



Abstracts of the 29th Annual Meeting of the TN Herpetological Society, Union City, TN

Student Oral Presentations

The Importance of Early Successional Habitat for Conservation of Herpetofaunal Assemblages in Tennessee

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Increasing anthropogenic influence has exacerbated climate change and ecosystem degradation, leading to global biodiversity loss, specifically within herpetofauna. However, snake population status is exceedingly difficult to determine due to cryptic and elusive behaviors, brief and patchy movement patterns, and use of inaccessible habitats. The Western Pygmy Rattlesnake (*Sistrurus miliarius streckeri*) is classified as Threatened within Tennessee (TN) and primarily occupies floodplain habitat adjacent to early successional habitats (ESH) along the western portion of the Interior Plateau ecoregion. ESH support unique flora and fauna but have declined over the last few decades mostly as a result of urban encroachment and disturbance suppression. We used a combination of drift fence and box trap arrays, road cruising, and radio telemetry to evaluate the importance of ESH for herpetofaunal conservation, with an emphasis on Pygmy Rattlesnakes. Preliminarily, we observed robust recaptures of common herpetofauna species, more frequently occurring at forest edges nearest ESH. We found box trapping to be an effective method for passive sampling of highly cryptic Pygmy Rattlesnakes, with all individuals captured within 150 meters of field edges. Evidence from this work suggests importance of forested edge habitat for herpetofaunal conservation.

Living la vida local: philopatry results in consistent patterns of annual space use in a long-lived lizard

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Some reptiles can live decades in the wild and experience extreme environmental variation that influences patterns of habitat use. Individuals may modify their use of space over time, reducing the utility of single-year home range estimates. VHF telemetry data were collected for Gila monsters at three Mojave Desert sites in Clark County, Nevada and home ranges were calculated using an autocorrelated KDE. Home range size was consistent within individuals and populations and did not vary across years. To measure home range fidelity, we calculated Bhattacharyya's Coefficient (BC) for each combination of years in which an individual was tracked and averaged estimates across individuals and populations. Average BC was 0.86 (scale from 0-1) and did not vary among populations. Analysis of the frequency of movement by individuals, average distance traveled per movement, and cumulative distance traveled per active season revealed that

movement patterns vary considerably by year. Heterogeneity of space use among populations and individuals suggests that individual and local environmental variation, rather than annual variation in resource availability, may drive home range size and movement patterns of Gila monsters in southern Nevada. Annual variability in movement patterns did not translate to variability in home range size or location, and the species exhibits extremely high philopatry, using the same areas for periods of at least 3-5 years.

Incubation temperature has population-specific effects on the Streamside Salamander (*Ambystoma barbouri*)

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Climate change will impact all animals but especially developing embryos of vertebrate ectotherms which rely on environmental temperature for physiological activities and receive little parental care. The effects of a warming climate on non-avian reptile development have been studied extensively; however, there is comparatively less known about impacts on amphibians, particularly salamanders. The Streamside Salamander (*Ambystoma barbouri*), a Tennessee state endangered species, has a disjunct range across relatively wide latitudes which may have resulted in population-specific responses to environmental temperature. To determine how thermally sensitive traits vary across populations and, thus, how populations may respond to climate change, we collected eggs from across the Streamside Salamander's range and incubated them at various temperatures. At each location, we deployed temperature loggers to characterize the thermal environment of nests. We measured developmental rates and recorded resultant survival and morphology of larvae and metamorphs in the laboratory. We report nest temperatures and the effects of temperature during embryonic development, highlighting trends between disjunct populations. These results will aid in targeted conservation efforts as they will demonstrate if and how endangered Tennessee populations differ phenotypically from more robust populations in the northern portion of the range.

