

26th Annual Meeting of the Tennessee Herpetological Society



Photo credit: Matthew Grisnik

Monday, December 6, 2021

11:00 am –12:00 pm: Registration

12:00 –12:10 pm: Opening Remarks – Dr. Donny Walker

12:10 – 1:00 pm: Keynote speaker: Christopher L. Jenkins, PhD, Chief Executive Officer, The Orianne Society

Indigo Snakes as a Template for Developing Comprehensive Species Conservation Programs

1:00 – 1:15 pm: Brief restroom and coffee break

Session 1 – Oral Presentations

1:15 – 1:30 pm: **Effects of Urbanization on Amphibian and Reptile Species Richness in Metro-Atlanta Parks.**

Tristan Clark, Tobias Landberg, and Mark L. Mandica

1:30 – 1:45 pm: **The effects of Pyrophytic Pine Restoration on Herpetofaunal Community Assemblages in Bankhead National Forest.**

Rachel Brubaker and William B. Sutton

1:45 – 2:00 pm: **Benefits to Amphibians and Reptiles through the Mud Creek Stream Mitigation Project.**

Anthony Brais

2:00 – 2:15 pm: **Effects of Watershed Degradation on Survival, Behavior and Physiology of *Cryptobranchus alleganiensis* Larvae.**

Alex Funk, Caleb Keoho, E. Davis Carter, Joseph Patrick W. Cusaac, and Matthew Gray

2:15 – 2:30 pm: **Escape Performance and Desiccation Rates in Cope's Grey Tree Frog Metamorphs: Is Bigger Always Better?**

Connor D. M. Pogue, S. Casey Perkins, Mark L. Mandica, and Tobias Landberg

2:30 – 2:45 pm: Coffee break

Session 2 – oral presentations

2:45 – 3:00 pm: **Thermoregulation of Pregnant and Non-Pregnant *Nerodia sipedon* in Middle Tennessee.**

Alexis Hamous and Vincent Cobb

3:00 – 3:15 pm: **The Effect of Snake Ecdysis on the Epidermal Microbiome and Snake Fungal Disease: Microbes are Responsive to Host Processes.**

Alex Romer and Donald Walker

3:15 – 3:30 pm: **Physiological Ramifications of SFD Infection: Shifts in Endocrine Allocation.**

Cody Davis Godwin, Donald M. Walker, and Christopher M. Murray

3:30 – 3:45: **Highly Convergent Species of *Anolis* Lizards Exhibit Divergence in Fundamental Life-History Traits.**

Joshua M. Hall, Christopher J. Thawley, and James T. Stroud

3:45 – 4:00: **Osteological Comparisons of the Eastern Newt (*Notophthalmus viridescens*) Between the Terrestrial Eft and Adult Stage.**

Aaron Hardgrave and Richard Carter

4:00 – 5:00 pm: Business meeting and annual elections

5:00 – 6:00 pm: Behind the scenes Aquarium tour

6:00 pm: Poster session prior to banquet

7:00 – 9:00 pm: Banquet and auction!

Tuesday, December 7th

Registration 8:00 – 8:45 am

Session 3 – Oral Presentations

8:45 – 9:15 am: **Floyd's Legacy, Dr. A. Floyd Scott; Jan 10, 1944 – April 25, 2021,**
John Byrd, William H. Redmond, Jessica Grady, and Angelo Bufalino

9:15 – 9:30 am: **Sampling Biases during Long-Term Study of Diamondback Terrapins at Kiawah Island, South Carolina.**

Kristen Cecala, Philip Gould, Cris Hagen, and Whit Gibbons

9:30 – 9:45 am: **Phylogeography of the *Mauremys mutica* Complex and the Implications for Conservation Management.**

Daniel Gaillard, Liu Lin, Huaiqing Chen, Shu-Jin Luo, Torsten Blanck, Yangchun Gao, Shiping Gong, and Haitao Shi

9:45 – 10:00 am: **Testing for Associations between Boldness and Thermoregulation in the Eastern Box Turtle, *Terrapene carolina carolina*.**

Natalie Foster, Matt Klukowski, and Clinton Warren

10:00 – 10:15 am: **Fighting to Save the World's Most Endangered Turtles: the Turtle Survival Alliance.**

Nathan Haislip, Carol Alvarez, Kelly Currier, Clinton Doak, Cris Hagen, Rachael Harff, and Sheena Koeth

10:15 – 10:30 am: Coffee and restroom break

Session 4 – Oral Presentations

10:30 – 10:45 am: **Osteohistological Analysis of *Alligator mississippiensis* across its Natural Range: Climate, Photoperiod, and Fossils.**

R. Davis Gunnin and Blaine W. Schubert

10:45 – 11:00 am: **Climate Change Impact on Climatic Suitability for Tennessee Reptiles and Amphibians.**

Matthew Grisnik and William B. Sutton

11:00 – 11:15 am: **Applications of Species Distribution Modeling (SDM) with Regards to the North American Quaternary Herpetofaunal Stability Hypothesis.**

Matthew W. Bushell and Blaine W. Schubert

11:15 – 11:30 am: **Effects of Estimator and Sampling Regime on Home Range Size of a Long-Lived Lizard.**

Jocelyn B. Stalker, Connor J. Hughes, Jason L. Jones, and C. M. Gienger

11:30 – 11:45 am: **Urban turtle community in an Atlanta Nature Preserve**

John A. Martin, Tristan M. Clark, S. Casey Perkins*, Connor D. M. Pogue, Mark L. Mandica, Tobias Landberg

11:45 – 11:50 am: Student Awards – Stephen Nelson and Dave Withers

11:50 am: Closing remarks – Dr. Donny Walker

12:00 – 1 pm: lunch on your own

1 pm: Tour of the Aquarium Conservation Center (**Please sign up on 12/6 during registration**)

~2:30 pm: Happy hour event at Naked River Brewing. The Tennessee Aquarium formed a partnership with Naked River for a beer that raises funds for turtle conservation programs by both the Aquarium and the TSA. The beer is a blood orange hazy IPA called Cosmic Turtle. Come join us to chat about results and future projects!

Abstracts – Oral presentations

Effects of Urbanization on Amphibian and Reptile Species Richness in Metro-Atlanta Parks.

