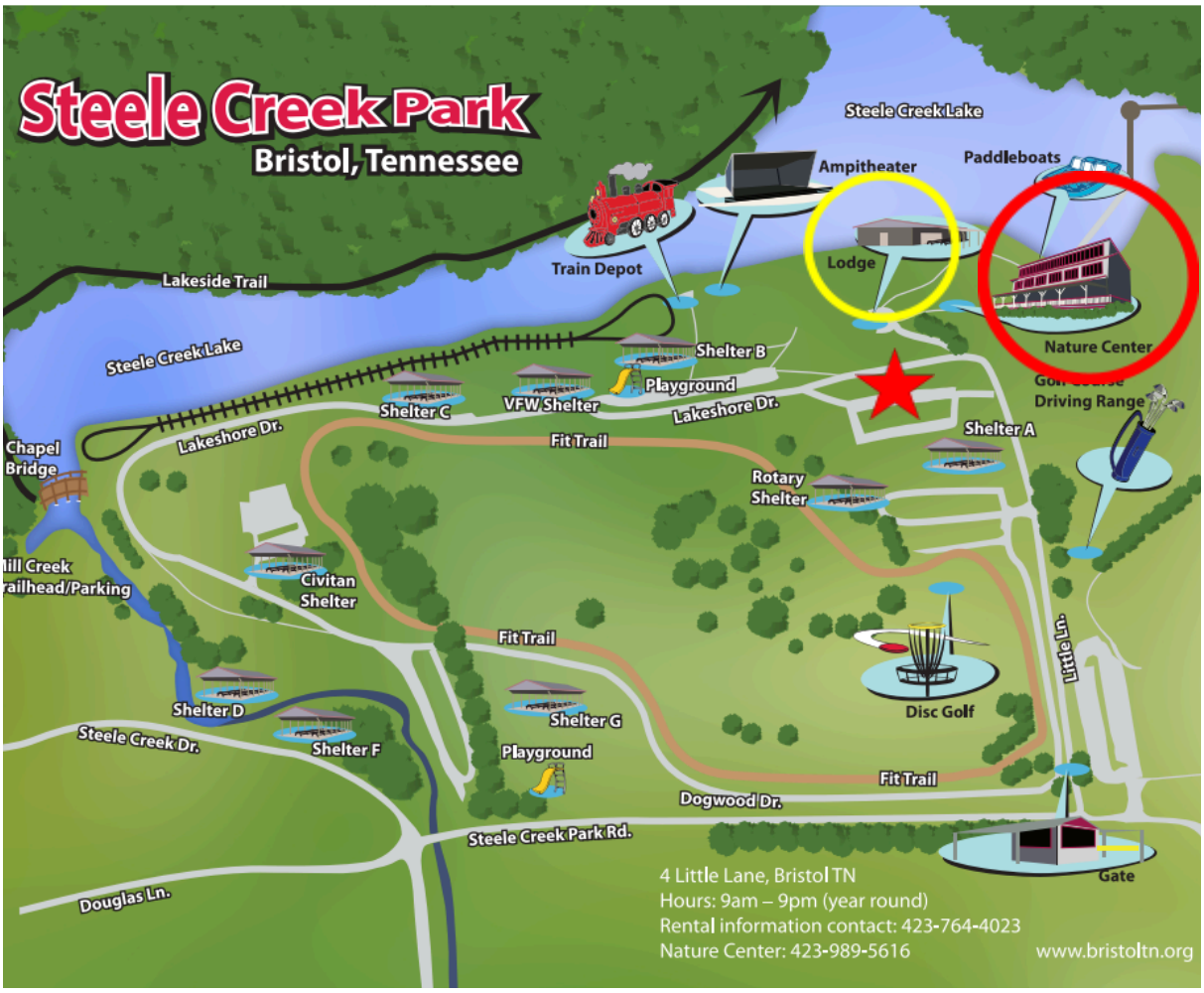
THE NATURE CENTER
At Steele Creek Park

TENNESSEE WILDLIFE RESOURCES AGENCY

TENNESSEE HERPETOLOGICAL SOCIETY
Research, Conservation & Education

Front Cover: Eastern Box Turtle, Lance Jessee, The Nature Center at Steele Creek Park

Artwork for T-shirts: Spotted Salamander, Lisa Powers



Campus map: https://www.bristoltn.org/540/Steele-Creek-Nature-Center

Attendees should park in the main parking lot, denoted by the star above, adjacent to the Nature Center.

All conference presentations will take place in the Nature Center classroom. The posters, banquet, and live auction will be held at The Lodge (circled in yellow)

Thursday Afternoon, September 26, 2024

Nature Center Classroom	
11:00 AM - Nature Center Opens	
11:00-12:30	Registration/Mix and Mingle. View Nature Center Exhibits. Snacks and beverages provided by THS. *****If you are presenting a talk on Thursday, you can upload your presentation.***** *****If you are presenting a poster, you may hang your posters during this time.*****
12:30-12:50	Opening Remarks (**Jeremy Stout, Nature Center Manager**)
Student Session I	
12:50-1:05	Can variable amounts of poly(I:C) quantifiably affect behavioral fever (**Noah Bowen**)
1:05-1:20	How do Spring Peeper advertisement calls signal Bd infection status? (**Trina Chou**)
1:20-1:35	Assessing stress hormones in Green Frogs (*Lithobates clamitans*) throughout the mating season (**Abby M. McIver**)
1:35-1:50	The influence of symbiotic algae on ranid embryonic survival and development (**Zachary T. Vegso**)
1:50-2:05	Ecological connectivity and in-kind mitigation: A case study with Four-toed Salamanders in Oak Ridge, TN (**Bryce S. Wade**)
2:05-2:20	Monitoring the response of herpetofaunal assemblages to longleaf pine forest restoration in William B. Bankhead National Forest (**Sam M. Robinson**)
2:20-2:50	15 minute break. Snacks and coffee provided by THS. ***** Session II Speakers, please upload your presentation if you have not done so.***** *****If you are presenting a poster, you may hang your posters during this time.*****
Student Session II	
2:35-2:50	Where the rubber eels meet the road: mapping the potential range of *Typhlonectes natans* in Florida using ecological niche modeling (**Derek den Ouden**)
2:50-3:05	Weller's wannabe? Investigating possible mimicry between *Desmognathus orestes* and *Plethodon welleri* (**Morgan Crum**)

