

# 25<sup>th</sup> Annual meeting of the Tennessee Herpetological Society



*Photo credit: Matthew Grisnik*



**Thursday, September 26<sup>th</sup>**

Registration opens 11:00–12:00 pm

12:00 pm: Opening remarks – Donny Walker

12:00 – 12:50 pm: Keynote speaker: Pete Wyatt

**"Watershed Events in Tennessee Herpetology: (circa 1980-2015) A Perspective from a Nongame Warden"**

12:50 – 1:00 pm: Coffee break

**Session 1 – oral presentations – Chair: Reed Alexander**

1:00 – 1:15 pm: **A new genus of desmognathan salamander (Plethodontidae) from the early Pliocene Gray Fossil Site of Northeast Tennessee.** R. Davis Gunnin

1:15 – 1:30 pm: **The effect of shedding on snake fungal disease progression in Common Watersnakes (*Nerodia sipedon*).** Alexander Romer

1:30 – 1:45 pm: **Characterization of the bacterial cutaneous microbiome of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) before and after translocation.** Emilly Nolan

1:45 – 2:00 pm: **Demographic traits of freshwater turtles in a maritime forest habitat.** Ryan J. Hanscom

2:00 – 2:15 pm: **Investigating the proximate mechanisms of habitat constraints in two species of Plethodontid salamanders in the Southern Appalachian Mountains.** Trevor Chapman

2:15 – 2:45 pm: Coffee break

**Session 2 – oral presentations – Chair: Olivia Bowers**

2:45 – 3:00 pm: **Testing the febrile response of snakes inoculated with *Ophidiomyces ophiodiicola*, the causative agent of snake fungal disease.** Cody D. Godwin

3:00 – 3:15 pm: **Assessment of turtle and leech (Hirudinea) parasite-host assemblage variation in Middle Tennessee wetlands across a disturbance gradient.** Laura Horton

3:15 – 3:30 pm: **Variation in the Slimy Salamander (*Plethodon* spp.) skin and gut microbial assemblages is explained by geographic distance and host affinity.** Matthew Grisnik

3:30 – 3:45 pm: **Geometric morphometric analysis of Eastern North American Pitviper vertebrae with implications for fossil identification.** Lance D. Jessee

3:45 – 4:00 pm: **Oxygen consumption during development in embryos of the oviparous snake, *Pantherophis guttatus*.** Celeste Gallardo

4:00 – 4:05 pm: Tennessee Valley Authority award for outstanding commitment to amphibian conservation - Dr. Keven Hamed

4:05 – 4:35 pm: Fill your glasses for the business meeting

4:35 – 5:35 pm: Business meeting and annual elections

5:35 – 6:30 pm: Poster session and social (Beer provided by East Nashville Beer Works)

6:30 pm: Banquet and Silent Auction

7:30 pm: Live Auction

### **Friday, September 27<sup>th</sup>**

Registration 8:00 – 10:00 am

8:00 – 8:30 am: **Amphibians and Reptiles of the ~5-Million-Year-Old Gray Fossil Site.** Blaine Schubert.

8:30 – 10:00 am: Behind the scenes tour of Gray Fossil Site.

### **Session 3 – oral presentations – Chair: Matthew Grisnik**

10:00 – 10:15 am: **Cohabitation patterns between stream salamanders and crayfish in the southern Appalachians.** Susan L. Cragg

10:15 – 10:30 am: **Constantly fluctuating in an inconsistent way: comparing the effects of sinusoidal and naturally fluctuating incubation temperatures on embryo development.** Joshua M Hall

10:30 – 10:45 am: **Assessing short-term effects of translocation on the spatial ecology of eastern hellbenders.** Bradley D. Nissen – *Recipient of the Niemiller Travel Award*

10:45 – 11:00 am: **Using physiological conditions to assess patterns of subarctic Wood Frog (*Rana sylvatica*) habitat use.** Thomas P. Hastings

11:00 – 11:15 pm: **The effects of food web complexity and phenological variability of pond breeding salamanders.** David Burton

11:15 – 11:30 pm: Coffee Break

### **Session 4 – oral presentations – Chair: Alex Romer**

11:30 – 11:45 pm: **Nest-site selection of an isolated population of Bog Turtles (*Glyptemys muhlenbergii*) in Johnson County, Tennessee.** Amy Turpin – *Recipient of the Chadwick Lewis Award*

11:45 – 12:00 pm: **Macro-ecological scale geographic variation of a keystone predator in a common garden environment.** Kenzi M. Stemp

12:00 – 12:15 pm: **Response to a predator cue from a nonnative fish by a larval predator-naïve salamander (*Desmognathus quadramaculatus*).** Brian Lee Dempsey

12:15 – 12:30 pm: **Application of eDNA for rapid assessment of potential novel populations of *Ambystoma barbouri* in Middle Tennessee.** David Ian Withers

12:30 – 12:45 pm: **Using a passive sampling technique to survey for reptiles and amphibians.** Josh Campbell

12:45 – 12:55 pm: Student Awards – Stephen Nelson

12:55 pm: Closing remarks – Donny Walker

### **Abstracts – Oral presentations**

#### **A new genus of desmognathan salamander (Plethodontidae) from the early Pliocene Gray Fossil Site of Northeast Tennessee**

R. Davis Gunnin<sup>1,2</sup>, Blaine W. Schubert<sup>1,2</sup>, Joshua X. Samuels<sup>1,2</sup>, Keila E. Bredehoeft<sup>1</sup>

<sup>1</sup>*Don Sundquist Center of Excellence in Paleontology*

<sup>2</sup>*Department of Geosciences, East Tennessee State University*

Tennessee is regarded as one of the most herpetologically biodiverse states, largely due to the high endemism of salamanders along the Eastern Highlands. Our state is home to at least 41 species of salamander, mostly belonging the family Plethodontidae, the lungless salamanders. This family reaches its highest global sympatric diversity in the Southern Appalachians (Blue Ridge) from West Virginia to northern Georgia. While these salamanders have long been studied as models for developmental biology and phylo-biogeographical analyses, the fossil record for plethodontids is surprisingly sparse. Few fossils older than ~15,000 years have been recovered, complicating our understanding of the historical distribution of this group and the timing of key evolutionary events within the family. Discovery of fossil forms to contextualize molecular phylogenetic analysis is important, especially in a family that has historically been under much phylogenetic scrutiny. Recently discovered salamander fossils from the Gray Fossil Site provide the foundation of this project. We describe these exceptionally large plethodontid remains to a new genus that belongs to the dusky salamanders, or desmognathans. The morphology of the fossil material resembles *Phaeognathus hubrichti*, an extant burrowing species from southern Alabama with a suite of primitive characteristics. Comparison of the fossil material to modern desmognathans using geometric statistical methods has revealed that the extinct form was likely similar in lifestyle to *P. hubrichti*, but considerably larger. It was unparalleled in the southern Appalachians in terms of size and ecology and reveals a more complex evolutionary history for desmognathan salamanders.