Tristan Clark, Tobias Landberg and Mark L. Mandica.

Amphibian Foundation, 4055 Roswell Rd NE, Atlanta, GA 30342

Urban areas are the fastest growing ecosystems in the world. Urbanization has many negative effects on habitat including loss, fragmentation, and altering wetlands. Urban streams become degraded through stormwater run-off from surrounding impervious surfaces which increases litter, pollution, erosion, and sedimentation. Reptiles have been hypothesized to tolerate urbanization better than amphibians due to their protective scaly skin which prevents desiccation and direct absorption of pollutants. We measured habitat availability, diversity and quality for 110 parks inside the I285 perimeter of metro-Atlanta. Using a variety of methods, we surveyed parks at least 3 times in different seasons and spent at least 40 minutes per hectare. Park size and number of wetland types had positive effects on species richness. Amphibians had higher occupancy than reptiles at small park sizes but not at large park sizes. Stream urbanization had a negative effect on amphibians but not reptiles. Impervious surface area and terrestrial habitat quality had no statistical effect on species richness. Atlanta is located in a hilly portion of the Piedmont and has an abundance of small creeks. Atlanta might function differently from other urban areas in lowland or coastal regions where protected headwaters are not abundant. We conclude that amphibians and reptiles are robust to urbanization if their habitat needs are provided and even degraded habitat can be home to resilient species. This gives us hope for urban ecological restoration and there is a surprising amount of biodiversity left in one of the most urbanized parts of the United States.

The Effects of Pyrophytic Pine Restoration on Herpetofaunal Community Assemblages in Bankhead National Forest

Rachel Brubaker, William B. Sutton

Tennessee State University, Nashville, TN 37209

The southeastern United States historically contained upwards of 92 million contiguous acres of *Pinus palustris* habitats, but less than five percent of these forests remain today. The US Forest Service is restoring *P. palustris* stands in the William B. Bankhead National Forest (BNF) located within the northern terminus of the *P. palustris* historic distribution. This study aims to investigate the effects of the four distinct forest structural stages of *P. palustris* restoration on herpetofaunal community assemblages within the BNF. We sampled herpetofaunal communities within 16 BNF forest stands using passive drift fence, box trap, and pitfall trap arrays for 880 trap nights during summer 2021. We captured over 1200 individuals comprising 45 species of herpetofauna. We recorded daily temperature and humidity throughout the trapping season and measured forest stand structural characteristics. We will compare species assemblages within restoration stages using nonmetric multidimensional scaling and will develop habitat models for individual species using a mixed models approach. My research will help answer questions regarding the effects of *P. palustris* restoration management on southeastern biodiversity. Additionally, it will investigate the relationship between forest structure, microhabitats, and disturbance on species richness within the northern terminus of *P. palustris* forests. Collectively, this study will assist managers in future forest management decisions, expand species records of Alabama herpetofauna, and clarify the effects of *P. palustris* restoration on herpetofaunal communities in the Southeast.

Benefits to Amphibians and Reptiles Through the Mud Creek Stream Mitigation Project

Anthony Brais

Resource Environmental Solutions, LLC, 103 Continental Place, Suite 100 Brentwood, TN 37027

Mitigation restoration projects offset environmental impacts by providing functional uplift in a similar ecoregion and hydrologic unit. Although not typically tied to restoration criteria, these projects benefit amphibian and reptile species through habitat protection, habitat enhancement and species monitoring. The Mud Creek stream restoration site is located in the foothills of the Cumberland Mountains, Morgan County, Tennessee. Long-term protection from anthropogenic disturbance benefits amphibian and reptile species utilizing riparian and upland habitats. A 380 acre conservation easement was established permanently protecting a forested riparian buffer surrounding 9 miles of stream channel. Restoration of wetland and stream hydrology through natural channel design creates the aquatic habitats beneficial for breeding by amphibian species. Although the focus of the project was approx. 4 miles of stream restoration, plugs within existing channel segments created offline floodplain wetland habitat. These features were discovered by amphibian species within 1 to 3 months of restoration. To date 9 species of *Anaxyrus*, *Gastrophryne*, *Hyla*, *Lithobates*, *Pseudacris* have utilized this habitat for breeding. Lastly, mitigation restoration projects are often located on private lands which affords the opportunity to conduct species assessments on new sites. The Mud Creek site is privately held and contains lower elevation upland and wetland habitats lacking on adjacent state property (Frozen Head State Natural Area complex). To date, a total of 35 species of amphibians and reptiles have been documented on the project site including 7 county records.

Effects of Watershed Degradation on Survival, Behavior and Physiology of *Cryptobranchus alleganiensis* Larvae

Alex Funk, Caleb Keoho, E. Davis Carter, Joseph Patrick W. Cusaac and Matthew Gray

University of Tennessee at Knoxville

Eastern Hellbender (*Cryptobranchus alleganiensis*) populations have experienced steep declines and local extirpations over the last century. Surveys of declining *C. alleganiensis* populations routinely document mature individuals and egg masses, but rarely observe larvae and juveniles approaching sexual maturity. Given that *C. alleganiensis* population declines and extirpations are often associated with degraded watersheds in agricultural areas, we hypothesized that sediment and chemical inundation would significantly decrease larval survival and use of interstitial refugia. We collected and reared a single *C. alleganiensis* egg mass until larvae reached the age at which they would normally disperse from nests and randomly assigned one larva to each of 54 replicated streambed mesocosms that were treated weekly with varying levels of sediment and atrazine in a crossed design (n=9 treatments). We then monitored larval survival and behavior for five weeks. We observed no mortality across treatments. However, larval use of interstitial spaces in the gravel/cobble layer decreased significantly as sediment accumulated ($p < 0.05$), with less time spent in these spaces associated with moderate and high sedimentation treatments. We observed sediment adhered to gills and skin, which could affect respiration and other physiological processes, and atrazine was detected within 55.5 and 16.6 percent of all tail and liver tissues, respectively from atrazine exposed animals ($\mu=0.368$ mg/mL). These results suggest that recruitment gaps in declining *C. alleganiensis* populations may be a product of increased predation rates associated with exclusion from interstitial refugia and that prolonged atrazine exposure may negatively impact development and reproductive success.

Escape Performance and Desiccation Rates in Cope's Grey Tree Frog Metamorphs: Is Bigger Always Better?