Professional Oral Presentations

Stream Restoration Design Considerations for the Streamside Salamander (*Ambystoma barbouri*) at a Sumner County, Tennessee Mitigation Site

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The state endangered streamside salamander (*Ambystoma barbouri*) is known from a single reach of a stream & wetland

mitigation site in Sumner County, Tennessee. Permitting & design work associated with the project was conducted from 2018-2023. Existing conditions within the reach occupied by *A. barbouri* found high bank erosion and poor floodplain connectivity. Hydrology was intermittent with flow only during the winter & spring. The existing riparian buffer was absent and/or largely composed of invasive species. Proposed stream restoration followed Natural Channel Design (NCD) methodologies which catered to the ecology of *A. barbouri*. The design incorporated a Rosgen “C” stream type with riffle pool geomorphology consistent with reference sites occupied by *A. barbouri*. A pool depth ratio >2.5 and % pool of >50% provided developmental areas for larvae in the spring & early summer. Constructed riffles will be top-dressed with native slab rock currently used by *A. barbouri* for breeding activities at this location. Invasive species will be cleared from the restoration reach. The project conservation easement will be re-planted with native tree and shrub species to establish an ecologically appropriate riparian buffer. Construction activities started in July 2023 and work on the occupied reach is planned to continue through the fall of 2023.

Early-life Manipulations of the Gut Microbiota in a Vertebrate Ectotherm Affect their Heat Tolerance

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The gut microbiota is known to influence and have regulatory effects in diverse physiological functions of host animals, but only recently has the relationship between host thermal biology and gut microbiota been explored. Here, we examined how early-life manipulations of the gut microbiota in larval amphibians influenced their critical thermal maximum (CT_{max}) at different acclimation temperatures. We removed the resident microbiome on the outside of wild-caught wood frog egg masses via an antibiotic wash, and then either maintained eggs without a microbiota or inoculated eggs with pond water or the intestinal microbiota of another species, green frogs, that have a wider thermal tolerance. We predicted that this cross-species transplant would improve the CT_{max} of the recipient wood frog larvae relative to the other treatments. In line with this prediction, green frog-recipient larvae had the highest CT_{max} while those with no inoculum had the lowest CT_{max}. Both the microbiome treatment and acclimation temperature significantly influenced the larval gut microbiota communities and alpha diversity indices. Our results are the first to show that cross-species gut microbiota transplants alter heat tolerance in a predictive manner. This finding has repercussions for the conservation of species that are threatened by climate change and demonstrates a need to further explore the mechanisms by which the gut microbiota modulates host thermal tolerance.

There and Back Again; A Herpetologist's Tale

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My professional research began in Tennessee as an undergraduate student, but graduate school took me south to Cajun country. Luckily, I gained the opportunity to return as a new faculty member at Cumberland University. I would like to share some of my past research experiences related to the

physiological ecology and functional morphology of reptiles, as well as talk about my future Tennessee herp research plans. At the University of Louisiana at Lafayette I worked on a variety of research projects related to the functional morphology of reptiles. I collaborated on several projects that measured the striking kinematics of ratsnakes, cottonmouths, and western diamond-backed rattlesnakes. My dissertation research focused on the feeding mechanics and digestive physiology of microcephalic and megacephalic map turtles (*Graptemys* spp.). I measured voluntary bite forces, and compared those with stimulated maximal bite forces, and theoretical models of maximum bite force derived from the craniofacial muscle anatomy of adult map turtles. To develop those theoretical models, I took a classical anatomy approach to dissect and describe the muscle fiber arrangements of each portion of the jaw adductors in map turtles. I also measured the standard metabolic rate and the differences in the energetic costs of feeding on different prey bases by map turtles with differing trophic morphologies. Now I am back in Tennessee at a small, teaching institution. I want to work to share my expertise and establish new partnerships and research collaborations.

Status and Update of Amphibian and Reptile Atlases of Tennessee

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The David H. Snyder Museum of Zoology at APSU houses the largest amphibian and reptile collection in Tennessee. The specimens in the collections were the basis for the biogeographic, ecological, and taxonomic information used in the publication of the Atlas of Amphibians in Tennessee (1996) and the Atlas of Reptiles in Tennessee (2008; Redmond and Scott). Data from the atlases are important for conservation planning, including the Tennessee State Wildlife Action Plans. For 23 years online versions of the atlases were updated quarterly (Redmond and Scott), but updating the static content of those legacy atlases is no longer possible. Here we present recent changes to the Tennessee Herp Atlas system including migrating all content to a database-driven webpage and a more contemporary digital framework drawing species occurrence information from multiple online data sources. A live demo of the new atlases will be given.