#### **The effect of shedding on snake fungal disease progression in Common Watersnakes (*Nerodia sipedon*)**

Alexander Romer<sup>1</sup>, Jackson West<sup>1</sup>, Emma Phipps<sup>1</sup>, Donald M. Walker<sup>1</sup>

<sup>1</sup>*Middle Tennessee State University, Biology Department, PO Box 60, Murfreesboro, TN USA*

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#### **Characterization of the bacterial cutaneous microbiome of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) before and after translocation**

Emilly Nolan<sup>1</sup>, Brad Nissen<sup>1</sup>, Rebecca Hardman<sup>2</sup>, Michael Freake<sup>3</sup>, William Sutton<sup>1</sup>

<sup>1</sup>*Tennessee State University, Nashville, TN 37216*

<sup>2</sup> University of Tennessee Knoxville, Knoxville, TN

<sup>3</sup> Lee University, Cleveland, TN

Eastern Hellbenders are declining across historic ranges and are listed as “Endangered” in Tennessee. To augment declining populations, we have conducted a telemetry and translocation project for this species in eastern Tennessee. In 2018, 10 hellbenders from Tumbling Creek and 17 hellbenders from Hiwassee River were implanted with radio-transmitters and were translocated respectively to Rough Creek and Citico Creek in 2019. Disease monitoring is an essential step in translocation projects, specifically in amphibians where emerging pathogens such as *Batrachochytrium dendrobatidis* are linked to population declines. Species like the hellbender may be resilient to *Bd* due to disease resistant bacteria found on amphibian skin. However, the amphibian cutaneous microbiome is sensitive to disturbance, and changes to microbial composition may lead to an increase in disease susceptibility. To test for *Bd* and collect skin bacterial samples, skin swabs off all hellbenders were taken before transmitter insertion, before translocations, and every 45 days post-translocation. Quantitative real-time polymerase chain reaction (qPCR) will be used to test for presence of *B. dendrobatidis*. To characterize skin bacterial communities, the bacterial 16S ribosomal RNA V3 and V4 regions will be amplified and sequenced using an Illumina MiSeq System. Variation in infection prevalence and bacterial communities will be compared before and after translocation to account for any changes that may occur. This project benefits conservation efforts to restore declining populations of the Eastern Hellbender and provide insight as to what role the cutaneous microbiome may have in the success of amphibian translocation projects with regards to disease dynamics.

### **Demographic traits of freshwater turtles in a maritime forest habitat**

Ryan J. Hanscom<sup>1</sup>, Stephen A. Dinkelacker<sup>2</sup>, Aaron J. McCall<sup>3</sup>, Adam F. Parlin<sup>4</sup>

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Local demographics of freshwater turtle populations are necessary in order to understand their natural history and assess population status. To examine whether maritime forest populations display different demographic traits compared to other populations, we studied the freshwater turtle community at Nags Head Woods Ecological Preserve (NHWEP), located on a barrier island of North Carolina. We determined specific demographic traits such as sex ratio, size distribution, somatic growth, annual survival ( $\phi$ ), annual abundance ( $\hat{n}$ ), and population growth rate ( $\lambda$ ) of Yellowbelly Slider (*Trachemys scripta*), Common Snapping Turtle (*Chelydra serpentina*), Chicken Turtle (*Deirochelys reticularia*), and Northern Red-bellied Turtle (*Pseudemys rubriventris*). *Deirochelys reticularia* showed apparent annual survivorship similar to that of a typical turtle species, contradicting previous studies reporting low annual survival due to reduced longevity. We determined that most demographic traits of freshwater turtle populations at NHWEP are structured similarly to other populations studied. We found that the population growth rates for three out of the four species analyzed are increasing, indicating expanding populations (with the exception of *T. scripta* ( $\lambda = 0.99 \pm 0.04$ )). Analyzing multiple demographic traits of different freshwater turtle species within a maritime forest community on a barrier island provides an important baseline on freshwater turtle demographics in unique habitats that are subject to intense environmental stochasticity.

### **Investigating the proximate mechanisms of habitat constraints in two species of Plethodontid salamanders in the Southern Appalachian Mountains**

Trevor Chapman<sup>1</sup>, Dr. Joe Bidwell<sup>1</sup>

<sup>1</sup>*East Tennessee State University*

Research aimed at identifying sensitive habitats has revealed complex interactions between biotic and abiotic variables in montane salamander communities. This study examines the behavioral and physiological mechanisms that restrict the northern gray-cheeked salamander (*Plethodon montanus*) to high elevation habitats. Slimy salamanders (*Plethodon glutinosus*) serve as a potential competitor with *P. montanus* where their distributional boundaries overlap. Plot surveys at Lamar Alexander Rocky Fork State Park were used to identify elevation limits and habitat environmental conditions for each species. A novel system was constructed to test behavioral preference for temperature and relative humidity in a controlled setting for each species. Habitat environmental conditions were significantly different between the species. Furthermore, peak annual activity was correlated with a significant increase in temperature and relative humidity. In controlled trials, *P. montanus* behavioral preference was strongly correlated with average night temperature of collection day. The next phase of this study will involve a reciprocal transplant experiment using mesocosms. Physiological stress in response to sub-optimal environmental conditions will be examined using a recently developed, non-invasive method of measuring cortisol from dermal swabs. Preliminary results indicated that standard enzyme immunoassay kits can detect cortisol from dermal swabs in *P. glutinosus*.