3:05-3:20	Investigating the genomic variation among Green Salamanders (*Aneides aeneus*) in Bays Mountain Park and Planetarium (**Brianna Drake**)
3:20-3:35	Geometric morphometrics of the Eastern Newt's limbs across polyphenic life stages (**Aaron Hardgrave**)
3:35-3:50	Salamander distribution over a 65-year period on Mount Rodgers in southwestern Virginia (**Charlie Holguin**)
3:50-3:55	Long term impacts of roadbed contamination on salamander communities in the Great Smoky Mountains (**Ethan Furr**) - *Lightning Talk*
3:55-4:00	Determining niche overlap of two woodland salamanders in Great Smoky Mountains National Park (**Bailey C. Sauls**) - *Lightning Talk*
4:00-4:05	Caging Four-toed Salamander nests reduces nest predation in northeastern Tennessee (**Kalin J. Ferguson**) - *Lightning Talk*
4:05-4:10	Diet of 3 Co-occurring Large Plethodon Salamanders (**Maxwell Ramey**) - *Lightning Talk*
4:10-4:15	Hot Genes, Cool Salamanders: Thermal Response and Comparative Gene Expression in Streamside Salamander Populations (**Miranda J. Gaupp**) - *Lightning Talk*
This concludes the student oral presentations.	
4:15-4:25	10 minute break. Snacks/beverages provided by THS.
4:25-5:15	THS Business Meeting; Agenda is listed after the conference schedule below.
Nature Center - The Lodge	
5:15-6:30	Poster Session and mix and mingle *****Please remove posters at the end of the poster session*****
Nature Center - The Lodge	
6:30-9:30	Banquet and Live Auction

Friday, September 27, 2024

Nature Center - Classroom	
8:00-9:10	Registration/Mix and mingle; Snacks and coffee provided by THS ***** Speakers, please upload your presentation***
9:10-9:20	Announcements (**Mr. Lee Barton, THS President**)
Professional Session I	
9:20-9:35	More complex choice environments level the playing field for inconsistent or unattractive treefrog males (**Jessie C. Tanner**)
9:35-9:50	Machine Learning for Bioacoustics Analysis: The Frogs and Toads of Steele Creek Park (**John McMeen**)
9:50-10:05	A brief history of the Tennessee Herpetological Society (**Joshua M. Hall**)
10:05-10:20	*Ex Situ* Dinosaur ichnites from the Late Triassic Trostle Quarry (Newark Supergroup), Adams County, Pennsylvania (**Jeremy Stout)**
10:35-10:50	Status and Update of Amphibian and Reptile Atlases of Tennessee (**Jessica Grady**)
This concludes the conference presentations.	
10:50-11:05	*15 minute break*
11:05-11:20	Society Awards (Best presentations, Chad Lewis Award, Travel Award, Bob Hatcher)
11:20-11:30	Closing Remarks (**Mr. Lee Barton**)
11:30-12:30	THS Annual Field Trip: Nature Center - Hemlock Hollow Trail
12:30	Lunch on your own. Feel free to continue exploring the Nature Center and Steele Creek Park.

Tennessee Herpetological Society Business Meeting Agenda (9/27/2024)

Reading of the 2023 Meeting Notes

Treasurer's Report

Committee Reports

- Conservation Committee
- Chad Lewis Memorial Grant Committee
- Website/Social Media Committee
- Publication/Newsletter (Society Journal)
- Other Committees

New Business

Elections - Positions to be elected during the 2024 meeting:

- Vice President
- Secretary
- Treasurer
- East TN Representative
- Middle TN Representative

Potential 2025 Meeting sites

Current Tennessee Herpetological Society Board Members

President: Lee Barton
Vice President: Stephen Nelson
Secretary: Julia Thulander
Treasurer: Chris Ogle
West TN Representative: Brian Butterfield
Middle Tennessee Representative: Matt Grisnik
East Tennessee Representative: Scott Dykes
Sergeant at Arms: Darrell England

Abstracts of the 30th Annual Meeting of the TN Herpetological Society

Student Oral Presentations

Can variable amounts of poly(I:C) quantifiably affect behavioral fever

Noah Bowen, Joe Bidwell, Trevor Chapman
East Tennessee State University, 1276 Gilbreath Dr, Johnson City, TN 37614

A variety of threats are harming amphibian populations worldwide. Disease outbreaks being of major concern. While metabolically-induced fever may be part of the endothermic response to a pathogen, ectotherms are unable to increase body temperature by similar means. However, several studies with ectotherms indicate they may seek out warmer microclimates in response to a pathogen exposure through a phenomenon called "behavioral fever". An understanding of the extent to which amphibians exhibit this behavior could provide insight on their capacity to deal with disease exposure. The goal of this project is to explore how treatment with a chemical pyrogen (a substance that can mimic pathogen exposure) affects behavior and dermal corticosterone of Slimy salamanders (*Plethodon glutinosus*). Salamanders were randomly assigned to a treatment of the pyrogen poly(I:C): control, 7.5 µg/g, 15 µg/g, and 21.5 µg/g. Individuals were injected and placed within a behavioral arena. Dermal corticosterone was swabbed at three points: once upon capture, once before being placed in the arenas, and once after 36-hours had passed.

How do spring peeper advertisement calls signal Bd infection status?

Trina Chou and Jessie Tanner
University of Tennessee, Knoxville, Department of Ecology and Evolutionary Biology, Dabney Hall, 1416 Circle Dr, Knoxville, TN 37996

Males of many frog and toad species advertise in leks. In these systems, female choice is based on male advertisement calls. Calls are thus evolutionarily selected to convey information about an individual's quality. Disease status may affect the energy a male can allocate toward calling behavior, although it is unclear in what way and whether this is beneficial for males or females. The Hamilton-Zuk hypothesis of sexual selection posits that female selection for parasite resistance has shaped the ornamentation of male sexual signals, meaning that males with low infection levels will have more elaborate calls. Conversely, the terminal investment hypothesis predicts that individuals facing death allocate more energy into immediate reproductive investment. Evidence for these hypotheses is mixed across animal systems, and there is no clear consensus in the frog literature. To test these hypotheses, we sampled 63 male spring peepers (*Pseudacris crucifer*) and natural infections of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd) around Knoxville, TN. We analyzed 30 calls per male to test whether vocal performance was associated with fungal load and found that while Bd infection did not influence most call parameters, males with high fungal loads tended to have shorter calls with narrower bandwidths. These findings support the Hamilton-Zuk hypothesis and suggest that information about infection status is conveyed in the calls of spring peepers. Future studies will test whether female spring peepers pay attention to such differences when making mating decisions.

