

Abstracts of the 28th Annual Meeting of the TN Herpetological Society Student Oral Presentations

What's in a 'Game'? A new way of teaching evolution.

Jacob Botello

Auburn University, Biological Sciences, 350 S College St, Auburn, AL 36830

Teaching evolution by natural selection represents a special challenge for educators in biology because of its natural complexity as well as rampant misconceptions in popular culture. Using the Unity engine, we have developed a free, browser-based video game (2D platformer) as an intervention to supplement instruction of evolution by natural selection in university classrooms. Within this game, students control a population of organisms subject to the forces of selection, set narrow-sense heritability, and observe the response to selection at the population level. We aim to investigate the effects of this intervention on student performance outcomes, student engagement, and general attitudes toward science. To do this, we plan to assess students' knowledge of evolution by natural selection in pre- and post-gameplay surveys composed of questions from the concept assessment of natural selection (CANS), questions evaluating attitudes toward science from the Scientific Attitude Inventory (SAI), and additional questions which assess student engagement.

Bog Turtle Movement and Demographics in Northeast Tennessee

Timothy Calhoun

University of Tennessee, Knoxville, Department of Forestry, Wildlife, and Fisheries; 2621 Morgan Cir, Knoxville, TN 37996

The bog turtle (*Glyptemys muhlenbergii*) is a federally threatened freshwater turtle species found primarily in wetlands within the Appalachians. There is only one known natural meta-population of these turtles in Tennessee, a handful of wetlands within one valley in northeast Tennessee. In recent decades the Nature Conservancy has worked to restore and expand these wetlands. This summer (May – August 2022), I conducted capture-mark-recapture surveys at two of these wetlands. I used live traps, camera traps, and random walk probing surveys to capture individuals. I also attached radio transmitters to six individual turtles to assess movement through the wetland using a passive telemetry grid of receiver nodes installed at one of the

wetlands. I captured 19 individuals and found a diverse age structure including a 2-year-old juvenile and adults over 30 years old. Additionally, I found individuals had dispersed into the restored wetland areas where previously there had been no records of captures. I am currently conducting deeper analysis of this data using spatially explicit capture recapture modeling to assess population density.

Thermoregulation of pregnant and non-pregnant *Nerodia sipedon* in Middle Tennessee

Alexis Hamous, Vincent Cobb

Middle Tennessee State University, 1301 E Main St, Murfreesboro, TN 37132

Snakes of several species select and maintain higher body temperatures (T_{bs}) 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 a warmer and relatively constant body temperature could be more beneficial than costly to a pregnant snake. In 2021 we used radiotelemetry and temperature loggers in gravid and non-gravid *Nerodia sipedon* in Middle Tennessee to continuously record their body temperatures throughout the typical gestation period. With a sample size of 10 (6 gravid, 4 non-gravid) and total of 6240 T_{bs} (3744 gravid, 2496 non gravid) in 2021, we found that overall 24-hour mean T_b for all snakes was 27.1 ± 0.03°C. The effect of pregnancy did not lead to differences in 24-hour mean snake body temperature (non-gravid snakes = 27.0 ± 0.05°C, gravid snakes = 27.2 ± 0.04°C). Even after using a generalized additive mixed model where body temperature was the response variable and pregnancy status was the main predictor variable and accounting for time of day (in hours), air temperature, water temperature, month, snake ID (individual snakes) and site as random variables, we found that pregnancy had no effect on body temperature. Therefore, our study with *N. sipedon* does not appear to support the idea of snakes maintaining higher body temperatures during pregnancy.

What are the genomic causes of aging variation? An examination of divergent garter

snake ecotypes.