**Testing the febrile response of snakes inoculated with *Ophidiomyces ophiodiicola*, the causative agent of snake fungal disease.**

Cody D. Godwin<sup>1</sup>, Christopher M. Murray<sup>1</sup>, Alejandro Grajal-Puche<sup>2</sup>, Matthew Grisnik<sup>2</sup>, Alexander S. Romer<sup>2</sup>, Donald M. Walker<sup>2</sup>

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Snake Fungal Disease (SFD) is a fungal pathogen of wild snake populations, predominantly in the Eastern and Midwestern United States. SFD is characterized by heterophilic granulomas that can form around the mouth and eyes with severe cases causing weight loss, impaired vision and eventual death. Researchers, making field observations, have noted early season basking from severely infected snakes. This may suggest that snakes are attempting to raise their body temperature, inducing a febrile response, to combat the mycosis. This study tested the hypothesis that the causative agent of snake fungal disease (*Ophidiomyces ophiodiicola*) induces a febrile and behavioral response of seeking differential basking temperature to regulate body temperature. Eastern ribbon snakes (*Thamnophis sauritus*, n=30) were sham or *O.o* inoculated. Seven days after inoculation, snakes were tested on a thermal gradient that ranged from 40°C to 18°C. The internal body temperature of each snake was measured every 30 minutes for eight hours with a thermal probe inserted into the cloaca of each snake. Additionally, substrate temperatures, where the snake was basking, were measured every 30 minutes, using a laser temperature gun. Snakes inoculated with *O.o* did not demonstrate significantly different internal body temperatures but did bask at significantly higher temperatures.

**Assessment of turtle and leech (Hirudinea) parasite-host assemblage variation in Middle Tennessee wetlands across a disturbance gradient**

Laura Horton<sup>1</sup>, William Sutton<sup>1</sup>

<sup>1</sup>*Tennessee State University Department of Agricultural and Environmental Sciences*

Prior research has established clear links between decreased reptile biodiversity in degraded or disturbed habitats, including chelonian groups. There are negative impacts associated with high parasite loads on hosts, and previous studies found parasite loads increase with habitat disturbance, however there have been no published attempts to evaluate detectable sublethal health effects associated with this potential increase in chelonian ectoparasite (leech) load. Thus we aimed to assess if leech loads varied across a landscape disturbance gradient in Middle Tennessee wetlands and if they follow measurable patterns of increased sublethal health effects on chelonians by assessing heterophil:lymphocyte ratios, packed cell volume, and host body condition. We sampled 19 wetlands from June-October 2018 and obtained data from three host species; *Trachemys scripta elegans*, *Sternotherus odoratus*, and *Chelydra serpentina*. Collectively, the interpretation of these data may be used to understand how anthropogenic disturbance affects wetland turtle-leech communities and potential associated health implications.

### **Variation in the Slimy Salamander (*Plethodon* spp.) skin and gut microbial assemblages is explained by geographic distance and host affinity**

Donald M. Walker<sup>1</sup>, Aubree J. Hill<sup>2</sup>, Molly A. Albecker<sup>3</sup>, Michael W. McCoy<sup>3</sup>, Matthew Grisnik<sup>1</sup>, Alexander Romer<sup>1</sup>, Alejandro Grajal-Puche<sup>1</sup>, Carlos Camp<sup>4</sup>, Crystal Kelehear<sup>5,6</sup>, Jessica Wooten<sup>4</sup>, Justin Rheubert<sup>7</sup>, Sean P. Graham<sup>6</sup>

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A multicellular host and its symbiotic microorganisms are recognized as a metaorganism—a composite unit of evolution. Microbial communities have been shown to have a variety of positive and negative effects on the host life history, ecology, and evolution. This study used high-throughput amplicon sequencing to characterize the complete skin and gut microbial communities, including both bacteria and fungi, of a terrestrial salamander, *Plethodon glutinosus* (Family Plethodontidae). We assessed salamander populations, representing nine mitochondrial haplotypes ('clades'), for differences in microbial assemblage across 13 geographic locations in the Southeastern United States. We hypothesized that microbial assemblages were structured by both host factors and geographic distance. We found a strong correlation between all three microbial assemblages at close geographic distances, whereas, as spatial distance increases, the patterns became increasingly discriminate. Network analyses revealed that gut-bacterial communities have the highest degree of connectedness across geographic space. Host salamander clade was explanatory of skin-bacterial and gut-fungal assemblages but not gut-bacterial assemblages, unless the latter were analyzed within a phylogenetic context. We also inferred the function of gut-fungal assemblages to understand how an understudied component of the gut microbiome may influence salamander life history. We concluded that dispersal limitation may in part describe patterns in microbial assemblages across space and also that the salamander host may select for skin and gut communities that are maintained over time in closely related salamander populations.

## **Geometric morphometric analysis of Eastern North American Pitviper vertebrae with implications for fossil identification**

Lance D. Jessee<sup>1</sup>, Blaine W. Schubert<sup>1</sup>, Austin R.J. Gause<sup>1</sup>, Joshua X. Samuels<sup>1</sup>

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Within Pleistocene cave deposits, snake fossils tend to be relatively common and generally occur as disarticulated vertebrae. However, identification of vertebrae is often hindered by significant intra- and interspecific morphological variation along the preloacal vertebral column, a subject that has largely been neglected in fossil snake identifications. Such identifications are typically based on mid-trunk vertebrae, comparison to disarticulated modern specimens, and an over reliance on modern distributions. This study examines vertebral variation and the utility of specific viperid vertebrae in making identifications. We use 2-D geometric morphometrics to examine vertebral variation along the preloacal vertebral column of four sympatric crotalines in the eastern United States: *Crotalus horridus*, *C. adamanteus*, *Agkistrodon piscivorus*, and *A. contortrix*. The focus here is to examine morphological regionalization patterns of the preloacal vertebral column. One individual of each species was chosen and every third vertebra was analyzed in anterior view using a set of 13 landmarks. The relative warp analyses show continual variation along the column, with mid-trunk vertebrae showing less variation than anterior and posterior trunk vertebrae. Both genera show the same trends in columnar variation and that mid-trunk vertebrae can be readily distinguished from other vertebrae. Preliminary discriminant function analyses also show that vertebrae can be accurately classified to genus level based on their shape. We plan to incorporate other viperids into our analyses to further assess specific and generic identifications. If this is successful, we will apply these methods to fossil specimens to better understand distributions of these snakes through time.