Weller's wannabe? Investigating possible mimicry between *Desmognathus orestes* and *Plethodon welleri*

Morgan Crum, Charlie Holguin, Kevin Hamed, Holly Kindsvater
Virginia Tech, 600 Appalachian Drive Apt 31, Blacksburg, Virginia 24060

Batesian mimicry occurs when a model species displays warning coloration to signal an unfavorable trait to predators, and a mimic species possesses the same coloration, but no such trait. This phenomenon has been observed in salamander species such as *Plethodon jordani*, the model, and *Desmognathus imitator*, the mimic. In this case, *P. jordani* produces a noxious, unpalatable secretion that is sticky and acts as a deterrent to predators while *D. imitator* does not. A mimetic relationship has been hypothesized between two species found in the Mount Rogers National Recreation Area: *Plethodon welleri* (model with noxious secretions) and *Desmognathus orestes* (mimic with no secretion). In this study, we surveyed sites separated by 30.5m in elevation, in Virginia's Smyth, Grayson, and Washington counties. Bidirectional transects of 10m by 5m were completed at each site. For each *P. welleri* and *D. orestes*, we matched their color to a swatch in the Herpeton Color Catalogue for Field Biologists. We then calculated the proportions of overlapping colors for *D. orestes* and *P. welleri* for each elevation. Our results suggest that *D. orestes* appears to be mimicking *P. welleri* based on the similarity of dorsal colors in areas of co-occurrence and a significantly reduced similarity in areas without co-occurrences.

Investigating the genomic variation among Green Salamanders (*Aneides aeneus*) in Bays Mountain Park and Planetarium

Brianna Drake, Joe Bidwell, Trevor Chapman
East Tennessee State University, 1276 Gilbreath Drive, Johnson City, TN 37614

Anthropogenic and environmental constraints have contributed to the global decline of amphibians. Comprehensive taxonomic information on threatened species is crucial for identification and provides valuable insights on crafting conservation strategies tailored to species' distinctive attributes and needs. Green salamanders (*Aneides aeneus*) are found throughout the Southern Appalachian Mountains and are labeled as vulnerable or imperiled cryptic species. This study aimed to characterize the genetic diversity in *A. aeneus* at Bays Mountain Park (BMP) in Kingsport, Tennessee to better understand the *aeneus* complex and increase the demand to recognize them as an evolutionary significant unit (ESU). Previous mitochondrial and nuclear phylogenetic analysis determined four distinct lineages within the complex: Northern Apps, Southern Apps, Hickory Nut Gorge (HNG), and Blue-Ridge Escarpment (BRE). Data from molecular studies investigating the genetic relationship among *aeneus* individuals are delimited making it difficult for researchers to assess their conservation status accurately. Tail tips were collected from individuals in BMP from April to October 2024. Genetic variation was assessed by targeting and comparing two mitochondrial genes with high mutation rates to address the presence or absence of gene flow. It was hypothesized that individuals in BMP would be genetically similar and exhibit a close genetic relationship to individuals in the characterized northern lineage. It was also hypothesized that individuals in BMP could be genetically isolated from the northern lineage.

Geometric morphometrics of the Eastern Newt's limbs across polyphenic life stages

Aaron Hardgrave and Richard Carter
East Tennessee State University, 1276 Gilbreath Dr, Johnson City, TN 37614

Eastern Newts (*Notophthalmus viridescens*) are a ubiquitous member of eastern North America's caudate fauna. Unlike the common amphibian with two major life stages, their life cycle is typically split into three phases, commonly called a triphasic life cycle. The larvae of Eastern Newts are fully aquatic, eventually metamorphosing into terrestrial juveniles called efts. Upon sexual maturity, the eft will metamorphose into a semi-aquatic adult whose external morphology resembles other aquatic salamander species. The Eastern Newt is considered polyphenic and possesses alternative life cycle strategies that are less common, including an aquatic juvenile stage and a facultatively paedomorphic adult stage. Since the different life stages of these salamanders occupy different ecological niches (terrestrial vs. semi-aquatic vs. fully aquatic) throughout their lives and, therefore likely experience various physical forces on their skeletons, they provide a unique model to study musculoskeletal changes across ontogeny and ecology. We hypothesize that ontogenetic niche shifts and the associated shifts in locomotion biomechanics will coincide with shifts in the morphology of limbs. Using micro-computed tomography (μCT) and geometric morphometrics (GMM), we quantified shape changes of limb bones across different life stages (terrestrial juvenile, aquatic juvenile, paedomorph, adult). Our findings indicate a correlation between the form and function of specific bones in the limbs, with ecological differences and the associated biomechanics.

Salamander distribution over a 65-year period on Mount Rodgers in southwestern Virginia

Charlie Holguin, Kevin Hamed, Holly Kindsvater, Matthew J. Gray, Joel W. Snodgrass
Department of Fish and Wildlife Conservation, Virginia Tech, 310 West Campus Dr. Ste 11, Blacksburg, VA 24061, United States

Climate change is hypothesized to pose a significant risk for species whose ranges are restricted by barriers to dispersal, potentially preventing organisms from shifting their distribution to track favorable climatic conditions. High-elevation species may be vulnerable as movement to their preferred climatic niches might not be possible. This may be particularly true for species that are high-elevation endemics, restricted to climatic conditions found only on mountains. We took advantage of past sampling efforts in a portion of the southern Appalachian Mountains, Mount Rogers National Recreation Area, Virginia, to investigate changes in the occurrence of 12 Plethodontid salamanders in relation to elevation over a 65-year period. Surveys of occurrence were initially conducted in the late 1950s and early 1990s, and we repeated these surveys from 2008-2012 and 2024 including repeated surveys at a subset of sites to allow estimation of detection probability. Multi-season occupancy models identified significant relationships between salamander species occurrence and elevation. We observed slight range changes from the 1990s to 2012. Additionally, preliminary data from 2024 suggests salamander populations of which species are moving upward on southward facing slopes.