Connor D. M. Pogue, S. Casey Perkins, Mark L. Mandica, and Tobias Landberg
Amphibian Foundation, 4055 Roswell Rd NE, Atlanta, GA 30342

Newly metamorphosed frogs vary greatly in size and shape and must face many novel dangers simultaneously. When transitioning from aquatic to terrestrial habitats, metamorphs must avoid drying out and escape predators. Body size and shape change may affect jumping performance and rates of evaporative water loss. While larger frogs may desiccate slower, lighter frogs may jump farther. To test for this trade-off, we examined the effects of dehydration on escape performance by measuring mass and jump distance of metamorph Cope's Grey Tree Frogs (*Dryophytes chrysoscelis*) before and after brief desiccation. As predicted, larger frogs lost less water than smaller frogs during desiccation trials. Additionally, frogs that lost up to 20% body mass generally increased jump performance. However, frogs that lost over 20% of their body mass generally decreased jump performance. It appears that slight water loss results in increased performance due to water weight load reduction, whereas losing over 20% of body mass in water loss results in decreased performance due to the deleterious physiological effects of dehydration. Since larger frogs lost less water than smaller frogs, their jumping performance tended to increase whereas small frogs tended to lose more water and were more likely to suffer decreases in performance. Consequently, in this case, larger frogs seem to be better suited to both of these environmental challenges than small frogs. When rearing frogs for release into the wild, producing bigger frogs may increase survival as opposed to creating a variety of sizes in crowded tanks that limit growth.

Thermoregulation of Pregnant and Non-Pregnant *Nerodia sipedon* in Middle Tennessee

Alexis Hamous, Vincent Cobb
Middle Tennessee State University

Snakes of several species select and maintain higher body temperatures (Tb's) during pregnancy. The leading hypothesis for this behavior is that the rate of embryonic development is facilitated by warmer temperatures and there are optimal temperature ranges for successful embryonic development. Therefore, maintaining an optimal and relatively constant body temperature could be more beneficial than costly to a pregnant snake. We used radiotelemetry and temperature loggers in gravid and non-gravid *Nerodia sipedon* to continuously record their body temperatures throughout the active season. With a sample size of 10 (6 gravid, 4 non-gravid) and total of 14284 Tb's (8848 gravid, 5436 non gravid), we found that overall 24-hour mean Tb for all snakes was $26.4 \pm 0.02^\circ\text{C}$. Non-gravid snakes overall 24-hour mean Tb ($26.4 \pm 0.04^\circ\text{C}$) was essentially the same as that of gravid snakes ($26.3 \pm 0.03^\circ\text{C}$) (RMANOVA $F=0.276$, $P=0.603$). The only difference observed was that gravid snakes were more precise thermoregulators during daytime-only (10:00-19:00) hours ($F=1.126$, $P<0.05$). Our data agrees with other studies of *Nerodia sipedon* in northern latitudes, therefore does not appear to support the notion of snakes maintaining higher body temperatures during pregnancy.

The Effect of Snake Ecdysis on the Epidermal Microbiome and Snake Fungal Disease: Microbes Are Responsive to Host Processes

Alex Romer and Donald M. Walker
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Ecological disturbance is a key factor in structuring biotic assemblages. As with macroscopic communities, microbial assemblages are subject to disturbance and react predictably to it. This study aimed to determine if a host-associated process (ecdysis; i.e., skin shedding) acts as a perturbation to the host microbiome. Additionally, ecdysis has been proposed as a host mechanism to reduce pathogen load in snakes infected with Snake Fungal Disease (SFD). SFD is an emerging infectious disease of snakes which is caused by *Ophidiomyces ophidiicola*. To study the effects of ecdysis on snake-associated microbes, we collected 22 Northern Watersnakes (*Nerodia sipedon*) and observed them over 82 days. During this time snakes were housed in experimental mesocosms where we provided an environmental reservoir of microbes using a homogenised mix of soil from snake capture locations. We successfully sampled 8 shedding events over the course of this experiment using a timeseries (0, 1-, 2-, 4-, 8-, 12-, & 24-hours post-shedding). Samples were taken of pathogen load and the microbiome using sterile swabs. DNA was extracted from these swabs which was used for qPCR (pathogen load) and high-throughput sequencing (microbiome). Ecdysis was correlated with a significant decrease in pathogen load (GLMM, z -value = - 3.271, $p < 0.05$, marginal $R^2 = 12.4\%$). Additionally, turnover of microbial taxa was significantly higher during periods of shedding than when measured pre- and post- shed ((MMDMR, t -stat = 8.40, $p < 0.0001$). These results suggest that both pathogenic and commensal microbes are responsive to ecdysis with unknown functional consequences.

Physiological Ramifications of SFD Infection: Shifts in Endocrine Allocation

Cody Davis Godwin¹, Donald M. Walker², Christopher M. Murray³

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³ Southeastern Louisiana University, Department of Biological Sciences. Hammond, LA 70402

While the lethal and acute effects of mycosis are primary concerns, organisms that survive infections can incur sublethal effects associated with a mounting immune response. Allocation of energy to the immune response can reduce an individual's fitness by interfering with sex hormone physiology eventually translating to population wide effects. Within the last decade a novel fungal pathogen *Ophidiomyces ophidiicola* has been identified and threatens wild snake populations with Snake Fungal Disease (SFD). The immune response elicited by *Ophidiomyces ophidiicola* infection may interfere with normal endocrinological function, including the hypothalamo-pituitary-gonadal (HPG) axis. We hypothesized that *Nerodia sipedon* infected with *Ophidiomyces ophidiicola* have lower sex steroid hormone concentrations (17 β -estradiol in adult females and testosterone in adult males) than non-infected snakes. Additionally, we hypothesized that increased fungal load would correlate with a decrease in respective sex steroids. Three populations of *N. sipedon* were sampled for SFD and circulating plasma hormone concentrations with the use of cutaneous swabs and ELISA hormone assays. SFD-positive female *N. sipedon* had significantly lower 17 β -estradiol concentration than SFD- negative females, while SFD positive and negative male *N. sipedon* did not differ in testosterone concentrations. An analysis of covariance detected a negative correlation of 17 β -estradiol concentration as fungal load increased in female *N. sipedon* when seasonal variation was accounted for but did not detect a correlation between testosterone concentration and fungal load in male *N. sipedon*. Our results suggest infected female snakes may have reduced reproductive energy allocation, if steroids are considered a proxy for energy investment, than non-infected female snakes. This reduction in sex hormones, related to disease state, could translate to population levels effects.