## **Oxygen consumption during development in embryos of the oviparous snake, *Pantherophis guttatus***

Celeste Gallardo, Joe Bidwell, James Stewart

<sup>1</sup>*Department of Biology, East Tennessee State University*

In the amniotic egg of oviparous reptiles (turtles, lizards, crocodylians, snakes and birds) all sources of energy and nutrients required for embryonic development must be available within the egg. Oxygen; and water for parchment-shelled eggs, must be accessible from the environment. Variation in the metabolic patterns between different species of developing birds, crocodylians, and lizards have been described. However, snakes, have remained underrepresented in this type of research. The aim of this experiment was to generate an oxygen consumption curve for the corn snake, *Pantherophis guttatus*, over the course of embryonic development to test the hypothesis that *P. guttatus* embryos will exhibit an exponential increase in oxygen consumption over time. Recently oviposited eggs were placed in chambers for 24-hour periods where percent oxygen was recorded. Siblings were sampled periodically throughout development. Embryos were staged after oxygen measurements and the compartments of the egg were separated and weighed (eggshell, yolk, embryo). The results indicate a steady incline in oxygen consumption during the earlier stages of development. As the embryos enter the final stages, the amount of oxygen being utilized increases substantially, signifying a rise in energy dependent processes and tissues towards hatching. These results are consistent with limited data regarding several species of desert snakes and may facilitate future comparisons of embryonic metabolism across multiple animal groups as well as establish patterns within Squamata.



## **Cohabitation patterns between stream salamanders and crayfish in the southern Appalachians**

Susan L. Cragg<sup>1</sup>, Joshua R. Ennen<sup>2</sup>, Shawna M. Mitchell<sup>2</sup>, Kristen K. Cecala<sup>3</sup>, Jon M. Davenport<sup>1</sup>

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Competitive interactions can determine the distribution and abundance of species within communities. If cohabitation occurs among species with similar requirements for refugia, then the strength of competitive interactions may not be strong. Stream salamanders and crayfish are both numerically dominant taxa within stream communities and often use similar cover objects for refuge. Therefore, we evaluated the abiotic (i.e. substrate composition, water flow, refuge size, and channel morphology) and biotic (i.e. competition) factors that may explain spatial patterns of cohabitation and body condition of these organisms using a two-pronged approach (both field-based and experimental methods). Cohabitation, frequency of refuge use, and body condition were not affected by refuge density or species identity in our experiment. However, crayfish cohabitated more frequently with salamanders than with other crayfish. Our field surveys found that stream salamanders exhibited higher rates of cohabitation when fewer boulders were surrounding the refuge being occupied. Overall, cohabitation for all study organisms was neither affected by competition, stream flow, channel morphology, nor the surrounding proportion of substrate (silt, sand, cobble, and bedrock). Our results failed to detect any competitive interactions between stream salamanders and crayfish for refuge. Cohabitation for salamanders, however, was dependent on the substrate composition surrounding their refuge. Thus, the cohabitation of stream salamanders and crayfish in the southern Appalachians is potentially driven by the abiotic environment, not competitive interactions.

## **Constantly fluctuating in an inconsistent way: comparing the effects of sinusoidal and naturally fluctuating incubation temperatures on embryo development**

Joshua M Hall<sup>1</sup>, Daniel A Warner<sup>1</sup>

<sup>1</sup>*101 Rouse Life Sciences Building, Auburn University, AL 36849*

Temperature is a commonly studied environmental factor influencing embryo development in reptiles. Though most studies use constant temperature incubation conditions, researchers are aware of the effects of fluctuating temperatures on development. Daily-repeating sinusoidal fluctuations are now commonly used in studies of developmental plasticity; however, thermal fluctuations in natural nests are highly variable from day to day. Thus, using repeated, uniform fluctuations (e.g. sine waves) may still provide an incomplete picture of how embryos develop in the wild and generate inaccurate predictions of how species will respond to future thermal conditions (e.g. climate change). We used eggs from the brown anole lizard (*Anolis sagrei*) to test the effects of realistic nest temperature fluctuations vs constant temperatures and sinusoidal fluctuations in the lab. We used temperature data from nests to create 4 incubation treatments: a constant mean temperature, a daily-repeating sine fluctuation, a daily-repeating asymmetrical fluctuation (i.e. mean, hourly nest temperatures), and a treatment that allowed each day's thermal fluctuation to differ from all other days as in real nests. These 4 treatments were created for both early-season (March-April, relatively cool) and late-season (June-July, relatively warm) nest temperatures (2 by 4 factorial design; season x incubation treatment). We report results for developmental rates, physiology (VO<sub>2</sub> and heart rate), embryo survival, as well as morphology, performance, growth, and survival of hatchlings. By comparing the effects of several commonly used thermal regimes with those of natural fluctuations, our study assesses the importance of using ecologically relevant incubation conditions when studying developmental plasticity of reptiles.