Randy L. Klabacka, Anne M. Bronikowski, Suzanne E. McGaugh, Dawn Reding, Andrew Lithio, Daniel Nettleton, Laurie S. Stevison, Jessica Judson, Tonia S. Schwartz

Auburn University, Biological Sciences, 350 S College St, Auburn, AL 36830

Identifying the genetic mechanisms that contribute to senescence is necessary to understand the targets of natural selection in facilitating divergence in life history strategies. Examination of genetic dissimilarity in natural populations with variation in senescence provides a window to genes and gene networks involved with senescence. Two divergent ecotypes of terrestrial garter snakes (*Thamnophis elegans*) are present in populations around Eagle Lake in Northeastern California (USA). These ecotypes are on opposite ends of the “pace of life” spectrum, with one ecotype having a higher rate of senescence, shorter lifespan, and higher metabolic rate compared to the other. Targeting gene networks associated with senescence in model organisms, we sought to identify patterns in gene expression and sequence variation associated with senescence. Between ecotypes, we identified networks with enriched expression and genes with significant sequence variation. We also examined the functional implications of sequence variation (e.g., effect of mutation on peptide sequence and protein function). Our results largely corroborate those found in model systems, with variation in the same gene networks (and some of the same genes) associated with variation in senescence.

Comparison of Survival Rate from Two Field Seasons of Reintroduced Zoo-raised Eastern Hellbenders

Marley E. Machara, William Sutton, Sherri Doro Reinsch, Dale McGinnity, Rebecca Hardman, Brian Flock

Tennessee State University, 3500 John A Merritt Blvd, Nashville, TN 37209

Zoo reintroduction programs are becoming necessary to re-establish historic ranges or bolster wild populations of species whose numbers have declined due to anthropogenic disturbances. The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is an ideal candidate for captive reintroduction due to their fragmented and aging populations across their historic range. In Tennessee, Hellbender populations have

declined considerably, especially in stream habitats within the Interior Plateau ecoregion. Due to these declines, our study evaluated the potential of reintroductions to bolster declining populations over the course of two field seasons. During June and July 2021, we released 6 year-old, zoo-raised hellbenders (N=29, Cohort 1 [13], Cohort 2 [16]). During May 2022 we released 4 and 6 year-old hellbenders (N=34, 6 year-olds [15], 4 year-olds [19]). We used a combination of radiotelemetry and PIT-tag technology to assess hellbender movements, habitat use, and survival. We hypothesized that 2022’s release would have greater survival rates than 2021, as the 2022 release animals were exposed to earlier environmental conditioning. We conducted survival analysis to compare the survival rates of the two field seasons after the same number of days in the field. After analysis we found that the survival rate for all the 2022 cohorts was higher than the 2021 cohorts. These results suggest exposure to release conditions such as local water and food items as soon as possible, as well as earlier release dates, is key to the success of released captive-reared animals.

Xenobiotic estradiol-17 β alters gut microbiota of hatchling American Alligators (*Alligator mississippiensis*)

Kaitlyn M. Murphy, Madison M. Watkins, John W. Finger Jr., Meghan D. Kelley, Ruth M. Elsey, Daniel A. Warner, Mary T. Mendonça

Auburn University, 330 Funchess Hall, Auburn, AL 36849

Environmental estrogens pose serious concerns for ecosystem and wildlife population sustainability through their influence on a broad range of organismal functions. The gut microbiome is highly vulnerable to environmental influence, yet the specific effects of estrogens on gut homeostasis are unknown because they are poorly studied in wildlife populations. To determine the influence of environmental estrogens (i.e. xenoestrogens) on the diversity and abundance of gut microbiota, we randomly assigned 23 hatchling American alligators (*Alligator mississippiensis*) to three treatments (control, low, and high estrogen concentrations). We predicted that xenoestrogen exposure would decrease microbial diversity and abundance within the digestive tract and that this effect would be dose-dependent. Microbial samples were collected following diet treatments and microbial diversity was determined using 16S rRNA gene-sequencing. Individuals in estrogen-treatment groups had decreased microbial diversity, but a greater relative abundance of operational taxonomic units than those in

the control group. Additionally, this effect was dose-dependent, whereby as individuals were exposed to more E2, their microbiota became less diverse, less rich, and less even. Findings from this study suggest that estrogen contamination can influence wildlife populations at the internal, microbial-level, which may lead to future deleterious health effects.