Highly Convergent Species of *Anolis* Lizards Exhibit Divergence in Fundamental Life-History Traits

Joshua M Hall¹, Christopher J Thawley², James T Stroud³

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Convergence is considered powerful evidence for the predictability of evolution. However, for many convergent species, it is unclear if convergence extends to relatively cryptic aspects of biology, especially those with major fitness consequences (e.g. reproductive strategies). By measuring multiple key reproductive traits across a full annual cycle, we discover divergence in life history traits in two otherwise highly convergent species of *Anolis* lizards. One species (*A. sagrei*) produces many, small eggs during a concentrated reproductive season, while the other (*A. cristatellus*) produces comparatively fewer, larger eggs over a longer period. Thus, despite being constrained to a single-egg clutch and being highly convergent in ecology and morphology, these species appear divergent in the trade-off between offspring size and number. Our results indicate that evolutionary pathways for life-history and ecomorphological traits are uncoupled in *Anolis* lizards, and there may be important variation in life-history evolution across this otherwise well-studied adaptive radiation.

Osteological Comparisons of the Eastern Newt (*Notophthalmus viridescens*) Between the Terrestrial Eft and Adult Stage.

Aaron Hardgrave, Richard Carter

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Eastern Newts (*Notophthalmus viridescens*) are a ubiquitous member of eastern North America's caudate fauna. Like many salamandrids, their life cycle is split into three phases, commonly called a triphasic life cycle. The larvae of *N. viridescens* are fully aquatic, eventually metamorphosing to become terrestrial juveniles, called efts. Upon sexual maturity, the eft will metamorphose into an aquatic adult where its external morphology is typical of an aquatic salamander. We compared 10 aquatic adults and 10 juvenile efts using micro-computed tomography and hypothesized that there would be differences in skeletal morphology between the two stages due to differences in locomotory requirements between aquatic and terrestrial environments. Qualitatively, we found similarity between the skeletons of the eft and adult stages. Quantifiable shape analyses between certain bones is discussed.

Floyd's Legacy, Dr. A. Floyd Scott Jan 10, 1944 – April 25, 2021

John Byrd, William H. Redmond Jr

For future generations of biologists and nature enthusiasts, especially in Tennessee, Dr. Scott's legacy includes many achievements. In addition to the many students guided and influenced, perhaps his most enduring legacy was the development and implementation of two Austin Peay State University (APSU) websites, *Atlas of Amphibians in Tennessee* and *Atlas of Reptiles of Tennessee*. Currently, APSU Biology Department's website lists Dr. Scott as coauthor and website manager of these two atlases. Dr. Scott was much more than manager of these two websites. The title "website-master" seems more appropriate. Soon after the 1996 *Atlas of Amphibians in Tennessee* was printed, Dr. Scott proposed that it be developed into a website resource. His goals were to make a widely available web tool that would be easy

to use, stimulate interest in the distribution of amphibian species in Tennessee, and promote amphibian conservation. This website went online in 1999. In 1998, he initiated and led efforts to develop a complimentary website for reptiles that was launched online in 2008. In total, these two websites have been operating and achieving Dr. Scott's original goals for over 20 years. Both have proven to be valuable reference and learning tools for biologists and the general public. Both have stimulated significant advances in understanding distributions of amphibian and reptilian species in Tennessee. Dr. Scott was the primary force behind implementation and subsequent long-term success of both websites. At his insistence, both websites were refreshed quarterly to add new data, make needed taxonomic changes, and correct errors. He was the scientific gatekeeper and spent an enormous amount of time learning the software intricacies of website management. He was truly website-master of both sites. At the time of his death, he was in the initial stages of developing a strategy to transfer these responsibilities. Hopefully, this legacy will continue and be improved as new technology becomes available.

John Byrd – I would like to thank Floyd's Family and Friends for their help and allowing me the honor to share and celebrate a few highlights of Floyd's life. I also thank Bill Redmond for his summary of Floyd's legacy

Sampling Biases During Long-Term Study of Diamondback Terrapins at Kiawah Island, South Carolina

Kristen Cecala¹, Philip Gould², Cris Hagen³, and Whit Gibbons⁴

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Long-term data are necessary for understanding the population dynamics of organisms, but even more so for long-lived species like turtles. Recent modeling efforts have demonstrated that monitored populations should be expected to decline due to biases associated with selecting sampling locations. Therefore, it should be unsurprising that the methodology used to sample terrapins during long-term study may also be subject to biases. Using the long-term dataset of diamondback terrapin captures at Kiawah Island using seines, we evaluated environmental factors that may influence the detection of terrapins and potentially bias the conclusions we may draw about the current status or trajectory of populations. Recent surveys on Kiawah Island have detected more young and new individuals than previous sampling, and environmental factors such as tide amplitude and time of year significantly influence our ability to capture terrapins with seines. We are optimistic that these data signal a potential recovery of terrapin populations at Kiawah Island, but more years of data will be necessary to determine if this trend will continue. Furthermore, these data indicate that we should interrogate our capture method for bias and carefully use that information to either adjust our methods or incorporate additional modeling terms to provide more accurate estimates of ongoing population trends.

Phylogeography of the *Mauremys mutica* Complex and the Implications for Conservation Management.