## Assessing short-term effects of translocation on the spatial ecology of eastern hellbenders

Bradley D. Nissen<sup>1</sup>, Emily Nolan<sup>1</sup>, Michael Freake<sup>2</sup>, Rebecca Hardman<sup>3</sup>, William Sutton<sup>1</sup>

<sup>1</sup>Tennessee State University

<sup>2</sup>Lee University

<sup>3</sup>University of Tennessee Knoxville

Due to population declines and habitat fragmentation throughout its range, the Eastern Hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*) is a strong candidate for translocation in Tennessee. We evaluated the short-term success of translocation on wild Eastern Hellbenders by comparing the spatial ecology of individuals pre- and post-translocation using radio-telemetry. We studied the home range sizes, movements and multi-scale habitat use of individuals (N =27) in two sustainable populations (Tumbling Creek and Hiwassee River) for one year and then subsequently collected similar data from a portion of these individuals (N =17) that were recently translocated (May-July 2019) into two nearby streams with declining populations (Rough Creek and Citico Creek, respectively). We hypothesized that translocated individuals would show similar spatial ecology patterns, after an initial exploration period. We collected over 1,300 location data points (824 prior to translocation and 435 post-translocation) from our four study sites. Home ranges of recently translocated individuals decreased when moved from Tumbling Creek to Rough Creek, while they increased when moved from Hiwassee to Citico Creek ( $p < 0.05$ ). Long distance movements ( $>100$  m) were observed in 58% of translocated individuals (7/12) at Citico Creek, compared to only 20% (1/5) at Rough Creek. The greater rate of “exploration” amongst individuals in Citico Creek could be due to increased competition for prey items with other predators (e.g. large fish, otters) that are not present in Rough Creek. This on-going study will serve to inform managers about the potential for translocation as a conservation strategy for Eastern Hellbenders.

## Using physiological conditions to assess patterns of subarctic Wood Frog (*Rana sylvatica*) habitat use

Thomas P. Hastings<sup>1</sup>, LeeAnn Fishback<sup>2</sup>, Jon M. Davenport<sup>1</sup>

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Arctic regions are especially susceptible to the effects of climate change. Habitat alterations and changing environmental conditions can be particularly harmful to wildlife that are dependent on the environment. The wood frog (*Rana sylvatica*) is one of only two Subarctic amphibian species that must maintain appropriate water balance conditions in a terrestrial environment. We hypothesized that the cutaneous water loss (CWL) rates of *R. sylvatica* will be significantly lower at specific frog locations than at paired random locations due to greater moisture availability and vegetative cover. To address this hypothesis, we selected seven sites in northern Manitoba and radio-tracked 64 adult frogs throughout the active season (June-September). To estimate CWL rates, fully hydrated plaster models were deployed at specific frog and random locations. Environmental conditions were measured at all model locations. We found that CWL rates were significantly lower at frog locations than at paired random locations. For every one unit increase in CWL ( $mg * min^{-1} * cm^{-2}$ ), there was a 1.8 percent decrease in the odds of a frog using a microhabitat location. For every one percent increase in the cover of sedge or shrubs, there was a 2.7 percent increase in the odds of a frog using an area. Lastly, greater relative humidity and surface moisture increased the odds of a frog using an area while increased wind speeds decreased the odds. Identifying current patterns of physiological and environmental conditions are critical to accurately predict future behavior and range expansion or contraction of Subarctic wildlife.

## **The effects of food web complexity and phenological variability of pond breeding salamanders**

David Burton<sup>1</sup>, Thomas Anderson<sup>1</sup>, Jon M. Davenport<sup>1</sup>

<sup>1</sup> *Department of Biology, Appalachian State University, Boone, NC 28608*

Variability in phenological shifts (seasonal changes in biological events) is becoming more common in nature. As phenological variability increases due to climatic factors, species interactions within communities can be affected. Shifts in breeding times can lead to body size variability within a population resulting in more complex food webs. Larval ambystomatid salamanders are important in many wetland communities as they often serve as top predators. To gain insight into the effects of shifting phenology, we conducted a mesocosm experiment manipulating breeding synchrony of *Ambystoma maculatum* across three dates (3/19/19, 4/2/19, 4/16/19) and food web complexity (presence of *Ambystoma opacum*, *Ambystoma talpoideum*, both present, neither present). We hypothesized that predation on *A. maculatum* would be highest in the lowest synchrony treatment due to predator satiation effects in high synchrony additions. We also predicted that *A. talpoideum* (top predator) would impose greater predation than *A. opacum* (intermediate predator) on the *A. maculatum*. *A. maculatum* in food webs with *A. talpoideum* present had the lowest survival rates for compared to other food webs. *A. maculatum* size at metamorphosis was also highest in the *A. talpoideum* food webs suggesting a thinning effect. *A. maculatum* synchrony treatments did not have a significant impact on survival or size at metamorphosis except in treatments where intermediate and top predators were not present. Shifts in phenology are an understudied aspect that have the potential to alter species interactions. These results show that predation may be enough to suppress these effects but unchecked can alter food webs.

## **Nest-site selection of an isolated population of Bog Turtles (*Glyptemys muhlenbergii*) in Johnson County, Tennessee**

Amy Turpin

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In order to provide effective conservation strategies for the southern population of Bog Turtles, it is essential to understand their life history and in extension, nesting behavior. It was hypothesized that environmental conditions influence Bog Turtle nest-site selection. Bog Turtles were caught by trapping, visual surveys, probing, and muddling. All captured turtles were measured and weighed. New turtles received permanent markings, and reproductive females were palpated to determine if they were gravid. The males ( $n = 3$ ) had an average carapace length of 101 mm ( $SD \pm 5$ ), max carapace length of 102.7 mm ( $SD \pm 4.2$ ), carapace width of 71.8 mm ( $SD \pm 3.6$ ), plastron length of 81.4 mm ( $SD \pm 4.3$ ), and max plastron length of 88.5 mm ( $SD \pm 4.2$ ). The females ( $n = 5$ ) had an average carapace length of 84.4 mm ( $SD \pm 7$ ), max carapace length of 85.8 mm ( $SD \pm 6.8$ ), carapace width of 63.8 mm ( $SD \pm 3.8$ ), plastron length of 72.8 mm ( $SD \pm 5.3$ ), and max plastron length of 77.8 mm ( $SD \pm 6.0$ ). Three turtles from the site were killed by predators during the field season. Three reproductive females were fitted with radiotransmitters that allowed their movements to be tracked throughout the nesting season via radiotelemetry. While two of the females did not nest, one female's nest was predated after egg deposition. The nest-site was found in habitat dominated by grasses, bulrushes, and sphagnum moss. This followed nest-site selection trends of Bog Turtles from other parts of the southern population.