Among- and within-population variation in reaction norms of brown anole embryos

Mike C Norris, Joshua M. Hall, Daniel A. Warner
Auburn University, 330 Funchess Hall, Auburn, AL 36849

Developmental plasticity refers to the capacity of a single genotype to express more than one phenotype in response to early-life developmental environments. Phenotypic responses to the environment are quantified as reaction norms (e.g., shape of the relationship between the environment and phenotype), which often vary among individuals and populations. Importantly, variation in reaction norms among individuals (i.e., genotypes) within populations provides an opportunity for natural selection to operate on plasticity. Moreover, differences between populations could indicate local adaptation to environmental conditions. In this experiment we examined variation in plasticity within and between populations in the brown anole lizard (*Anolis sagrei*). Lizards were collected from two island populations in the Matanzas River (Crescent Beach, Florida), and their eggs (n=413) were incubated under one of two treatments that mimicked nest sites found in shaded versus open habitat). After hatching, we measured offspring morphology, sprint speed and desiccation rates. Preliminary results demonstrate considerable plasticity in developmental rate and offspring morphology, and the shape of the reaction norms differ among family groups (i.e., genotypes) within populations.

Interactive Effects of Phenological Shifts and Experimental Warming on Larval Wood Frogs

Reese Sloan, Jon M. Davenport
Appalachian State University, Department of Biology, Boone NC 28608

Climate change is an anthropogenic-induced shift to warmer average temperatures globally. As temperature increases, so does variability in precipitation, which directly affects the timing of phenological events. In

response many amphibian species have begun to breed earlier. Many larval amphibians experience faster growth in response to higher temperatures and faster drying of temporary ponds. To measure the interactive effects of hatching phenology and temperature on larval wood frogs (*R. sylvatica*), we performed a mesocosm experiment. We deployed 2 treatment levels of hatching phenology (early and late) and temperature (ambient and +1°C). Tadpoles in late hatching treatments metamorphosed faster than those in early hatching treatments. Mean size at metamorphosis was reduced in +1°C treatments compared to ambient treatments. Across treatments, survival was highest in +1°C treatments compared to ambient treatments. There were no interactive effects between hatching time and temperature on growth, time to metamorphosis, or survival. Larval wood frogs experienced faster growth in response to higher temperatures; however, there was a tradeoff in smaller size at metamorphosis. Exposure to freezing temperatures early in the larval period likely decreased survival and resulted in delayed metamorphosis. Responses to environmental variation can lead to plastic responses but could be difficult to predict without further research.

Investigating summer occupancy of small terrestrial salamanders (genus *Plethodon*) in the Southern Appalachian Mountains

Elyssa Winterton, Jon Davenport, Zachary Farris
Appalachian State University, 565 Yosef drive. Boone, NC 28607

As habitats become more fragmented and amphibian assemblages change, understanding what environmental factors determine the detection and occupancy of *Plethodon* salamanders is becoming increasingly important. Previous research has found that salamander habitat requirements may be restricted to specific environmental parameters. We investigated the regional occupancy of *Plethodon cinereus* and *P. richmondi* in the southern Appalachian Mountains, on 47 plots surveyed three times during the months of June, July, and August of 2021. We evaluated 16 covariates to explain both occupancy probability and detection probability for each species. Detection probability for both *P. cinereus* and *P. richmondi* was best explained by Julian date with detection becoming more likely earlier in the field season. The top occupancy model for both *P. cinereus* and *P. richmondi* was aspect. Specifically, occupancy has a negative association with northness. Meaning that higher occupancy occurs on southern facing slopes and lower occupancy on north facing slopes. Across all sites

occupancy was $0.35 + 0.09$ for *P. richmondi* and $0.35 + 0.08$ for *P. cinereus*. Ultimately, detection and occupancy of both species appear to be influenced by date surveyed and slope direction most heavily. This data will serve as the foundation for future models across seasons and to understand how these species coexist.