Assessing stress hormones in Green Frogs (*Lithobates clamitans*) throughout the mating season

Abby M. McIver, Joe Bidwell, Trevor Chapman
East Tennessee State University, 1276 Gilbreath Dr, Johnson City, TN 37614

Amphibian populations are declining due to combinations of abiotic and biotic stressors throughout their annual active periods. Understanding how these stressors affect the physiology of amphibians is vital to conservation efforts. The goal of this study was to investigate corticosterone in Green Frogs (*Lithobates clamitans*) in response to an acute stressor and throughout the mating season. To collect corticosterone, dermal swabbing was performed in the field from May to September of 2024 in ponds at Bays Mountain Park in Kingsport, Tennessee. Data were collected for six consecutive days each month including dermal swabs, body metrics, water chemistry, weather conditions, and observational variables. The acute stress response was assessed by capturing individual frogs and taking repeated swabs over the course of a one hour holding period. The collected swabs were later analyzed in the lab using enzyme-linked immunosorbent assay (ELISA) kits to quantify corticosterone levels. It was expected that corticosterone levels will be greater in males with both sexes experiencing a peak in August.

Where the rubber eels meet the road: Mapping the potential range of *Typhlonectes natans* in Florida using ecological niche modeling

Derek den Ouden and T. Andrew Joyner
Department of Geosciences, East Tennessee State University, Johnson City, TN 37614

Florida is a globally recognized hotspot for introduced species, with nonnative herptiles being especially abundant. Among the most recently documented introductions is the neotropical aquatic caecilian *Typhlonectes natans*, which is currently found in man-made canals around the Miami-Dade area. Other introduced species have expanded from these localized urban habitats into surrounding regions, suggesting a similar possible trajectory for *T. natans*. To inform how a potential range expansion could proceed, we used maximum entropy niche modeling to establish the extent of currently suitable habitat for *T. natans* in Florida and projected future suitability under three different carbon emission scenarios. Current model results suggest habitat suitability is constrained by low temperatures and moisture availability, with suitable habitat predicted south of the frost line in Florida. This potential habitat encompasses areas of Everglades National Park and Big Cypress National Preserve, both critical conservation areas for native species. Future projections suggest that under all emission scenarios, suitable habitat for *T. natans* will likely remain restricted to South Florida. While continued range expansion is possible without human management, the negative impacts of increasing seasonality and competition from both native and nonnative species could hamper the spread of *T. natans*. These results suggest that South Florida is likely to remain a suitable habitat for *T. natans* into the future, but its invasive potential across the rest of Florida may be limited.

Monitoring the response of herpetofaunal assemblages to longleaf pine forest restoration in William B. Bankhead National Forest

Sam M. Robinson, Rachel Brubaker, Allison Cochran, William B. Sutton
Tennessee State University, 3500 John A. Merritt Blvd. Nashville, TN 37209

The United States Forest Service is working to restore longleaf pine (*Pinus palustris*) forests across the southern range of the William B. Bankhead National Forest through strategic thinning, replanting, and prescribed burning. To monitor how herpetofaunal assemblages responded to these efforts, we deployed 16 drift fence arrays with funnel traps and pit fall traps across a chronosequence of forest restoration stand types: Control (no restoration), Early-stage, Late-stage, and Desired Forest Condition (DFC). Early, Late, and DFC stands are burned on a 2–5 year rotation. Stands were monitored from May to August for four years (2021-2024). We used an ordination technique, non-metric multidimensional scaling, to examine patterns in community composition across stand types. Restoration treatments had a significant effect on reptile assemblages (p-value = 0.001), but not on amphibian assemblages (p-value = 0.52). The presence of emergent ephemeral wetlands is likely more significant than restoration treatments in determining amphibian assemblages. For reptiles, the primary ordination axis revealed a distinct gradient between control stands, dominated by generalist species, and DFC stands, characterized by specialist species. Both Early and Late-stage restoration treatments were characterized by a mixture of generalist and specialist species. Overall, the abundance and richness of herpetofauna was greatest in Early, Late, and DFC stands, apart from salamanders which had the greatest abundance in Control stands.

The influence of symbiotic algae on ranid embryonic survival and development

Zachary T. Vegso, Ryan Kerney, Sharyn B. Marks
University of Tennessee, Knoxville, Knoxville, Tennessee, USA, 37996

The single-celled *chlamydomonad* alga *Oophila amblystomatis* is known to colonize the inner egg capsules of certain pond-breeding amphibian species. Previous experiments with ambystomatid salamanders indicate a positive correlation between algal density and embryonic growth, survival, hatching synchrony, and hatchling body size, suggesting a mutualistic relationship. However, the nature of this symbiotic relationship in other documented host taxa, such as ranid frogs, remains unclear. We raised Northern Red-legged Frog (*Rana aurora*) and Wood Frog (*Rana sylvatica*) egg masses under three light treatments (24-hour light, 12:12 light:dark cycle, and 24-hour darkness) to test whether embryonic ranid hosts benefit from the symbiotic association with *Oophila amblystomatis*. Due to previously documented differences in egg mass oxygen transport and embryonic responses to hypoxia between ranid and ambystomatid hosts, we hypothesized that light treatment will have less of an influence on *R. aurora* and *R. sylvatica* embryos compared to results from similar experiments with ambystomatids. We found that eggs raised in 24-hour darkness experienced decreased survival compared to those raised in lighted treatments, but hatchling body size, stage at hatching, or rate of development was not influenced by light treatment. This differs from previous experiments with ambystomatid hosts and suggests greater diversity in embryo-algal relationships within pond-breeding amphibians.