Daniel Gaillard^{#1,4}, Liu Lin^{# 1}, Huaqing Chen², Shu-Jin Luo², Torsten Blanck⁵, Yangchun Gao³, Shiping Gong^{*3,6}, Haitao Shi^{*1}

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² *The State Key Laboratory of Protein and Plant Gene Research; School of Life Sciences; Peking-Tsinghua Center for Life Sciences; Peking University, Beijing 100871, China*

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⁴ *Department of Life Sciences, Dalton State College, 650 College Drive Dalton, GA. 30720*

⁵ *Cuora Conservation Center, Austria.*

⁶ *College of Life Science and Technology, Jinan University, Guangzhou, 510632, China*

For more than three decades, the Asian turtle crisis has resulted in the decline of every native species in China. For some species, such as the yellow pond turtle (*Mauremys mutica*), wild populations have dwindled to near functional extinction. Previous studies show there is deep genetic divergence of *M. mutica* sensu lato between populations north and south of the Pearl River Drainage but no data to show if phylogeographic structure occurs within these two main types. In this study, we found clear phylogeographic structure. In northern types, we found two main clades, corresponding to mainland China and island clades (Taiwan and Yaeyema Islands) with uncorrected *p* values of 0.00-2.0% divergence in our 2353 bp concatenated mtDNA data set. For the southern types, we found three main clades corresponding to Hainan, Mainland (Vietnam/Guangxi) and the Annam pond turtle (*Mauremys annamensis*) with divergence ranging from 1.0-1.8% among these three groups. Moreover, the identification of northern and southern types by phenotype was roughly 98% accurate, which, coupling with the deep genetic divergence in mtDNA (5.5-6.7%) and in the 6056 bp nuDNA data set (0.16-0.37%) provide sufficient evidence for northern *M. mutica* to be an independent species, and individuals from the southern clade should be regarded as subspecies of *M. annamensis*. Finally, we provide the most comprehensive database to date which can be used to determine the region of origin for captive stock. Making the large captive populations of *M. mutica*, under the right conditions, potentially valuable for restocking or augmentation of wild populations.

Testing for Associations Between Boldness and Thermoregulation in the Eastern Box Turtle, *Terrapene carolina carolina*

Natalie Foster, Matt Klukowski, and Clinton Warren
Middle Tennessee State University

The study of animal personality in non-mammalian species is an active area of research. Due to the rise in identified animal personality traits and recognized species with personalities, many studies now aim to explore the ecological relevance and fitness implications of animal personality. Recently, box turtles have been shown to exhibit consistent individual boldness behaviors. It has been speculated that thermoregulation could be an important driver for the persistence of boldness personality in box turtles via the life-history trade-offs that are typically associated with thermoregulation in reptiles. However, some temperature-oriented studies of box turtles suggest that *T. carolina* are largely thermoconformers and select habitats based on non-temperature related features. This study sought to determine whether a pattern between boldness personality and thermoregulation occurs in an eastern box turtle population of a Middle Tennessee wetland. Behavioral and thermal data were repeatedly collected from 15 adult, mixed-sexed *T. carolina* during the summer of 2020 and 2021. The box turtles demonstrated consistent individual differences in boldness scores and were classified as either ‘bold’ or ‘shy’. However, no correlation occurred between boldness and the turtles’ shell carapace temperatures. This could reflect that *T. carolina* are not heavily dependent upon behavioral thermoregulation during the summer in Middle Tennessee, and that boldness is uncoupled from thermoregulatory behavior in these turtles. If correct, boldness may have fitness implications other than enhanced thermoregulation. Future studies should

consider other alternative life-history trade-offs involved in the evolution and persistence of boldness personality within box turtles.

Fighting to Save the World's Most Endangered Turtles: The Turtle Survival Alliance

Nathan Haislip, Carol Alvarez, Kelly Currier, Clinton Doak, Cris Hagen, Rachael Harff, and Sheena Koeth

Turtle Survival Alliance, Charleston, South Carolina, 29407

The Turtle Survival Alliance (TSA) was established in 2001 in response to the “Asian Turtle Crisis” in which unsustainable numbers of turtles were being harvested from the wild for the food trade, pet trade, and traditional Chinese medicine. Initially a joint taskforce with the IUCN Turtle and Tortoise Specialist Group, the TSA began to establish programs throughout the world in areas where chelonians were threatened with extinction. Currently, the TSA is active with *in situ* conservation in 13 countries as well as a U.S. based facility in South Carolina, impacting 20 of the top 25 most endangered turtles in the world. The goal of the TSA is “zero turtle extinctions” and utilizes relationships with zoos and aquariums, government organizations, other non-profit organizations, and the private sector to help chelonian species continue to persist both *in situ* and *ex situ*. The TSA’s Turtle Survival Center (TSC) is the focus of *ex situ* conservation efforts. Species housed here are difficult, if not impossible, to work with abroad due to their population size, collection pressures, or other factors such as lack of suitable habitat or legal protection. The TSC is establishing assurance colonies of these species in the hopes that one day, future generations can be repatriated where these species once occurred.

Osteohistological Analysis of *Alligator mississippiensis* Across its Natural Range: Climate, Photoperiod, and Fossils

R. Davis Gunnin, Blaine W. Schubert

Center of Excellence in Paleontology and Department of Geosciences, East Tennessee State University, 325 Treasure Lane, Johnson City, TN

Histological analysis of long bone thin sections has long been used to study the population dynamics of extant herpetofauna as well as growth rates and ecology in extinct vertebrates. Interpreting bone histology in living and fossil taxa requires a robust understanding of the factors influencing bone growth and histology. Among the most widely histologically studied fossil vertebrates are archosaurs, and as such, much of our knowledge of histology is derived from the two extant lineages: avian dinosaurs and crocodylians. The American alligator (*A. mississippiensis*) is widely available for study in the United States and several osteohistological analyses have been published. These studies focused on intraskeletal variation, histovariability, and skeletochronology in one or a few specimens. No published studies address large-scale geographic and climatic variation in bone histology. To fill this gap in our knowledge of archosaurian osteohistology, we plan to assess histovariability against climactic and geographic variables in a large sample of *A. mississippiensis* from seven states across its range, including relatively recent invasions into continental habitats. Additionally, we plan to sample bone from histological thin sections for oxygen isotope analysis with the goal to compile a dataset for comparison to other archosaur taxa. Finally, we will apply our findings to Pliocene *Alligator* fossils from the Gray Fossil Site, Washington Co., Tennessee. Assessing variation in this fossil taxon’s closest living relative (*A. mississippiensis*) will provide insight into the paleoecology and growth rates of the GFS *Alligator*, as well as the climate of the Southern Appalachians during the earliest Pliocene.