Professional Oral Presentations

eDNA Sampling for the Streamside Salamander (*A. barbouri*) at a Sumner County, Tennessee Mitigation Site

Anthony Brais

Resource Environmental Solutions, 103 Continental Place, STE 202 Brentwood, TN 37027

The state endangered streamside salamander (*A. barbouri*) is known from a stream & wetland mitigation site in Sumner County, Tennessee. Individuals are difficult to locate given restricted surface activity within intermittent streams during the Dec.– Apr. breeding season. Environmental DNA (eDNA) passively surveys for presence / absence. Samples were collected at 14 locations in Mar. 2022 to establish baseline reach occupancy. Four control samples were collected at a reference location in Mar. 2022. At each sample point, we attempted to extract a ~1000 mL sample volume through a Sterivex™ filter. Samples were analyzed by a third-party lab against a Tennessee specific *A. barbouri* assay from Witzel et al. 2020. Surveys were conducted at each sampling point to identify adults, larvae, or egg masses. Preliminary eDNA results failed to detect *A. barbouri* at the mitigation site; a positive detection occurred at the control site. Adults, larvae and egg masses were observed at both sites. Water quality allowed extraction of 1000 mL per sample at the control site. Increased turbidity prevented collection of 1000 mL from all samples at the mitigation site. Lower concentrations of DNA may have been present due to late season sampling or a less robust population of *A. barbouri* given current agricultural land use. Additional sample volume may have been required to achieve positive detection at the mitigation site. Work is ongoing to determine if genetically distinct clades of *A. barbouri* within Tennessee may have influenced detection results.

Multi-Species Survey and Ecosystem Conservation within The Elephant Sanctuary

Jesse Eaker,

Tennessee Wildlife Resources Agency, 5107 Edmondson Pike Ellington Agricultural Center Nashville, TN 37211

Conservation Opportunity Areas (COA's) as identified by The State of Tennessee's State Wildlife Action Plan (SWAP) identifies lands and waters significant for restoring species of greatest conservation need (GCN). The Elephant Sanctuary in Tennessee consists of 3,000 acres that lie within the Western Highland Rim COA. This property provides a significant amount of land that serves as a sanctuary to not only the resident elephants, but to wildlife native to the Western Highland Rim COA. Several species of greatest conservation need have been observed or are suspected to be within the sanctuary, including the four-toed salamander (*Hemidactylium scutatum*), eastern hog-nosed snake (*Heterodon platirhinos*), pygmy rattlesnake (*Sistrurus miliarius*) and eastern pine snake (*Pituophis melanoleucus*). Tennessee Wildlife Resources Agency has implemented an ongoing species inventory project in 2020 to document native wildlife within The Elephant Sanctuary in Tennessee. Between June 2020 and September 2022, 511 individuals representing 42 species of herpetofauna have been identified.

The Protobiome: Identifying Amoebae and Other Protozoans on Salamander Skin

Aubree J. Hill, Nathan Owens, Emily Lannom, and John Gunderson

Tennessee Technological University, Department of Biology, Cookeville, TN 38505

During previous work, high-throughput DNA sequencing was used to characterize the structure of the skin-bacterial microbiome of salamanders. This work, along with other similar studies, identified chlamydia as relatively abundant members of the skin-bacterial community. Furthermore, ecological interaction networks pinpointed chlamydia as influential members of the community. Because chlamydia are obligate intracellular symbionts, interactions involving chlamydia must be explained by the presence and ecological influence of their eukaryotic hosts (e.g., amoebae). However, the presence of such protozoans in the cutaneous microbiome has not been explored. Protozoans are known to play important roles in the soil rhizosphere and the mammalian intestinal tract. We hypothesize that skin-dwelling protozoans play similar roles in the salamander microbiome and, therefore, could impact host health and response to invading pathogens such as *Batrachochytrium*

salamandrivorans. Here we report preliminary findings of an ongoing endeavor to identify members of the so-called “protobiome” using traditional microbiological isolation techniques, PCR amplification of 18s rRNA genes using a peptide nucleic acid (PNA) to block amplification of salamander genes, and high-throughput DNA sequencing.