Ecological connectivity and in-kind mitigation: A case study with Four-toed Salamanders in Oak Ridge, TN

Bryce S. Wade, Evin T. Carter, R. Trent Jett, Sarah E. Darling, Jamie M. Herold, Christopher R. DeRolph, Greg Byrd, M. Kitty McCracken, Lindsey E. Hayter, and Teresa J. Mathews
University of Tennessee, Knoxville, 310 Ferris Hall 1508 Middle Dr Knoxville, TN 37996

Ecological connectivity is critical to the survival and long-term viability of populations but is often overlooked in regulatory frameworks. We integrated landscape-level processes into a mitigation strategy for impacts to aquatic resources on the U.S. Department of Energy (DOE) Oak Ridge Reservation (ORR) in eastern Tennessee. Wetlands on the ORR, which contain significant breeding populations of the imperiled Four-toed Salamander (*Hemidactylium scutatum*), will be impacted by construction of an environmental waste disposal facility. We emphasized habitat connectivity through models that prioritized an area's importance to natural area connectivity and maintenance of population structure for an affected habitat specialist (Four-toed Salamanders). We also emphasized in-kind mitigation through the preservation and enhancement of ecologically similar resources and the translocation and establishment of a new subpopulation of four-toed salamanders elsewhere on the ORR. We translocated dozens of four-toed salamander nests, reared larvae in a unique outdoor mesocosm setup, and We ultimately released over 500 juvenile salamanders that originated from the impacted site into the chosen mitigation wetlands. This study helps show that ecological connectivity and the conservation of species that are not afforded explicit regulatory processes can be effectively and efficiently integrated into environmental decision-making and land use planning.

Professional Oral Presentations

Status and update of Amphibian and Reptile Atlases of Tennessee

Jessica Grady, Jesus Miranda, C.M. Gienger, Rebecca Blanton
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 web page 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.

A brief history of the Tennessee Herpetological Society

Joshua M. Hall
Tennessee Tech University, 1100 North Dixie Avenue, Cookeville, TN 38505

As the Tennessee Herpetological Society (THS) celebrates its

30th anniversary, it's important to recall the society history. Using materials from Lisa Powers and Chris Ogle, I wrote a new narrative of THS history for the society website and added a diversity of historical documents. THS began as a loosely organized group in 1992, but first held a conference in October 1994 at Lincoln Memorial University with keynote speaker Dr. George Folkerts. In 1995, the conference members helped pass legislation designating the Tennessee Cave Salamander and Eastern Box turtle as the state amphibian and reptile. In 1996, the society began sequestering funds for the Chad Lewis Memorial Grant, in honor of a graduate student who passed unexpectedly. The grant has grown to a $1000 award, supported by a live auction at each meeting. In 1999, during the 5th annual conference at Tennessee Tech, attendees established a formal organization, and the THS was born! The first meeting as a formal society was held in 2000 at Chickasaw State Park. In 2017, a generous donation enabled the creation of the Niemiller Travel Scholarship for students, and the first scholarship was delivered in 2018. That same year, the society published the first volume of the Tennessee Journal of Herpetology. Across the 30-year history of THS, it is clear the society has been unwavering in its commitment to research of natural history and conservation of southeastern reptiles and amphibians, with particular focus on supporting the efforts of undergraduate and graduate students.

Machine learning for bioacoustics analysis: the frogs and toads of Steele Creek Park

John McMeen
Northeast State Community College, 2425 TN-75, Blountville, TN 37617

Close monitoring of amphibian populations is essential for making informed conservation decisions. Amphibian population trends are often markers for ecosystem health, particularly in the face of habitat loss, climate change, and noise pollution, as they are highly susceptible to population decline due to environmental changes. Currently, amphibians are experiencing a global population decline, necessitating more research on the ecological impacts of these changes. For vocalizing taxa, bioacoustics monitoring has emerged as a valuable tool for gaining insights into biodiversity by studying the acoustics of an ecosystem. Passive acoustic monitoring allows researchers to collect data without necessitating their full time presence in the field, but often results in large datasets that are labor-intensive to analyze manually. To offset the time needed to analyze this data, machine learning and artificial intelligence tools can be trained to identify and classify audio collected from field research. From April through September 2023, researchers deployed passive audio monitoring devices to capture frog and toad calls at Steele Creek Park in Bristol, Tennessee. Custom artificial neural networks were implemented to aid in the acoustic analysis and audio classification of over 2700 hours of audio data. This presentation will highlight the continuing efforts of researchers at Steele Creek Park, and how these methods can be applied to research, conservation, and education in Tennessee and beyond.

Ex Situ dinosaur ichnites from the late Triassic trostle quarry (Newark supergroup), Adams County, Pennsylvania

Jeremy B. Stout, *The Nature Center at Steele Creek Park, 80 Lakeshore Drive, Bristol, TN 37620*

The Newark Supergroup is a series of Upper Triassic – Lower Jurassic age rocks existing as a north-south band across much of the eastern slopes of the Appalachian Mountains in eastern North America from Nova Scotia to South Carolina, and preserves a rich record of evolution and extinction during a tumultuous period in Earth History. The Gettysburg Shale is overlain by the York Haven Diabase sill, a course-grained diabase intruded as part of the rifting of the supercontinent Pangaea and is indicative of the late Carnian/early Norian stages of the Late Triassic. Described here are tetrapod ichnofossils from *ex situ* quarried blocks of Gettysburg Shale, observed in Adams County (south-central), Pennsylvania. At least three ichnotaxa are present: **Atreipus milfordensis**, **Anchisauripus** sp, and a manus/pes combination from a large, quadrupedally locomoting, tetrapod similar to **Eosauropus cimarronensis**. The pes of the latter is circular in shape and measures approximately 14 cm in diameter. The manus is ovate and approximately 10 cm by 3 cm. **Eosauropus cimarronensis** is known from from Arizona, Wales, and Italy, and likely represents a sauropodomorph trackmaker. The presence of an **Eosauropus**-like trace was not previously known from the Newark Supergroup and suggests a more derived sauropodomorph (and more diverse dinosaurian fauna overall) was present in Late Triassic North America than was previously thought.