Climate Change Impact on Climatic Suitability for Tennessee Reptiles and Amphibians

Matthew Grisnik and William B Sutton
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The Southeastern United States represents a hotspot for reptile and amphibian diversity, containing approximately half of all the reptile and amphibian species in the US. However, climate change is predicted to cause shifts in suitable habitat which may result in the loss of many species. Therefore, identifying areas that will be impacted by climate change is vital to guide conservation activities. In this study we use species distribution modeling and vulnerability analyses to predict the effect of climate change climatic suitability for reptiles and amphibians across Tennessee with an emphasis on species of greatest conservation need. We generated species distribution models using Maxent and RandomForest algorithms for all the reptiles and amphibians found within Tennessee. Each model was then projected on forecasted climate data, specifically 12 Global Circulation Models, for two Relative Concentration Pathways (4.5 and 8.5), across two years (2050 and 2070) to predict the shift in distribution of suitable climatic conditions under climate change.

Applications of Species Distribution Modeling (SDM) with Regards to the North American Quaternary Herpetofaunal Stability Hypothesis

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The North American Herpetofaunal Stability Hypothesis states that the distributions of reptiles and amphibians were relatively stable during climatic and environmental fluctuations of the Quaternary, including glacial advances and retreats. However, this hypothesis has been criticized because species identifications were typically inferred using a combination of broad morphological criteria and current geographic ranges. Thus, identifications were taken too far, resulting in a self-fulfilling hypothesis based on erroneous circular reasoning. Since the recognition of this problem, some workers have focused on finding species specific osteological characters, but at this time most reliable identifications of Quaternary herps are at the genus or family level, not species. Here we take another approach to understanding the impact of Quaternary climatic change on extant herpetofauna using species distribution modeling (SDM). Using this technique, we determine the potential distributions of selected herpetofauna species over time using current environmental parameters. We compare these data to the late Pleistocene fossil record of northeastern, Tennessee, and discuss the potential implications for past distributions of North American herpetofauna. Finally, we provide some projection data on the same species based on predicted future climatic changes.

Effects of Estimator and Sampling Regime on Home Range Size of a Long-Lived Lizard

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Small sample sizes, irregular sampling regimes, temporal autocorrelation, and environmental variation are often unavoidable when studying the spatial ecology of animals in their natural environment and can introduce noise that make data challenging to analyze. We sought to examine the impact of factors influencing the home range size of Gila monsters (*Heloderma suspectum*), whose longevity (up to twenty years in the wild), highly variable and hostile environment, and secretive nature can create experimentally

noisy data. VHF telemetry data were collected from two Mojave Desert sites in Clark County, Nevada. Minimum convex polygon (MCP), kernel density estimator (KDE), and autocorrelated kernel density estimator (AKDE) methods were used to estimate home range area. We predicted that AKDE home range estimates (which account for autocorrelation, a source of negative bias in other estimators) would be larger than either MCP or KDE estimates and found that AKDE estimates are larger than MCP estimates only ($p=0.0001$). Our study sites differed in water availability and habitat and mean AKDE home range size differed between the sites ($p=0.0188$) and ranged from 7 to 418 hectares. We predicted annual home range size estimates for individuals would exhibit temporal instability due to long-term weather variation and found high individual variation ($p=3.01e^{-5}$) but low annual variation ($p=0.185$). Heterogeneity of spatial use among populations and individuals suggests that individual and local environmental variation drive home range size of *H. suspectum* in southern Nevada when sampling effects are accounted for.

Urban Turtle Community in an Atlanta Nature Preserve

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Urbanization threatens wildlife populations through many mechanisms. Long-lived species with delayed maturity like turtles are at risk of decline due to invasive species, road mortality, subsidized predators, and habitat degradation. We conducted a mark-recapture study for six months in 2020 and two months in 2021 on a 12 ha metro-Atlanta nature preserve to assess turtle diversity and abundance. Based on problems facing other urban turtle populations, we predicted this community would exhibit male-biased populations and that the invasive Red-eared Slider would be present on the preserve. Over 33 total weeks of study, we made 93 hand captures in >173 search hours and 84 trap captures in 225 trap nights. 123 individual turtles were identified with 54 recaptures. Only one Snapping Turtle and one Red-eared/Yellow-belly Slider intergrade hatchlings were found. The male:female ratio varied dramatically among species: 29:4 in Snapping Turtles, 3:12 in Pond Sliders, and 30:33 in Eastern Musk Turtles. Less abundant species included: Painted Turtles (0:3), Box Turtles (0:2), a River Cooter (1:0), and a Loggerhead Musk Turtle (1:0). Six invasive Red-eared Sliders and two Red-eared/Yellow-bellied Slider intergrades were removed from the population to be used in education and outreach programs. Identifiable sources of mortality included juvenile road mortality and territorial male combat. Both species richness and abundance of turtles exceeded our expectations for this small urban park, however the strongly male biased sex ratio of Snapping Turtles suggests significant problems with urbanization that may impact future generations.

Abstracts –Poster presentations

Acute Exposure of Roundup is Sufficient to Induce Behavioral Changes in Larval Stream Salamanders

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Glyphosate is a commonly used agricultural pesticide that can have non-target effects on a wide range of organisms. Although directly applied to agricultural fields, runoff during rainfall introduces the chemical into surrounding water bodies. In lotic systems, glyphosate accumulates and negatively affects the physiology, development, and behavior of amphibians. However, these known effects stem from chronic exposure relative to acute exposure experienced by amphibians inhabiting adjacent streams. Previous studies demonstrated a dose-dependent decrease in burst distance associated with glyphosate exposure in stream salamanders, but it is unclear whether and how long it might take for individuals to recover from acute exposure. Our objective was to describe the recovery of stream salamander larvae, *Eurycea wilderae*, to short-term exposure of glyphosate. We observed that exposed individuals recovered from glyphosate exposure between 2 and 4 hours post-exposure. Length of exposure did not affect responses by stream salamander larvae. Concurrently, we also observed decreased burst responses by unexposed animals that could be due to habituation or exhaustion during our trials, and we recommend that future trials minimize the frequency of sampling. Because burst distance is associated with swimming ability, impairment could temporarily increase predation risk, or more significantly, contribute to downstream displacement of larval individuals inhabiting streams in agricultural areas.