Tennessee Snake Identification and Education Facebook Group: What We Are Doing & What We Are Learning

Lisa Powers

Froghaven Farm, Bon Aqua, TN

The popularity of internet social media sites makes getting answers both quick and easy. There are many groups on Facebook that address herps; snakes in particular. The Tennessee Snake Identification and education FB group is one of the largest and most popular snake ID groups on FB. We not only identify the snakes and other herpetofauna from our region, we are able to provide education, promote conservation and help ease the fears of many. We also have the ability to give back to science via our interface with the public. I will present some of the things we do that make us different from the other groups, how we help the public, what we are learning and how we contribute to the scientific community.

Venomous Snake Research in Tennessee: The Past, Present and Future

Lisa Powers

Froghaven Farm, Bon Aqua TN

Tennessee has 4 endemic species of venomous snakes that are of medical significance to humans. They are the Eastern Copperhead (*Agkistrodon contortrix*), the Northern Cottonmouth (*Agkistrodon piscivorus*), the Pygmy Rattlesnake (*Sistrurus miliarius*) and the Timber Rattlesnake (*Crotalus horridus*). Tennessee scientists and research institutions have a long history of studying these venomous snakes in Tennessee, including their basic biology and life histories, distribution and ranges, ecological niches, diseases and conservation needs. In the past, researchers were under fewer restrictive policies as set out by the Tennessee Wildlife Resources Agency (TWRA). Have the newer policies currently in use made the researchers and

public any safer? Do they unduly restrict researchers and put the safety of the researcher and the snakes in jeopardy? How do these policies affect the treatment of snakebite for researchers currently required to maintain their own antivenin? Do these restrictions further put the public and our wild snake populations in danger because of the lack of current information due to the difficulties imposed by these restrictions? Do we need to re-examine these policies and propose fact-based information and suggestions that increase safety for the researcher, proper handling and care of the snakes, emergency protocols for snakebite treatment and proper safety precautions to protect the public, our scientific community and our venomous snakes?

The Good, the Bad, and Myatt Drive: Updates on recent *Ambystoma barbouri* surveys and a look at the fate of a fossorial amphibian in the urban interface.

David I. Withers

Tennessee Natural Heritage Program, Division of Natural Areas (DNA), Department of Environment and Conservation, William R. Snodgrass TN Tower, 2nd Floor 312 Rosa L. Parks Avenue, Nashville, TN 37243

The state endangered streamside salamander (*Ambystoma barbouri*) has been of intense conservation interest since it was first reported in Murfreesboro in 1996. The presence of the species in a rapidly developing part of middle Tennessee caused speculation as to its ability to cope with evolving suburban and urban habitats. A denizen of often undervalued seasonal channels, occupied sites frequently were reimagined into neighborhoods, commercial centers, and industrial properties. Due to its apparent rarity amid the growing shadow of the urban interface, the USFWS and TWRA broadened staff participation in surveys, outreach, and environmental review for the species, and have supported seasonal breeding and development inventories by DNA staff. Recent surveys have focused on potential breeding habitats in the Central Basin and a portion of the Highland Rim, all within a ten-county region dominated by Nashville. Surveys have primarily been undertaken in Davidson, Rutherford, Wilson, Sumner, and Robertson counties. With help from several collaborators new locations and novel drainages have been discovered, including the Barren River system. In urbanized Davidson County new populations have been found in the Dry Creek, Pages Branch, and Ewing Creek systems. A site discovered in the Myatt Drive industrial corridor revealed some unusual habitat

constructs and resilience that may enable this once robust population to recover and flourish at the newly developed tract. A review of some unusual finds 2020-2022 provides hope for the species even in the most challenging environments.