Natural History and Status of the Pygmy Rattlesnake (*Sistrurus miliarius*) in Tennessee

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Tennessee is home to 35 snakes, with 4 of these species belonging to the family Viperidae. One species within this family, the Pygmy Rattlesnake (*Sistrurus miliarius*), has a limited range in Tennessee, with historical detections occurring in habitats associated with the Tennessee River in the Western Highland Rim and Southeastern Plains regions. Historically, this species has been confirmed in seven Tennessee counties, with questionable reports from an additional six counties. Since 2016, the Tennessee State University Wildlife Ecology Lab and

Tennessee Wildlife Resources Agency have collaborated to better understand the natural history, ecology, and distribution of this species in Tennessee. We report data from 67 detections (59 new captures and 7 recaptures) of Pygmy Rattlesnakes in Tennessee, including body size, sex ratios, and activity patterns. In addition to noting important behavioral observations (e.g., courtship), we have also documented the presence of neonate snakes in one population. Our work in combination with citizen science, primarily the Tennessee Snake ID Page, has resulted in the documentation of the species in three previously unconfirmed counties. The Pygmy Rattlesnake appears to be patchily distributed throughout the known range in Tennessee; however, reliable detection of the species at suspected localities remains a difficult issue.

Poster Presentations

Fecundity Selection and Sexual Dimorphism in the Gila Monster (*Heloderma suspectum*)

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The Gila monster (*Heloderma suspectum*) is a large-bodied venomous lizard that inhabits deserts across the Southwestern United States and Northern Mexico. Previous studies have observed sexual dimorphism in this species; males have larger heads than females after accounting for body size. This trait is thought to be under sexual selection due to male-male combat in their mating system. Sexual dimorphism that could facilitate higher fecundity in females, like abdomen size, has not been previously tested. Female Gila monsters lay a clutch every 2-4 years, with each clutch being relatively large, suggesting they could benefit from having larger trunk sizes (relative to males) to accommodate large clutches and more offspring. Using morphometric data from preserved zoological museum specimens, analyses were conducted to determine if trunk size could be a target of selection and if a difference existed between males and females. Our results indicate that after accounting for body size, there is no significant difference between trunk sizes of males and females and that males are larger than females on a multivariate basis. Trunk size for both sexes had a significant effect on the amount of reproductive tissue (testes or eggs) in the specimens we measured, indicating that a larger abdomen could overall hold more eggs.

The search for a rare salamander: the use of eDNA in detection of *Eurycea junaluska* in the Great Smoky Mountains National Park

Ben F. Brammell, Sara A. Brewer, Elizabeth K. Strasko, Jarrett R. Johnson

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Eurycea junaluska is a small, semiaquatic plethodontid salamander with a very restricted range, much of which is encompassed within the Great Smoky Mountains National Park (GSMNP). *Eurycea junaluska* was first described in 1976 and

was initially only known from three creeks in Graham County, North Carolina. Currently, *E. junaluska* is known to exist in a total of five counties, all within TN and NC. The objectives of this study were to A) develop and validate a species-specific assay which detects *E. junaluska* and B) utilize this assay to reassess the distribution of *E. junaluska* within GSMNP boundaries. We obtained *E. junaluska* DNA (Swain Co., NC) and amplified and sequenced a 650 BP segment of cytochrome b (cytb). We aligned this sequence with the only *E. junaluska* cytb sequence published in public databases and designed an assay (primers and probe) utilizing conserved regions of these two sequences (95% similar). This assay was tested with sympatric species in silico (22 species) and in vitro (7 species) to confirm specificity. Fifty water samples were collected in July 2023 throughout the GSMNP; samples were collected in every major drainage, on both the TN and NC sides, and spaced as evenly as possible throughout the park. Samples were filtered in the field using sterile disposable filter funnels and extracted in the lab. qPCR analysis is currently in progress. These data should expand our understanding of exactly how rare *E. Junaluska* is and facilitate conservation efforts.