Detecting Fossorial Salamanders Using eDNA; Development and Validation of Quantitative and End-Point PCR Assays for the Detection of Five Species of Ambystomid Salamanders

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In the past decade environmental DNA (eDNA) has become firmly established as an effective method for detecting the presence of organisms of research and conservation interest and promises to greatly increase the ease, efficacy, and scope of ecological studies. Salamanders of the family Ambystomatidae are large, fossorial species; adults are rarely encountered aboveground outside of their brief reproductive season. Larvae develop rapidly, in ephemeral pools or streams, often with multiple species coexisting in a single habitat. A number of Ambystomatid species are of conservation interest in various portions of their range. We developed species-specific qPCR assays for *Ambystoma barbouri*, *Ambystoma jeffersonianum*, *Ambystoma opacum*, *Ambystoma maculatum*, and *Ambystoma tigrinum* and tested all in silico and in vitro. Additionally, all except *A. tigrinum* were tested in situ. Tissue tests confirm specificity of primers among all these species as well as the frequently sympatric *Notophthalmus viridescens*. In situ larvae surveys were conducted and water samples collected from known *Ambystoma* breeding sites in central and Eastern Kentucky. Initial probe-based qPCR eDNA results (nine sites) indicate detection at each site of each species collected via dipnet sampling. Additionally, two sites were positive for species not collected via dipnet. Replicate analysis of the field collected water samples using end-point PCR produced results identical to qPCR assays. These assays provide an effective means of determining

species present in particular habitats rapidly and definitively and therefore offer to increase the ease of range delineation and spawning habitat studies.

An Underutilized Tool in Conservation: A High School Herpetology Club Goals for Outreach, Education, and Fundraising

East Hamilton Herpetology Club, Julianne Evitts, Sonja Fabela, Avery Keown, Bliss Murphy, Elissa Nord, Ja'Toria Powell, Howard Swanson, Leah Tudor, Andrew Young, Paul-Erik Bakland
East Hamilton School, 2015 Ooltewah Ringgold Rd., Ooltewah TN, 37363

In a world with a rapidly worsening biodiversity crisis, a need for novel approaches which address outreach, education, and conservation funding has arisen. The East Hamilton Herpetology Club is a recently established high school club and is the first of its kind in Hamilton County and may be the only such club in Tennessee. The club is comprised of likeminded students that share a passion for reptiles and amphibians and wish to contribute to the conservation of these groups. Presented here are some short-term club goals that include plans to improve habitat for herps on East Hamilton's campus, engage in public outreach, generate a campus species inventory, and raise money for regional and global conservation efforts. Long-term goals include building partnerships with conservation organizations and local population monitoring projects. Additionally, this club will function to give students an introduction into the increasingly competitive world of professional herpetology and provide pre-college experience in data collection, analysis, presentation, and publication.

Current Host and Geographic Range of Snake Fungal Disease in Tennessee: An Update to Grisnik et al., 2018

Emily K. Stone, N. Reed Alexander, Tom Blanchard, Olivia Bowers, Danny Bryan, Josh Campbell, Kristen Cecala, Rob Colvin, Jesse Eaker, Josh Ennen, Matt Grisnik, Cody Godwin, Kevin Fouts, Alexis Hamous, Ryan Hanscom, Becky Hardman, Cory Holliday, Daniel Istavanko, John Lamb, Kenan Lockmueller, Brian Miller, Kylie Moe, Andrew Moore, Chris Murray, Chris Ogle, Emma Phipps, Helen Plylar, Alex Romer, Chris Simpson, Shawn Snyder, Thomas Stroud, Bill Sutton, Mallory Tate, Dustin Thames, Pandy Upchurch, Jackson West, Alexis Young, & Donald M. Walker

Snake fungal disease (SFD) is an emerging mycosis that has been associated with declines in snake populations across the eastern United States since 2005. The causative agent of snake fungal disease (SFD), *Ophidiomyces ophiodiicola*, was first detected in Tennessee in 2012. *Ophidiomyces ophiodiicola* has previously been detected in snakes collected from 15 of 28 (54%) counties surveyed statewide with an overall prevalence of 30%. Here, we present an updated account of *Ophidiomyces ophiodiicola* presence in Tennessee compiled from statewide sampling efforts. We report consistent positive detection of the pathogen in all eight Tennessee ecoregions. We also note seasonal patterns in both pathogen detection and host mortality as well as behavioral patterns associated with infection, including surface basking during hibernation months. We report a higher relative prevalence in aquatic snakes compared to other ecomodes and an overall statewide prevalence of 23% across all animals sampled. Lastly, we report positive detection of *Ophidiomyces ophiodiicola* in 74% (23/31) snake species sampled throughout Tennessee, including four species of greatest conservation need. To better illuminate patterns in disease progression and host mortality, regular sampling across Tennessee is necessary.

Status and Update of Reptile and Amphibian Atlases of Tennessee

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The David H. Snyder Museum of Zoology at APSU houses the largest amphibian and reptile collection in Tennessee. The specimens in the collections have contributed to and have been enhanced by the publication of the Atlas of Amphibians in Tennessee (Redmond and Scott, 1996), Atlas of Reptiles in Tennessee (Scott and Redmond, 2008), and the subsequent APSU websites, Atlas of Amphibians of Tennessee and Atlas of Reptiles of Tennessee, both websites initiated and managed under the leadership of Dr. Scott. For 23 years the atlases were updated quarterly, and provide information on the biogeography, ecology, and taxonomy of Tennessee amphibians and reptiles. Data from the atlases are important for conservation planning, including the Tennessee State Wildlife Action Plans. The collections and atlases are a resource for researchers seeking to verify state or county records and provide an important historical record of herpetological collecting in Tennessee and adjacent states. The online atlases receive thousands of page views every week from around the world. Since November 2019, the goal has been to update atlas content and modernize the web interface, thereby continuing Scott and Redmond's goal of making data on the reptiles and amphibians of Tennessee as widely available as possible. Some beta version examples of the new reptile atlas will be demonstrated and options discussed. Please come share with us how you have used the atlases, what changes you would like to see, and find out how you can help us continue this legacy.

Multiple Methods of Detection of Semiaquatic Salamanders in Small Lotic Systems: A Comparison of eDNA and Leaf Litter Bags.