## **Macro-ecological scale geographic variation of a keystone predator in a common garden environment**

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Geographic variation in species behavior and life history has been well documented in biology. Species with wide geographic distributions (i.e. across a continent) and small home ranges (i.e. <2km<sup>2</sup>) are especially likely to experience variability in abiotic environments across the entirety of their range, and possibly strong local adaptation. This can result in differences in species interactions. Understanding variation on a geographic scale is especially important when considering species of ecological importance, such as keystone species. Yet, few studies have compared the potential cascading ecological effects of keystone predators on a macro-geographic scale. To understand how keystone ability in pond food webs can vary across a large geographic range, we conducted an artificial pond experiment predator that is known to be a keystone predator in at least part of its range, the marbled salamander (*Ambystoma opacum*). To do so, we collected size-matched salamander larvae from three geographically distant populations (>650km apart) in Ohio, Mississippi, and North Carolina and placed them in mesocosms with a suite of spring breeding amphibian prey species. Additionally, we quantified zooplankton abundance and primary productivity to better understand ecosystem-level effects of this keystone predator. Preliminary results indicate differential survival of some tadpole species leading to differences in anuran diversity among the three predator populations. Tadpole diversity was lowest in the most northern populations (i.e. Ohio) of salamanders indicating that keystone function may vary at a geographic scale. Further understanding of large-scale variability in ecologically important predators is needed to direct future conservation efforts and preserve biodiversity.

## **Response to a predator cue from a nonnative fish by a larval predator-naïve salamander (*Desmognathus quadramaculatus*)**

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Nonnative fish introductions can pose a serious threat to the native amphibian fauna that rely on true identification of predators. Over the last 50-60 years in particular, the stocking and encroachment of rainbow trout (*Oncorhynchus mykiss*) into headwaters where the black-bellied salamander (*Desmognathus quadramaculatus*) and brook trout (*Salvelinus fontinalis*) naturally coexist has raised concern. Naïve prey are ones that lack a strong evolutionary relationship with a predator and are susceptible to predation due to their lack of an appropriate predator-avoidance response. Prey often encounter a variety of predators and nonpredators, and the ability to distinguish between them is important for managing energy that could be spent foraging rather than exhibiting predator-avoidance behavior. The dominant way aquatic prey detect predators, is through their heightened sense of smell via detection of predators through their natural odor, or predator cue. Therefore, it is hypothesized that larval black-bellied salamanders would fail to exhibit a predator-avoidance response to rainbow trout due to their lack of a strong evolutionary relationship with the fish. Preliminary results indicate that larval salamanders from streams with only brook trout have limited responses to cues from rainbow trout. Additional work is underway to test the response of salamander larvae from streams with brook and

rainbow trout, rainbow trout only, and no trout. These results indicate that nonnative fish introductions can be detrimental to native amphibians.

### **Application of eDNA for rapid assessment of potential novel populations of *Ambystoma barbouri* in Middle Tennessee.**

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The Streamside Salamander (*Ambystoma barbouri*) is a state endangered, winter-breeding amphibian known predominantly from limestone-bearing, intermittent streams in middle Tennessee. The species' predilection for using low gradient, small streams for breeding and larval development has resulted in undesirable outcomes at some locations where rapid anthropomorphic development has been undertaken. In order to provide landowners and other decision-makers the timeliest information about this species, rapid assessments are needed to determine its presence or absence. Nicole Witzel (USFWS) developed an effective methodology for determining the presence of *Ambystoma barbouri* at breeding sites via environmental DNA (eDNA) (Witzel, 2019). In partnership with TSU & TWRA, the DNA collected 54 water samples to augment visual encounter surveys. Samples were collected from five counties between 21 March and 25 April 2019, at a time when late-stage eggs or larvae remained available, but after the mid-February peak of free *Ambystoma barbouri* eDNA. Six samples were positive and six were negative for eDNA at sites where larvae or eggs were observed, one could not be analyzed, and one was strongly positive at a site where no *Ambystoma barbouri* were noted. Examination of the specific circumstance of each sample site suggests the sensitivity of the methodology but limitations based on numbers of animals present or other influences to stream integrity. The single strong positive site with no visible *Ambystoma barbouri* will be investigated this winter to determine presence of breeding adults. Additional eDNA sampling may be undertaken during winter 2019-2020 when adults also are available at breeding sites.

### **Using a passive sampling technique to survey for reptiles and amphibians.**

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Active sampling techniques for reptiles and amphibians can provide quality data, but these techniques are often prohibitively labor intensive and time consuming. To make matters worse, rare and cryptic species often require substantially more effort to successfully capture with active techniques than more common species. Passive sampling techniques, on the other hand, can provide occurrence and monitoring data at a fraction of the time required by active sampling. Camera traps are a popular passive sampling technique utilized for relatively large animals. The technique can also be adapted to capture reptiles and amphibians by funneling animals into an area where close-up photos can be taken. We built and installed camera traps at Laurel Hill Wildlife Management Area in Lawrence County, Tennessee in an attempt to passively

document occurrences of northern pine snakes (*Pituophis melanoleucus*). Trap design, cost, manpower, and success of this passive survey technique will be discussed.

### **Abstracts – Poster presentations**

#### **Habitat selection by Cottonmouths (*Agkistrodon piscivorus*) in Middle Tennessee.**

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Many snake species exhibit an ontogenetic shift in habitat preferences, as this can potentially reduce interspecific competition. We investigated the habitat selection of a Tennessee population of cottonmouths inhabiting an isolated wetland system adjacent to the Cumberland River. Field collection was by visual-encounter surveys, and individuals were measured and sexed. Principle component analysis was used to elucidate intraspecific trends in habitat selection between male, female, and juvenile cottonmouths (N = 96). We found that cottonmouths exhibited non-random habitat use, with respect to available habitat features, and there were significant habitat differences among intraspecific groups. The results suggest that cottonmouths do change habitat use through ontogeny as from drier habitats to wetter habitats. This may be a mechanism to reduce intraspecific competition. However, among the cottonmouths each group (male, female, and juvenile) had large variations in habitat use, emphasizing that they are habitat generalists utilizing a wide variety of resources. In addition, the results showed females and juveniles had the highest variations in habitat preferences compared to males. We suggest that trends in ontogenetic shifts in habitat use may be associated more with actively avoiding larger individuals, than specific differences in habitat preference.