Student Poster Presentations

Interactions between skin bacteria and the snake fungal disease pathogen (*Ophidiomyces ophiodiicola*) across a nutrient gradient mimicking host skin chemistry

Ori Bergman , Alexander S. Romer, Emily K. Stone, Claire Matzek, Donald M. Walker

Middle Tennessee State University, 1672 Greenland Drive, Murfreesboro, TN 37132

A salient question in microbiome community ecology is whether bacteria interact with fungi in response to specific host chemistries. The snake skin microbiome can be shaped by factors such as dysbiosis induced by a fungal pathogen (*Ophidiomyces ophiodiicola*), disease, and interactions with fungistatic bacteria in the skin microbiome. We explored interactions between bacterial members of the snake skin microbiome and *O. ophiodiicola* across a nutrient gradient mimicking host skin chemistry, hypothesizing that bacterial species would respond differently to *O. ophiodiicola*-modified skin chemistry as it secretes exoenzymes such as keratinases to metabolize host skin. We postulated that as the fungal pathogen modifies the skin environment, a bacterial growth response will be more pronounced. We modeled a time series interaction between bacteria and *O. ophiodiicola* on keratin minimal media as it is the most abundant carbon source composing host snake skin. Bacterial growth curves were measured using absorbance (OD_{620nm}) over a 48 hour time period in response to three different fungal treatments or a control. Preliminary results suggest that both competitive and cooperative interactions explain the growth of 40 different bacterial species across a gradient of fungal metabolism and exoenzyme/metabolite production. These results will inform the broader scientific community on bacterial-fungal interactions in response to host chemistry in non-mammalian systems.

Use of eDNA in the detection of semiaquatic salamanders in eastern Kentucky streams.

Sara A. Brewer, Florene G. Bell, Angie F. Flores, Chi

Jing Leow, Ben F. Brammell Thomas A. Maigret, Kenton L. Sena, Ben F. Brammell

Asbury University, 1 Macklem Drive, Wilmore, KY 40390

Environmental DNA (eDNA) utilizes DNA organisms release into the environment to detect their presence and provides an efficient, non-invasive method to determine organism presence/absence. Recent works have emphasized the need for high quality, carefully tested assays for use in species-specific marker studies and the need to validate these oligos in silico, in vitro, and in situ. We developed species specific qPCR assays for three widely distributed species of semiaquatic North American salamanders: Northern red salamanders (*Pseudotriton ruber*), Spring salamanders (*Gyrinophilus porphyriticus*), and Mud salamanders (*Pseudotriton montanus*). Primers and probes were designed based on sequences obtained from locally collected specimens and screened in silico for specificity against nineteen salamander species that occur sympatrically with these species in various parts of their range. Water samples were collected from field sites in eastern KY and used in field validation of these assays. This project provides thoroughly vetted tools that should be useful for future monitoring or range delineation studies of these species.

Adaptive Plasticity of Coloration in Response to Environmental Change

Karissa M. Coffield

Murray State University, 1375 Chestnut St, Murray, KY 42071

When rapid environmental changes occur, different selective forces can create phenotypic trade-offs in which a trait can provide fitness benefits or costs under different environmental conditions. Amphibians are particularly vulnerable to environmental change, and previous research has revealed that some species will plastically respond to variation in temperature and ultraviolet radiation (UVR) by altering their coloration. Divergent selection on coloration may change with elevation and climate induced shifts in temperature because high temperatures are likely to result in lighter color morphs, but as UVR exposure increases darker color morphs will be more common. I will evaluate the adaptive plasticity of coloration in *Ambystoma mavortium*, the tiger salamander, by testing the following hypotheses: 1) increased UVR levels will more strongly affect color plasticity than temperature; 2) older individuals will converge on similar coloration because color plasticity is more important to larval fitness; and 3) coloration affects an individual's fitness. I will compare variation in coloration metrics of wild *A.*

mavortium present at different developmental stages along an elevational gradient in western Colorado. Individuals will be photographed, and coloration metrics will be quantified using ImageJ to compare differences in coloration. The information I gather in this study can be used to further understand both phenotypic shifts organisms face under environmental changes and the consequences of those shifts.