More complex choice environments level the playing field for inconsistent or unattractive treefrog males

Jessie C. Tanner, Kim L. Hoke, Mark A. Bee
University of Tennessee, 569 Dabney Hall, 1416 Circle Drive, Knoxville, TN 37921

Receivers in many taxa attend to the repetition rate of signals, an emergent property requiring receivers to sample over a

period of time. However, signalers show remarkable within-individual variation or "inconsistency". This inconsistency makes decisions uncertain, and is also a trait to which receivers attend. In nature, receivers must discriminate among many signalers based on multiple signal components. We measured sexual selection on two traits, call rate and inconsistency in call rate, in the diploid Gray Treefrog, *Hyla chrysoscelis*. For each of 125 receivers, we generated 8 unique hypothetical male phenotypes (1,000 total). These hypothetical males were represented by sequences of calls whose mean call rate (calls per min) and within-individual variation in call rate (coefficient of variation) were chosen randomly and independently from the range of natural variation. We assayed signal discrimination in 2-, 4-, and 8-choice phonotaxis tests (1,500 tests). Receivers overwhelmingly chose faster, more consistent call rates in 2-choice tests, but markedly less often in 4 and 8 choices. Results suggest inconsistent signals and noisy surroundings shelter males from selection.

Lightning Talks

Caging Four-toed Salamander nests reduces nest predation in northeastern Tennessee

Kalin J. Ferguson and M. Kevin Hamed
Virginia Tech, 310 West Campus Drive, Blacksburg, VA, 24061

Four-toed Salamanders (*Hemidactylium scutatum*) have disjunct populations within the United States and Canada. An increase in predation could negatively affect their populations and further alter their distribution. Using nest cages to protect threatened populations can increase their survival by reducing the chance of predation. We installed cages around Four-toed Salamander nests in northeastern Tennessee to test the efficiency of nest cages. We used a table of random numbers to select 65/120 nests to cage at our study sites at the South Holston Weir Dam and Bouton Tract in Sullivan County, Tennessee. Based on our camera trapping survey, we found that raccoons (*Procyon lotor*) were the only predators of uncaged four-toed salamander nests. Raccoon predation events resulted in moss being removed from the clump and the absence of the female and her eggs. Our nest caging experiment found that twenty-six percent of uncaged nests were preyed upon, and none of the caged nests experienced predation. We found nest cages to be an effective conservation tool in improving the nest success of Four-toed Salamanders and a method for land managers to conserve threatened populations.

Long term impacts of roadbed contamination on salamander communities in the Great Smoky Mountains

Ethan Furr, Jon Davenport, Benjamin Fitzpatrick
Appalachian State University, 572 Rivers St, Boone, NC 28607

Beech Flats Creek (BFC), a high elevation headwater stream in the Great Smoky Mountains National Park, was exposed to contamination by Anakeesta rock formations in 1963 resulting in fish and salamander mortality. A previous study revisited the stream and found that salamander community and age structure was still impacted 30+ years after the initial contamination. In the summer of 2024, we visited impacted and unimpacted sections of BFC and adjacent watersheds to observe if salamander populations had recovered since the 90's surveys. We completed night searches for adult salamanders in and around streams, and deployed leaf litter bags to detect and count any larvae in the stream. Overall, we found a higher proportion of adult salamanders at our disturbed sites, and few/no larvae in our sites closest to the road contamination. Our findings suggest that BFC may have been recolonized by adult salamanders in contaminated sites but that recruitment may still be low.

Hot Genes, Cool Salamanders: Thermal Response and Comparative Gene Expression in Streamside Salamander Populations

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In light of rising global temperatures and the urban heat island effect, understanding the adaptive responses of ectothermic species like the Streamside Salamander (Ambystoma barbouri) is crucial for effective conservation. This study aims to elucidate the genetic mechanisms underlying thermal tolerance in A. barbouri populations across latitudinal gradients, offering insights into their resilience to climate change. We conducted a differential gene expression analysis using RNA sequencing, exposing individuals from different populations to two thermal treatments: 10°C (optimal conditions) and 20°C (thermal stress). This design allowed us to assess gene expression changes in response to temperature fluctuations during critical developmental stages. We employed DESeq2 to identify differentially expressed genes (DEGs) and compare populations. While analysis is ongoing, we anticipate revealing transcript abundance variations, highlighting unique and shared DEGs across populations. Functional annotation and enrichment analyses are expected to identify key biological pathways involved in thermal stress responses. Ultimately, this research aims to improve our understanding of the molecular mechanisms driving

phenotypic differences and adaptive responses in A. barbouri. The insights gained will inform conservation strategies and management practices to preserve this species amid environmental changes, while providing a framework applicable to other species facing similar challenges.

Diet of 3 Co-occurring Large Plethodon Salamanders

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Three species of large *Plethodon* salamanders co-occur in northwestern NC; *Plethodon yonahlossee*, *Plethodon montanus*, and *Plethodon cylindraceus*. They occupy similar habitats so it has been hypothesized that dietary niches may differ to allow co-occurrence. We performed gastric lavages on >10 individuals of each species at three different sites. All regurgitated prey items were preserved for identification and enumeration. Out of the 170 samples collected thus far, we've processed 60. From those initial 60 samples, there is a high degree of overlap in the diets of the three species. There is also high overlap among the most common prey items: ants, springtails, millipedes, mites, and beetle larvae.

Determining niche overlap of two woodland salamanders in Great Smoky Mountains National Park

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Plethodon salamanders have been identified as model organisms for ecological and evolutionary research. These amphibians are typically abundant where found and play vital roles in their ecosystems. Southern Zigzag Salamanders, *Plethodon ventralis*, and Southern Redback Salamanders, *Plethodon serratus*, are two small-bodied woodland salamanders with limited range overlap in the Great Smoky Mountains National Park and the surrounding area. These two species are ecologically similar, and research is lacking to explain how they coexist. The many potential mechanisms for coexistence are widely debated. This research focuses on niche partitioning as a possible explanation of *P. ventralis* and *P. serratus* coexistence. In particular, the partitioning of dietary resources and the related trophic morphology will be studied by sampling sympatric and allopatric populations of the two salamander species. Stable isotope analysis and geometric morphometric analysis will be employed to determine if niche overlap exists in isotopic space and head shape morphology. Based on preliminary results at nineteen sites, it appears there is overlap in the isotopic niche space of allopatric and sympatric populations of *P. ventralis* and *P. serratus*. Geometric morphometric data are awaiting analysis, but I hypothesize that overlap will be found in morphological space. Environmental parameters may play a bigger role in the coexistence of the two woodland salamanders in question. This research will expand our understanding on what factors may enable coexistence of ecologically similar species.