An improved assay for eDNA detection of four-toed salamanders (*Hemidactylium scutatum*): significance of mitochondrial genome region in primer development

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Environmental DNA (eDNA) is an emerging tool that 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 the value of thoroughly vetting eDNA primers using as many local sequences as available. We developed a species-specific assay (primers and probe) for use in qPCR eDNA detection of *Hemidactylium scutatum* and tested these primers in silico (22 species) and in vitro (7 species) against sympatric species to ensure specificity. We field tested our assay at 15 field sites in central and eastern Kentucky and detected *H. scutatum* DNA at 10/15 sites in a manner mostly consistent with field observations. Additionally, we collected *H. scutatum* tissue from specimens from four locations in central and eastern KY and obtained DNA from a New York collected *H. scutatum* specimen; our assay was successful in amplifying *H. scutatum* DNA from each collection locality. A previously published *H. scutatum* eDNA assay was used to detect specimens from NY, but failed to detect specimens from KY; this assay targets the intergenic spacer region (IGS), while our assay targets cytochrome b. We completed sequencing of the cytb and the IGS region from both a KY and NY *H. scutatum* specimen and confirmed conservation of cytb but significant sequence differences within the IGS region of the two specimens. These results are consistent with our understanding of mitochondrial evolution and highlight the importance of locus selection in assay development.

A Novel Application of Membrane Inlet Mass Spectrometry to Study Larval Physiology of the Streamside Salamander (*Ambystoma barbouri*)

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Urbanization often causes environmental temperatures to increase due to the replacement of vegetation with heat-absorbing substrates. Thus, the physiology of ectotherms inhabiting urbanized areas may be detrimentally affected. The Streamside Salamander (*Ambystoma barbouri*) in Tennessee is primarily threatened by urbanization, which, in addition to habitat destruction, causes temperature increases in breeding streams around the Nashville metro area. During early development, salamanders are particularly sensitive to temperature increase due to their limited thermoregulatory capacity. To determine effects of rising temperatures on larval physiology, we applied an experimental application of a Membrane Inlet Mass Spectrometer (MIMS) to examine oxygen consumption of larval *A. barbouri* at two distinct temperatures (10 and 20 °C). These temperatures span nearly the entire range of environmental temperatures experienced in the wild. Statistical analysis revealed a Q10 of 2.6 based on oxygen levels measured from individual larvae at the two thermal treatments. A Q10 of 2.6 is within the range expected for biological reactions and that observed in other species. Therefore, these data support the hypothesis that larvae function normally within this range of temperatures and warmer or cooler temperatures would be required to induce thermal stress. The results of this study offer crucial insights into the physiological adaptations of *A. barbouri* and provide a foundation for the use of MIMS to study larval physiology in the future.

Incubation moisture and temperature influence embryo physiology but not hatchling morphology in the Eastern Fence Lizard (*Sceloporus undulatus*), a model vertebrate for global change research

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There is great need to understand the physiological limits of organisms to predict responses to climate change. Researchers often incorporate organismal thermal limits into predictive models, but we know comparatively less about how factors like water availability interact with temperature to mitigate or exacerbate heat stress. Embryos of vertebrate ectotherms (e.g. lizards) are particularly vulnerable to fluctuations in temperature and moisture because eggs are left to develop under prevailing conditions. Therefore, to understand the independent and interactive effects of moisture and temperature on development, we incubated eggs of the Eastern Fence Lizard (*Sceloporus undulatus*) in a factorial experiment of two moisture and two temperature treatments. One temperature regime subjected eggs to stressful temperatures so we could understand how moisture influences embryo responses to heat stress. Effects of moisture and temperature were expected: dry conditions reduced water

absorption and survival, high temperatures increased embryo heart rate, developmental rate, and lowered hatching success. We observed no statistically clear interaction of moisture and temperature on any phenotype; however, egg survival was greatest under cool, moist conditions and lowest under hot, dry conditions indicating that high incubation moisture mitigates adverse effects of extreme temperatures. We highlight the need for additional work combining realistic temperature and moisture treatments to understand the relationship between multiple abiotic factors and global change.