#### **Development of a molecular genetic method for characterizing amphibian diets**

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Dietary studies provide key insights into the ecology and behavior of animals. Methods common in dietary studies of amphibians (e.g., gastric lavage, fecal analysis, dissection) often fail to identify prey beyond the level of Order, are time intensive, and can be biased against soft-bodied prey. To complement these methods, we adapted an existing DNA metabarcoding assay to characterize amphibian diets from non-invasive fecal samples. In DNA metabarcoding, barcoding loci from mixed community samples (e.g., feces, eDNA) are sequenced on a next-generation sequencing platform, and the resulting reads are used to identify members of that community (e.g., diet composition). To validate this method, we prepared COI amplicon libraries from 27 fecal samples collected from wild Blue Ridge two-lined salamander (*Eurycea wilderae*) during the breeding season. We sequenced these libraries on an Illumina MiSeq and then compared the resulting reads against a reference database of arthropod sequences of known identity in QIIME2 to characterize invertebrate diet composition. Here, we present these preliminary results and discuss our ongoing efforts to refine and apply these methods to study competition among sympatric dusky salamanders (*Desmognathus*). We hope that this method will prove to be more accurate and efficient than previous methods, providing a versatile tool with which amphibian diets can be characterized and compared.

## **Predicting Southern Zigzag Salamander (*Plethodon ventralis*) occupancy in a fragmented landscape**

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As humans continue to alter natural landscapes, conservation planning necessitates a better understanding of the natural and anthropogenic factors that determine the distribution of wildlife. The Southern Zigzag Salamander (*Plethodon ventralis*) is a relatively recently described species (1997) and has been the subject of little research. This species can be locally abundant in the Ridge and Valley—including in some urban and disturbed habitats—but populations often appear to be discontinuous across the landscape. Here, we used repeated sampling of random quadrats among forests in Knox County, Tennessee to better understand factors predicting the distribution of *P. ventralis*. We fit occupancy models with site-level covariates such as forest patch size and the presence of invasive ground cover and observation-level covariates such as temperature and precipitation. We recorded *P. ventralis* at 15 of 56 quadrats. The principle findings of our study were that the most important factors driving *P. ventralis* occupancy are the presence (or lack thereof) of two invasive ground cover plants—English Ivy (*Hedera helix*) and Wintercreeper (*Euonymus fortunei*)—and the presence of limestone as the primary layer of underlying rock at a site.

## **Characteristics of the turtle community in the Freed-Hardeman University research ponds**

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Aquatic turtles serve an important role as indicator species in the ecosystems they inhabit. As aquatic habitats come under increased pressure from human development, habitat alteration, and pollution, many turtle communities are in decline. We hypothesized that the turtle community inhabiting the Freed-Hardeman (FHU) research ponds may be in decline due to recent human encroachment and habitat alteration. For the past 27 semesters, research students monitored this community by capturing turtles with baited traps. All individuals were marked with a distinct number and released. Here we describe community characteristics of the turtle assemblage in the FHU ponds. We specifically describe species diversity and explore changes in community structure. A Shannon index did not reveal a significant change in diversity over time, with diversity only significantly fluctuating between the spring and fall seasons. Turtle community diversity in the FHU ponds has remained relatively stable over the past 14 years in spite of changes to the surrounding habitat.

## **Conservation genomics and population status of the Streamside Salamander (*Ambystoma barbouri*) in Tennessee**

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In Tennessee, the state endangered Streamside Salamander (*Ambystoma barbouri*) is of conservation concern due to loss of habitat from deforestation and urban development. Information regarding the current status of *A. barbouri* populations in Tennessee and the taxonomic status of these populations relative to more northerly widespread core populations have important implications for effective

management and conservation of this species. Here we surveyed 37 known historical populations of *A. barbouri* across Middle Tennessee. We used genotyping-by-sequencing (GBS) to discover and characterize single nucleotide polymorphisms (SNPs) in order to investigate patterns of genetic variation within Tennessee populations of *A. barbouri* and to assess their relationship to populations in Kentucky. We observed *A. barbouri* breeding activity at 23 of our surveyed field sites. The health of the observed populations varied, ranging from populations threatened by encroaching development to healthy populations in protected areas. Genotyping-by-sequencing generated a panel of >800 SNPs from 265 sampled individuals representing 21 stream populations. Early GBS results from five populations show genetic clusters on either side of the Cumberland River. Results from this study will be used to define management units for consideration in planning conservation efforts.

### **Helping Tennessee's native herpetofauna: An overview of local conservation efforts at the Nashville Zoo**

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The Herpetology Department at the Nashville Zoo (NZG) is an active participant in a variety of conservation-based projects, several of which focus on species of conservation concern in Tennessee. Since 2007, NZG has been developing reproduction technologies for eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*). Resulting from these efforts, two hellbenders were successfully produced via in-vitro fertilization in 2012 and cryopreservation techniques were developed for the species. NZG has been continuing to refine these methodologies and in 2015 were able to produce the first Cryptobranchid and second caudate ever produced with cryopreserved sperm. After receiving funding through State Wildlife Grants in 2011, the NZG has been collaborating with Tennessee Wildlife Resource Agency (TWRA) and several universities to document the current status of hellbender populations in Tennessee. NZG and collaborators initiated a head-starting project, where eggs are collected and raised at the zoo, after which they will be released in appropriate streams. Along with TWRA, the NZG is participating in a head-starting project for alligator snapping turtles (*Macrochelys temminikii*). Hatchling turtles were acquired from Tishomingo National Fish Hatchery and are raised at the zoo until reaching sub-adult size, after which they are given to TWRA. NZG has also begun the establishment of an assurance colony of streamside salamanders (*Ambystoma barbouri*). These salamanders were collected as eggs or larvae from sites that were being developed in the Mill Creek watershed, where they are nearly extirpated. The NZG intends to develop a breeding program for this species to allow for eventual augmentation at restored sites.