Poster Presentations

Pathogen prevalence and habitat selection implications of free-ranging Eastern Box Turtle populations in the Central Basin of Middle Tennessee

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This study investigates pathogen prevalence in Eastern Box Turtles (EBTs) within Tennessee's Central Basin, a region where habitat fragmentation, loss, and urbanization create significant environmental pressures. These pressures, coupled with limited green space, are expected to exacerbate pathogen susceptibility and alter the behavior of EBTs. Our hypotheses are: (1) EBTs will select macrohabitats composed of deciduous forests near water bodies and microhabitats with deeper litter cover compared to random points; (2) pathogen and co-pathogen prevalence will be greater in this study due to higher urban impacts compared to previous studies; and (3) EBTs with positive pathogen detections will exhibit altered habitat use patterns compared to those without pathogens. Turtles were notched, swabbed, and fitted with transmitters for radio telemetry tracking at both the Ellington Agricultural Center and Nashville Zoo. Detailed weather, habitat, and behavioral data were systematically collected during field surveys at each detection site. We expect results to show an increased incidence of co-pathogen detection, along with significant behavioral and movement changes in infected turtles. These outcomes will provide essential insights into disease ecology in EBTs and guide improved habitat management strategies to mitigate pathogen spread. Ultimately, the study aims to enhance conservation efforts for EBTs in the Central Basin by addressing critical knowledge gaps regarding pathogen interactions and microhabitat use.

Molecular detection of a cryptic salamander: Development of an eDNA assay for the detection of the mud salamander (*Pseudotriton montanus*).

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The mud salamander (*Pseudotriton montanus*) is a notoriously cryptic semi-aquatic Plethodontid found throughout much of the eastern United States; reports of decades passing between observations of this species in areas of known occurrence are common. Although it is listed as imperiled or in need of conservation throughout much of its range, with extirpation suspected in many areas, relatively little is known of its current distribution due to its secretive nature. We developed a species-specific qPCR assay for use in eDNA detection of *Pseudotriton montanus.* Primers and probe were designed based on cytochrome b sequences obtained from specimens collected in central and eastern KY, compared to published sequences throughout the species' range, and screened in silico (twenty-seven species) and in vitro (seventeen species) for specificity against sympatric salamander species. The developed assay was field tested via the collection of water samples at sites known or suspected to serve as *P. montanus* habitat in Kentucky, Ohio, and Tennessee. Of the 69 samples collected, *P. montanus* eDNA was detected in eight, including all sites (six) in which P. montanus larvae were observed in the field. Sequencing of each environmentally-obtained amplicon confirmed detection of *P. montanus*. This work provides thoroughly vetted tools that should prove useful for future monitoring and range delineation of this highly cryptic species.

Further evidence for a chemical mirror in socially naïve sibling common garter snakes (*Thamnophis sirtalis)*

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The visually based mirror mark test, which various animals have successfully completed, notably apes, has been used to study self-recognition, sometimes equated with self-consciousness or self-awareness. However, for non-visually dominant animals, a chemical mirror may exist. There are controversial studies on dog and wolf urine that support this hypothesis, but snakes are even more chemically reliant. A previous study with garter snakes showed a higher ratio of tongue flicks per movement on their own substrate compared to snakes fed with conspecific different diet, conspecific same diet, and clean substrates by males but not females. The current study quantified escape movements. We tested 12 male and 12 female garter snakes, *Thamnophis sirtalis*, with cage liners that were either clean, their own, or from same-sex siblings fed the same or different diet in order to test this possibility. The snakes were individually housed and fed either an earthworm or fish diet from birth. In videotaped 30-minute trials, escape movements from small enclosures were counted. Snakes of both sexes tried to escape significantly fewer times on their own substrate, both overall and in the first two 10-minute segments, compared to other stimuli. In light of this additional analysis, the possibility that squamate reptiles have a chemical "mirror" form of self-recognition is strengthened.

Analyzing sexual dimorphism and sex ratio in an East Tennessee population of Slider Turtles (*Trachemys scripta*)

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Understanding sex ratios and sexual dimorphic traits are important for monitoring and conserving wildlife populations around the world. For the past 17 years, the Clinch River Environmental Studies Organization has been collecting data on pond sliders (*Trachemys scripta*), including morphometric measurements and sex identification in Oak Ridge, TN. Here, we utilize this large data set to better understand changes in sex ratio over and sexually dimorphic traits. We found that male and female sliders are extremely sexually dimorphic, with females having longer and wider carapaces and plastrons and taller shells, while males have longer nails ($p<0.001$ for all comparisons). We also found a general trend to a more female-biased sex ratio over time, but especially in the last decade. This research helps us to better understand how long-term studies of aquatic turtles like *Trachemys scripta* can vary in sex ratios and morphological traits.

Comparative morphology of North American pitviper cranial bones with implications for fossil identification

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Snake fossils generally occur as isolated bones in fossil deposits with vertebrae being the most abundant element found. Cranial fossils are much less common, but could be more taxonomically informative than vertebrae which show more conservative morphology across related taxa. Hickory Tree Cave (HTC) in northeastern Tennessee, which includes deposits of possible Pleistocene age, has produced numerous snake remains, including some cranial elements. The majority

of those remains are from pitvipers (*Crotalinae*). The morphology of isolated cranial bones of snakes has received little attention compared to vertebrae. This project aims to describe the morphology of isolated cranial bones of North American pitvipers and find apomorphic features to distinguish genera and species. Preliminary results show that there could potentially be apomorphies (not yet described) for *Agkistrodon* and *Crotalus* in at least the maxilla, prefrontal, ectopterygoid, pterygoid, and palatine. Specific apomorphies have not been examined yet. Further work will involve including more species and more individuals of pitvipers to examine the full range of variation in each taxon and compare the HTC fossils to the modern specimens.