Environmental Influences on Gila Monster Spatial Ecology

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The home range concept is a valuable tool for understanding how individuals use available habitat in relation to resource abundance and individual behavioral decisions. As resource abundance is tied to environmental conditions (precipitation, temperature, productivity), organisms may adjust their home range during periods of resource scarcity and retract range size in times of resource abundance. These patterns may be particularly apparent in organisms that live in highly seasonal environments with a wide range of suitable habitat types. We compiled telemetry data for Gila Monsters at eight sites throughout the states of Arizona, Nevada, and Utah. Annual home range utilization distributions were calculated using a weighted autocorrelated kernel density estimator. Annual environmental variables quantifying different aspects of precipitation, temperature, and habitat productivity were extracted and calculated for each individual in each year it was tracked. Annual home range size was positively correlated with annual heat moisture index and thermal seasonality, and negatively correlated with annual precipitation during the current year and annual precipitation from the year prior to each annual home range. These findings suggest that temperature and precipitation are the primary environmental variables influencing resource availability, and thereby home range size, of Gila Monsters.

Transcriptome assembly, annotation, and comparative analysis for characterizing adaptive potential of the Streamside Salamander (*Ambystoma barbouri*)

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Transcriptome sequencing and annotation use next-generation technology to sequence transcribed regions of the genome (i.e. mRNA) and assign them biological significance using a reference genome. Transcriptome sequences and gene expression levels result from existing genetic variation and its responses to environmental conditions. Thus, populations may vary in adaptive potential due to their existing genetic variation which reflects long-term historical population size and historical selection pressures like climatic history. Therefore, comparing transcriptomes across latitudinally separated populations can produce insights into how populations have adapted to past climates which may indicate how species will respond to future

climate change. In this study, we use Streamside Salamanders (*Ambystoma barbouri*) in a common garden experiment combined with genomic technology to obtain RNA-sequencing data from 80 individuals representing 6 populations across the latitudinal gradient of the species' range and two thermal treatments (10 & 20 °C). This sequencing data will be used in differential gene expression analysis to quantify population-level variation in response to thermal conditions as well as transcriptome assembly and annotation. Our data will be used to create genomic resources for conservation purposes as well as bolster our general understanding of the underlying genetic architecture of the species which may help predict population responses to future climate conditions.

Effects of Food Availability on Energy Allocation in *Sceloporus undulatus*

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Individuals have a limited amount of energy they can spend on reproductive effort, growth, and maintenance. The amount of energy that is beneficial for an individual to invest in each of these is dependent on the amount of food available in the environment, however, food availability is subject to change seasonally and between years. My objective is to better understand energy investment in maintenance, growth, and reproduction for *Sceloporus undulatus*, the Eastern Fence Lizard, under differing food availability conditions. I collected pairs of *S. undulatus* from two populations, provided them a standard diet until they laid their first clutch and then, if they reproduced, placed them under one of two feeding treatments to simulate a good quality or poor quality environment. This design specifically considers energy allocation in the late season when reproduction is particularly costly. I measured clutch and egg size, egg composition, hatchling morphology, and adult growth and body composition via necropsy at the end of the experiment. My statistical analyses focus on achieving two major objectives: determining what factors influence early-season reproductive traits in *S. undulatus* and understanding how females invest in maintenance, growth, or reproduction based on food availability across the season. This study enhances our general understanding of life-history theory while bolstering our knowledge related to *S. undulatus*, a model species for understanding life-history trade-offs.

Monitoring herpetofauna and *Ophidiomyces ophiodiicola* in response to pine restoration in Bankhead National Forest.