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Environmental DNA (eDNA) utilizes DNA released from aquatic organisms into the environment to detect their presence and provides an effective, non-invasive method to survey organisms in an efficient manner. This has generated considerable interest in the relationship between traditional sampling methods and eDNA, considering the potential benefits of eDNA in enhancing the ease of organism detection. We are surveying the salamander community of three first order streams in central Kentucky (Garrard, Rockcastle, and Madison Counties) using both traditional (leaf litter bags) and molecular (eDNA) assessment methods. Briefly, leaf litter bags have been placed at 3 m intervals at each sampling location and water samples are being collected biweekly below the lowest leaf bag at each site. Water samples are filtered in the lab within 24 hours and eDNA extracted following established laboratory protocols. End point PCR is being used to detect salamander DNA utilizing assays developed in our lab from cytochrome b sequences obtained from locally collected specimens. Preliminary data indicates detection of *Eurycea cirrigera* (southern two lined salamander) in leaf litter bags in two of three sites but in all three sites using eDNA. The final results should both provide interesting insight into the relationship between traditional and novel methods of amphibian detection and useful data concerning the species present in these systems.

Relative Abundance and Diversity Estimates in a Preliminary Study of Three Urban Wetland Sites in Dalton, Georgia

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To characterize a herpetofaunal community, it is necessary to measure ecological attributes by which to quantitatively describe the aspects of that community and to make comparisons. This effort reports on a preliminary investigation of the diversity of reptile and amphibian species in three urban wetland sites in north Georgia relative to the following hypotheses: 1) ecologically generalized species will be the most numerically abundant forms present in these urban habitats and 2) diversity of species will decrease with a reduction in area of available habitat. To examine these hypotheses, variable trapping regimes were carried out during March through September, from 2013 to 2021, in the three urban wetland settings. Collection activities incorporated methods for surveying aquatic and terrestrial species and included baited and unbaited traps, drift fences, cover boards, visual surveys and walking surveys/ hand captures. The relative abundance of generalist species was greater in each study site with *Trachemys scripta*, *Sternotherus odoratus*, and *Chelydra serpentina* being the most relatively abundant aquatic turtle species across the sites. Habitat specialists, like *Apalone spinifera*, were detected in relatively low numbers and were absent in some study sites. Observed diversity generally decreased at study sites that were smaller in area with 26 species (Park Creek/~40ha), 13 species (Civitan Park/ ~6ha), and 11 species (Lakeshore Park/ ~5ha) being encountered respectively.

Development and in-vitro Validation of PCR Assays for Use in eDNA Detection of *Ambystoma texanum* and *Ambystoma talpoideum*

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Since the publication of the first macroinvertebrate environmental DNA study in 2008, the eDNA method has rapidly developed into a powerful tool that shows great promise in increasing the ease and efficiency of ecological studies. Salamanders belonging to the family Ambystomatidae are fossorial and rarely seen above ground outside of their brief reproductive season, making their range and density difficult to assess. Eggs are deposited in ephemeral ponds and streams, often with multiple species utilizing the same habitat. Several species from this family are classified as threatened or in need of conservation in part or all of their range, highlighting the need for efficient data collection methods that can be applied to this taxon. We developed species-specific PCR assays for *Ambystoma texanum* and *Ambystoma talpoideum* and tested them in silico and in vitro. Tissue tests confirm specificity with all seven *Ambystoma* species found in Tennessee and Kentucky as well as the frequently sympatric *Notophthalmus viridescens*. Primers were also validated using laboratory water tests to confirm detection of target species. These assays demonstrate great promise in providing an efficient method of identifying breeding habitats used by these species, contributing significantly to a wide variety of studies concerning their ecology.

Analysis of Natricine Cranial Material from the Earliest Pliocene (4.9 – 4.5 MYA) Gray Fossil Site, Washington Co., TN

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The earliest Pliocene (4.9 – 4.5 MYA) Gray Fossil Site (GFS) of Washington County, TN preserves fossilized plant and animal remains from a diverse subtropical forest in the Southern Appalachian region. The herpetofauna of this ancient sinkhole lake is particularly rich, with the presence of a helodermatid, the oldest occurrences of several salamander taxa, numerous turtles, and numerous squamates. Squamate vertebrae, especially snakes, are among the most common fossils at GFS. As the most numerous bones in a snake's body, vertebrae are often the only fossil elements preserved for study. However, in exceptionally rich fossil deposits cranial material may also be preserved, analysis of which can reveal aspects of feeding behavior, phylogeny, and ecology difficult or impossible to ascertain when studying vertebrae alone. Here we present the preliminary findings from the study of a parietal and basisphenoid of a natricine snake from GFS with a mosaic of morphological features found in durophagous crayfish snakes and large, piscivorous *Nerodia*. The depositional timeframe at GFS (~5 MYA) corresponds to a period of rapid evolution within new world Natricinae (especially *Nerodia*). Thus, the study of fossil natricines at GFS has considerable potential to clarify the evolutionary history of new world Natricinae. Additionally, the unique morphology of the GFS natricine cranial material suggest more diverse feeding ecologies among the southern Appalachian colubrid community during the Pliocene than seen among modern communities in the area today

Brown Back Salamander (*Eurycea aquatic*) Distribution in Georgia's River Drainages.

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Eurycea aquatic (Brown Back Salamander), a Plethodontid salamander, was originally distinguished from other closely related species in 1971 by Rose & Bush. Often getting mistaken with others of the “*bislineata* complex” (Kozak 2006), such as *Eurycea bislineata* and *cirrigera*, the brown back salamander is a distinctly different species with different morphological traits (broader body and head, and shorter tail to body length ratio), habitat preferences, and partially different endemic ranges. *Eurycea aquatic*'s endemic range has been represented as being secluded to the Coosa valley, Cumberland Plateau, Highland Rim, and Birmingham Valley of Alabama and Northwestern Georgia (Tempe et al. 2009). There has been minimal research done on the brown back salamander, and is a priority species in Georgia due to the limited knowledge on the species and its conservation status. We believe their endemic range and preferred habitat have been underrepresented, and with our study we intend to determine current range throughout Georgia. However, due to similarities in morphological traits, particularly within the larval stage, genetic analysis is needed for proper species identification. Throughout our study, we will be surveying the different river drainages of Georgia, such as the Coosa, Tennessee, Chattahoochee, Altamaha and Savannah. Our Preliminary results show evidence for them existing in the Tennessee, Chattahoochee, and Coosa river drainages in Georgia, and that they extend further south and southeast than previously known.