Incubation moisture influences embryo physiology in the Eastern Fence Lizard (*Sceloporus undulatus*)

Lydia C. Dudley, Haley Oakley, Joshua M. Hall

Tennessee Tech University, 1100 North Dixie Avenue, Cookeville, TN 38505

The effects of moisture and heat stress on embryo development have been extensively studied separately in vertebrate ectotherms but combined effects represent a critical knowledge gap given that climate change will alter both temperature and rainfall simultaneously. To understand the interactive effects of moisture and temperature on embryo development, we incubated eggs from Tennessee populations of the Eastern Fence Lizard (*Sceloporus undulatus*) at two moisture concentrations (-150 kPa and -550 kPa) and measured fitness-relevant phenotypes of embryos and hatchlings. We then incubated eggs in a 2 by 2 factorial design of moisture and temperature. One temperature treatment was suitable for development and the other induced thermal stress. Eggs in the moist treatment absorbed more water but developed more slowly than those in the dry treatment; however, there were no treatment differences in hatchling body size due to moisture. Thus, our moisture treatments influenced physiology but not morphology. The factorial experiment is still underway; however, current data indicate that interactions between moisture and temperature will result from moisture-induced changes in physiology related to water uptake. These foundational studies will establish Tennessee *S. undulatus* populations as a model for understanding the combined effects of altered temperature and rainfall patterns due to climate change. Such knowledge will benefit reptiles of Tennessee and beyond by enhancing our understanding of ectotherm responses to global change.

Student-led surveillance for *Batrachochytrium salamandrivorans*

Xavier A. Jackson, Aubree Hill

355 Mountain View Drive

Batrachochytrium salamandrivorans (Bsal), a deadly fungal pathogen causing declines in salamander populations, is currently spreading rapidly throughout Asia and Europe with the help of the exotic pet trade. Because it is difficult to tell if an animal is infected based on visual inspection in the field, biologists typically test skin swab samples using quantitative polymerase chain reactions (qPCR) to determine infection status. Collecting these samples is crucial for pathogen monitoring. Thus, our goal was to collect skin swabs from amphibians, to help monitor for the presence of Bsal in a global hotspot for biodiversity. Our team of undergraduate Biology students from Tennessee Tech University collected 30 swabs from species known to be susceptible to infection or that are carriers of Bsal. We were careful to keep swab tips sterile and prevent cross-contamination. After swabbing, we recorded snout-to-vent length, species of each animal, and returned them to the exact location of capture. Swabs were placed on ice and shipped to the National Wildlife Health Center for DNA extraction and qPCR. None of the samples collected at our site tested positive for Bsal, and thus far, no other surveys conducted in wild populations of N. American salamanders have confirmed the presence of Bsal. However, continued large-scale monitoring efforts, such as those conducted by the Student Network for Amphibian Pathogen Surveillance (SNAPS), will be critical for early detection of Bsal and conservation of ecologically vital biodiversity of the Western Hemisphere.

Development and validation of qPCR assays for detection of four toed salamanders (*Hemidactylium scutatum*).

Brendan C. Jeffrey, Sara A. Brewer, Florene G. Bell, and Ben F. Brammell

Asbury University, 1 Macklem Drive, Wilmore, KY 40390

Environmental DNA (eDNA) has rapidly become a firmly established method for detecting organisms of research and conservation interest and promises to greatly increase the ease, efficacy, and scope of ecological studies. Recent works have highlighted the need for carefully tested assays for use in species specific marker studies and thorough vetting of eDNA primers using as many local sequences as available. We developed species specific primers for use in qPCR eDNA detection of *Hemidactylium scutatum* and tested these primers in silico and in vitro against sympatric species to ensure specificity. Additionally, we tested these primers against *Hemidactylium scutatum* species

collected in New York and note that they successfully detect specimens from more northern portions of the range of this species. The assays presented here and tested, both in silico and in vitro, should serve as valuable tools enabling the detection of this widespread salamander species.