### **Spatial ecology of Gila Monsters in a nutrient subsidized environment**

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Animal movements are often defined using the home range concept. Consequently, home ranges are determined by temporal, spatial, and individual-level processes. Within the environment, one of the key factors influencing an animal's range and how it uses the environment is that of resources. Alterations to



the environment that affect resource distribution and availability can have profound consequences on an animal's spatial patterns. One of the best examples of this is that of golf courses. Some environmental modifications exhibited by some human altered environment can have positive effects on certain wildlife species by altering their movement patterns and foraging efforts. We analyzed data collected from 22 Gila Monsters *Heloderma suspectum* at a subsidized environment in Arizona from 2007 to 2013 and a non-subsidized environment. We performed both kernel density estimation and minimum convex polygons for comparability purposes. After calculating least square means, males in the subsidized environment had an average area of 11.4 ha while the females had an area of 10.1 ha. In the un-subsidized environment males had an average range of 43.2 ha while females had an area of 22.1 ha. This suggests that the home ranges may be smaller in subsidized environments than those of un-subsidized environments due to increases in available resources. Gila Monsters may not have to invest in wide ranging foraging efforts as those populations of the un-subsidized environments.

### **Wet rock crevices impact egg deposition and survival in Green Salamanders, *Aneides aeneus***

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Green salamanders, *Aneides aeneus*, occur in rock crevices that are humid but not wet. This is true for deposition and brooding of eggs. Females usually do not deposit eggs in crevices with standing water. Eggs are usually attached to the crevice ceiling in a horizontal plane with a space below the egg clutch. The brooding female often has her head in this space. Also, the space may allow water accumulation without damaging eggs. Females may move into and out of contact with eggs to minimize water uptake. In two crevices that produced hatchlings over ten years, young were not produced in summers of 2004 and 2005. Periods of extended rainfall led to wet crevices and thus failed egg clutches, or eggs were not deposited although gravid females were observed. Extensive rainfall during egg deposition in July 2011 resulted in some breeding crevices with standing water. In one crevice that had been monitored for several years, a female deposited eggs 10 cm away from the regular crevice to the ceiling of a larger more exposed crevice visible from outside the rockface. Due to the large space below the eggs, the female was unable to brood them. She was located to the left of the eggs with head oriented toward them and snapped at introduced probes. The clutch failed and the eggs had disappeared by Aug 10, 2011. Adaptations of *A. aeneus* to this microhabitat resulted in return of brooding females and eggs to these crevices in 2014 and 2015.

### **Experimental inoculation of *Ophidiomyces ophiodiicola* and resulting mortality in the Common Watersnake (*Nerodia sipedon*)**

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Snake fungal disease (SFD) is caused by the pathogen *Ophidiomyces ophiodiicola* (*Oo*) and affects snake populations from the Northeastern and Midwestern USA. Snakes with SFD display clinical signs such as necrosis and fungal granulomas. Recent research on a population of Massasauga rattlesnakes suggests that SFD contributes to an increased mortality rate in snake populations. From 2015–2017, we detected a high prevalence (38% of individuals sampled) of *Oo* in Common Watersnakes on the landscape. Our objective of this study was to determine if high field prevalence of *Oo* in Common Watersnakes correlates with mortality in a controlled lab experiment. We postulated that SFD correlates with weight loss, as energy

resources are diverted to immune function, rather than growth. We captured 22 Common Watersnakes that were confirmed *Oo* negative using quantitative PCR (qPCR). The snakes were randomly and equally divided into a sham inoculum control (-*Oo*) and experimentally inoculated group (+*Oo*). All snakes were placed into clear mesocosms at 25 C under a 12:12 light/dark cycle and observed for a total of twelve weeks. Standardized weekly feeding, weight/length measurements, and swab samples for qPCR took place. Clinical signs were observed in experimentally inoculated snakes and all eleven tested positive for *Oo* with qPCR throughout the study. During the first four weeks, the experimental group experienced a 36% decline, whereas, the control group declined by 18% of trial individuals. At the end of the twelve-week experiment there was a 9.1% higher mortality rate within the experimental group. A repeated measures ANOVA indicated that both control and experimental snakes significantly gained weight over time ( $\chi^2_1 = 5.32, p < 0.05$ ) as a result of successful weekly feeding, but weight did not differ by *Oo* treatment group ( $\chi^2_1 = 0.21, p > 0.05$ ). Our results suggest that patterns of high *Oo* prevalence on the landscape could translate into mortality events, but SFD might not affect body weight when food is plentiful.

### **Gut microbial function of *Plethodon cinereus*, the Red-backed Salamander**

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Microbes are among the most diverse species on earth and have a wide range of metabolic abilities. Microbes are known symbionts of many multicellular hosts and have been shown to contribute to host evolution, ecology, immunity, and metabolism. Given a salamander's food preference, they are likely to harbor gut microbes that metabolize macromolecules. We isolated culturable gut microbes from seven *Plethodon cinereus* and created a metabolic profile for 222 pure cultures. In order to accomplish pure cultures, we collected salamander feces deposited on sterile filter paper moistened with sterile deionized water. We performed serial dilutions of the fecal pellets and plated it on LB agar. After 24-hours at 37°C, each unique colony was isolated into pure culture. Pure cultures were tested in triplicate on spirit blue to screen for lipid metabolism, on lab-made chitin agar for chitin metabolism, and on potato-agar plates for starch metabolism. Metabolic activity was determined if a zone of clearing was visible around the culture. In order to genotype the pure cultures, we used a direct colony PCR protocol for a subset of pure cultures, followed by amplification of a barcoding gene (16S rDNA) to identify each isolate to, at least, the family level. Results of metabolic profiles were analyzed using discriminant function analysis in SPSS v. 25. There were eight possible metabolic profiles, but the pure cultures with no metabolism of lipids, chitin, and starch were disregarded from further analyses. The metabolic profile of the bacteria exhibited the ability to metabolize the following combinations in order of decreasing abundance: lipids and starch (n=79), lipids only (n=75), lipids, starch, and chitin (n=36), starch only (n=14), lipids and chitin (n=13), starch and chitin (n=3), and chitin only (n=2). Future research will investigate functional redundancy in the gut microbiome in this and other salamander species.