Aquatic turtle captures over time: Assessing the impact of sampling conditions on capture efficiency

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Hoop netting is a common method of trapping in aquatic turtle research studies. However, research involving the effects of environmental conditions on the success rates of hoop netting aquatic turtle species is scant. For the past 17 years, the Clinch River Environmental Studies Organization has collected data on captured aquatic turtle species including pond sliders (*Trachemys scripta*), common snapping turtles (*Chelydra serpentina*), eastern musk turtles (*Sternotherus odoratus*), and painted turtles (*Chrysemys picta*) in Oak Ridge, TN. We utilized this long-term data set to assess the impact of environmental conditions on capture efficiency. We used linear regression to measure the relationship between capture rates and environmental conditions such as water temperature, precipitation, time of year, and specific pond sampling. We did not find any effect of precipitation, or the specific pond sampled. Additionally, we found no significant effect of temperature was found from when testing all turtle species together. However, we did find that temperature influenced capture rates when assessing turtle species separately. Capture rates of *T. scripta* decreased as temperature increased $r^2 = 0.145$ while capture rates of *C. serpentina* increased with temperature $r^2 = 0.06$. This study informed about the impact of environmental conditions on capture rates of varied species of aquatic turtles and their reaction. Lastly, this study demonstrates the potential for different thermal optima in *T. scripta* versus *C. serpentina.*

Amphibian Elevational Biogeography

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Many amphibian species are at risk of extinction due to the invasive chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) as well as other anthropogenic influences. There is a lack of baseline data that can be used for the purpose of predicting how these species may respond to the rapidly changing ecosystems in which they live and mitigating further amphibian biodiversity loss. This project looks at amphibian elevational biogeography; specifically, how elevation affects species distribution of frogs and salamanders in the Southern Appalachian region. The hypothesis is that at lower elevations, frog biodiversity will peak while at higher elevations, salamander biodiversity will peak. Twelve herpetological inventory studies in the Southern Appalachians were analyzed; for each source/collection site, the corresponding elevation at which each species was determined via Google Earth. The results were compiled into a spreadsheet, encompassing 76 species of frogs and salamanders from the southernmost Appalachian region of Rabun County, Georgia to the northernmost region of Blair County, Pennsylvania. Efforts are still underway to analyze the results and determine if a correlation exists between elevation and amphibian species abundance.

The Long-Term Effects of Thermal Developmental Plasticity on the Endangered Streamside Salamander (*Amybystoma 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 time. The purpose of this study was to determine the long-term effects of developmental temperature during embryogenesis on adult fitness-relevant traits. Eggs were collected from streams of natural populations, incubated at naturally occurring temperatures (5°C, 10°C, and 20°C), and resultant metamorphs are currently being raised into adulthood to assess temperature effects on morphology (body size, head size, body

mass), performance (speed and endurance), and physiological (growth rate) traits. We present preliminary results for morphology at 6- and 12-months post-metamorphosis and discuss our future endeavors to assess how interactions between incubation temperature and environmental temperature shape salamander performance traits. With these results, we aim to assess the potential for incubation temperature to influence fitness of the streamside salamander via lasting impacts on phenotypes.

Variability in Male Advertisement Calls of *Hyla chrysoscelis* in Eastern Tennessee

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Understanding differences in variability and specific properties of advertisement calls in Cope's gray treefrog, *Hyla chrysoscelis*, helps to reveal population dynamics as well as give insight into individual mall calling strategy. Last summer, we recorded male call data at 4 sites in East Tennessee for 64 males with 20 calls per male for a total of 1280 calls. We then measured 14 temporal and 4 spectral characteristics of each call using Raven Software. Data were temperature corrected to 20°C. Principal component analysis (PCA) was used to determine variation in males across the 4 sites. Coefficients of Variation within males (CVw) were calculated to classify signal traits based on within-individual variation. We expected to find population differences and clustering of males based on location. We also expect that differences in the amount of variation for specific call traits will give greater insight into these within-individual differences. Our findings contribute to the growing knowledge of signaling strategies employed by individual male anurans as well as population data for several sites within Eastern Tennessee.

Stable isotope analysis of dietary niche space in three coexisting Plethodon salamanders in North Carolina

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The southern Appalachians harbor a high diversity of lungless salamanders. Upwards of 6 closely related Plethodontid species can coexist in certain terrestrial or aquatic communities. Despite the coexistence of multiple species, 1-2 species tend to be numerically more abundant. Interspecific competition may explain this dominance, and prior research with terrestrial salamanders suggests that competition may limit the distribution and abundance of Plethodon salamanders. However, environmental parameters likely effect the extent of any competitive interactions. Three species of large-bodied Plethodon salamanders (*Plethodon montanus, P. cylindraceous, P. yonahlossee*) can be found coexisting in forested ecosystems of northwestern North Carolina. Little is known about the factors that permit this coexistence, but diet may be one contributing factor. Therefore, we sought to understand overlap in dietary niche space of these 3 species in 4 separate communities. We calculated isotopic niche space and diets of each species using tail tips from field collected individuals in summers of 2021-2022. We found significant overlap in niche space among the 3 species across all 4 communities. We also found that one species, *P. yonahlossee,* appears to be consuming different forest floor invertebrate guilds in comparison to the other 2 species. Our findings suggest that our 3 focal species may be eating diets of the same trophic level but there might be partitioning within the trophic group being consumed. This may provide some explanation for current patterns of coexistence.

Estimating Alligator Body Size Based on Skeletal Remains, with Application to the Gray Fossil Site of Northeastern Tennessee

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Body size is linked to many aspects of an organism's biology, and estimation of size is regularly employed in paleontological studies to better characterize life histories of extinct animals. Several studies have examined correlation of total body length to skeletal measurements in the American alligator (Alligator mississippiensis), with some being better predictors of total length than others. Femur length has been found to be tightly correlated with total body length in A. mississippiensis, and this relationship has been used to estimate body size of extinct crocodilians. Using the femur, we estimated the body size of Alligator from the Gray Fossil Site (GFS), an early Pliocene sinkhole lake deposit in the southern Appalachians of northeastern Tennessee. Our results suggest that the GFS Alligator is smaller on average than modern A. mississippiensis. A relatively smaller size in the GFS Alligator could reflect phyletic size differences between the extant and extinct form, inadequate sampling of the fossil record, or phenotypic plasticity related to environmental conditions and/or food availability. New data shows other limb elements may also be tightly correlated with total body length and useful for body size estimation size in A. mississippiensis, which is the focus of ongoing research.