Quantifying Habitat Parameters for the Southern Appalachian endemic, *Plethodon welleri*

Rosemary G. Ronca, Jon M. Davenport

Appalachian State University, 572 Rivers St, Boone, NC 28608

Plethodon welleri is a small-bodied salamander species that is endemic to select mountain tops in the Southern Appalachian Mountains and considered threatened across its entire range. Initial descriptions indicated that this species was a high-elevation, spruce-fir specialist only found above 1500m. However, recent observations have documented populations as low as 620m. The goal of this study is to assess environmental parameters for habitat preferences and build a comprehensive dataset on species detection and occurrence. To do this, we conducted surveys across North Carolina and Tennessee. Species surveys were combined with environmental parameters to predict population presence. Preliminary data from the 2021 field season indicates that the top environmental covariates for *P. welleri* during summer months was the interaction between humidity and elevation. Specifically, occupancy in the summer months was typically found at higher elevations due to cooler temperatures and lower evaporative rates. In the fall, occupancy was most influenced by the temperature, as populations retreat below ground with freezing temperatures. We expect that the most influential environmental covariates will change by season, as lower temps allow populations to emerge with less threat of desiccation. This study will provide foundational data for this species and other salamander species in this region. Having access to baseline knowledge for threatened species is necessary for the development of effective conservation strategies and comprehensive management plans.

Assessing the health and behavioral effects of turtle racing on eastern box turtles (*Terrapene*

***carolina*)**

Michelle K Weaver, John Hewlett, Sharon L. Deem, Jamie Palmer, Maris Brenn-White, Kathleen Apakupakul, Alex Heeb, Christine Light, Christine Casey, and Andrea Darracq

Murray State University, 1375 Chestnut St Murray Ky 42071

Turtle racing involves the public capturing and using largely wild-caught box turtles (*Terrapene* spp.) to compete in races. Turtle races are generally legal in most states because of a lack of regulations and/or funding to enforce regulations that may exist. Currently we lack information on the potential effects (e.g. disease transmission, survival, movements, and general health) of races on the turtles that could help support the implementation and enforcement of regulations. Our objective was to understand the short-term and long-term health and behavioral effects of turtle races on eastern box turtles (*Terrapene carolina*). To address this, we collected 29 box turtles from a race held in Kentucky. We completed physical exams and collected blood samples and oral and cloacal swabs from each turtle. We quarantined the turtles for 2 weeks and then released 19 turtles (weight > 400 g) onto a national wildlife refuge in western KY following negative PCR test results for ranavirus (FV3) and the attachment of a transmitter. We also captured 8 free-living box turtles from the same release location, followed the same methods, and released them with a transmitter at their collection site. We have been tracking the turtles since release and have completed health evaluations. We will continue to monitor the turtles for up to 2 years. No turtles at the race tested positive for ranavirus and we are currently completing laboratory analyses associated with testing for other diseases. We present here the preliminary data on health metrics, disease surveillance, movement, and survival.

Does stress physiology mediate disease resistance?

Megan E. Zenger, Howard H. Whiteman

Murray State University, 1375 Chestnut St, Murray, KY 42071

Panzootic pathogenic fungi *Batrachochytrium dendrobatidis* (Bd) causes the deadly disease chytridiomycosis (chytrid), a primary driver of global amphibian declines and extinctions. The pathogenesis of chytrid is still unclear, as certain species and individuals within a species are differentially affected. Prolonged stress hormone activity deleteriously affects many of the same physiological processes as Bd infections; therefore, elevated stress in response to Bd

may help explain the susceptibility and lethality of this devastating disease. Corticosterone (CORT) is the primary glucocorticoid released by amphibians in response to stress. Life history morph (terrestrial vs. aquatic), sex, and body condition are traits that may influence CORT production within salamander populations because of differences in physiology and environmental conditions. Thus, the objective of our study was to assess how CORT concentration and Bd infection status varies within Arizona tiger salamander (ATS; *Ambystoma mavortium nebulosum*) populations in the Gunnison Valley of Colorado. We used non-invasive sampling methods to collect baseline and

elevated CORT from paedomorph and metamorph ATS in June and July 2021. In June and July 2022, we repeated these methods while additionally sampling individuals for dermal Bd spores. This study will provide a greater understanding of the pathogenesis of Bd, and how stress hormones can be used to assess population health and disease susceptibility. Future research utilizing this method could benefit the conservation of threatened species.