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The US Forest Service is working to restore open-canopied pine forest across the southern range of William B. Bankhead National Forest through the strategic thinning/replanting of select stands and reintroduction of fire. To monitor how herpetofauna are responding to these efforts, we deployed 16 drift fence arrays with funnel traps and pitfalls across four different stand types: control, early, late, and mature. Control stands are unburned and unthinned, resulting in dense canopies and hardwood encroachment. Early replanting stands

have high sunlight penetration and pine trees <10 years old. Late replanting stands have pine trees ranging from 20–30 years old with relatively less sunlight penetration. Mature stands are characterized by open canopies and mixed age pine trees ranging from seedlings to mature trees. Early, late, and mature stands are burned on a 2-3 year rotation. Preliminary results have shown that uncommon reptiles such as *Pituophis melanoleucus*, *Plestiodon inexpectatus*, and *Ophisaurus attenuatus* are more often found in mature and early replanting stands with open canopies. Conversely, amphibians such as *Pseudotriton ruber*, *Plethodon mississippi*, and *Pseudacris brachyphona* are more common in control and late replanting stands. In addition, skin swabs were taken from each snake captured to monitor for *Ophidiomyces ophiodiicola*, a pathogenic fungus known to cause Ophidiomycosis (Snake Fungal Disease). Early results indicate a prevalence rate of approximately 9% across all stands with mature stands having the highest prevalence (12%) among all treatment types.

Population Surveys of *Ambystoma barbouri* in Middle Tennessee

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Streamside salamanders (*Ambystoma barbouri*) inhabit small first and second order streams in Tennessee, Kentucky, Ohio, Indiana, and West Virginia. In Tennessee, these streams often are misidentified as wet weather conveyances and may have little to no protection against degradation from regulated activities. Anthropogenic development poses a recognized threat to *A. barbouri* as human populations within its range continue to rise. We surveyed 47 populations of *A. barbouri* from December 2022 to April 2023 to assess the current status of *A. barbouri* within Tennessee. We discovered previously unknown populations that extended the established range of *A. barbouri* in the state. The known distribution now includes additional sites in tributaries of the Duck River in Marshall County, unnamed tributaries to West Fork Drakes Creek (Barren River watershed), Sumner County, and an unnamed tributary to Cumberland River/Cheatham Lake in Ashland City, Cheatham County. Several sites were also noted from the Western Highland Rim. Populations of *A. barbouri* were observed to occupy habitats at various states of development. We noted that some populations at disturbed sites exhibited reproductive strategy previously unreported for *A. barbouri* in Tennessee. In the absence of slab rock on bedrock habitat, females were observed utilizing unconventional substrates including grass, coarse woody debris, earthen bottomed channels, and human-made refuse. Results from this survey will be used to prioritize populations for conservation and management efforts.

Assessing the interaction of stress physiology and Bd infection in Arizona tiger salamanders (*Ambystoma mavortium nebulosum*)

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Amphibian biodiversity has greatly diminished in recent years due to panzootic pathogenic fungi *Batrachochytrium dendrobatidis* (Bd), the cause of the deadly disease chytridiomycosis (chytrid). The pathogenesis of chytrid is still

unclear, as certain species and individuals within a species are differentially affected. Susceptibility and mortality of Bd are influenced by prolonged corticosterone activity, which deleteriously affects many of the same physiological processes as Bd infections. Thus, the objective of our study is to assess the relationship of corticosterone variation and Bd spore load in Arizona tiger salamanders (ATS; *Ambystoma mavortium nebulosum*) of Colorado. In 2022 and 2023, we used a dermal swabbing method to collect baseline (resting) corticosterone from paedomorph (aquatic morph) and metamorph (terrestrial morph) ATS within three minutes of capture. Bd samples were then collected via skin swabbing. These samples will allow us to consider corticosterone and Bd spore load variation by morph, sex, location, and body condition. This study will provide a greater understanding of the pathogenesis of Bd and the interacting effects of glucocorticoid production and polyphenic life history on disease resistance. These results will develop the use of corticosterone as a predictor of Bd susceptibility and severity.