

2020 SE PARC

Poster Abstract Booklet

Abstracts are listed alphabetically by first author's last name.

Presenting author is denoted with an asterisk.

EFFECTS OF FOREST MANAGEMENT ON SNAKE FUNCTIONAL DIVERSITY.

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Accelerated human activity is a major contributing factor to global land-use change that affects the structure and function of biological diversity. For example, forest management is a form of land-use that is commonly applied to forested landscapes throughout the southeastern United States. However, knowledge of how this land-use drives community assembly is limited. Understanding the underlying mechanisms that affect assembly processes and result in novel communities is increasingly important. In addition, functional approaches can provide a better understanding of these mechanisms that drive habitat changes and species assemblages, and can be utilized to make comparisons between varied management regimes. Here we present results on the functional organization of snake communities in the context of their feeding ecology, foraging mode, and habitat use at two shortleaf pine sites under different management regimes (high-frequency [A] vs. low-frequency [B]). We captured snakes from May-July in 2018 and 2019 using box traps with drift fences, and measured 7 habitat variables at each trap. We observed differences in species richness and relative abundance between sites. Analyzing metrics of functional diversity from the community-weighted means of measured traits, we found greater functional richness, but lower functional divergence and evenness between species at site A. Furthermore, we observed that increased functional dispersion at site A was linked to greater variation in functional traits in terms of foraging mode and habitat use. Our results imply that forest management regulates the assembly of snake communities, and the lack thereof may result in the loss of rare, functionally distinct species.

NATIVE AND INVASIVE LEAF LITTER CAUSES DIFFERENTIAL BEHAVIORAL CHANGES IN A LARVAL ANURAN.

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Recent studies have demonstrated that Chinese tallow (*Triadica sebifera*) leaf litter can cause changes in behavior in anuran larvae by causing drastic changes in water chemistry. These changes are attributed to rapid leaf decomposition causing a microbial bloom, which causes a high biological oxygen demand. This reduction in oxygen impacts the behavior of tadpoles, forcing them to increase surfacing frequency to obtain aerial oxygen. It is unclear if the decomposition of native tree species leaf litter will have a similar effect on water chemistry and tadpole behavior. To test this, we compared the sub lethal effects of invasive Chinese tallow leaf litter to seven native tree species leaf litter. We exposed Bronze frog larvae (*Lithobates*

clamitans) to three concentrations (0.15g/L, 0.25g/L and 1.0g/L) of Chinese tallow leaf litter and to one concentration (1.0g/L) of 7 native tree species leaf litter and measured water chemistry and tadpole behavior. We found significant differences in water chemistry between the species of leaf litter. Tadpoles exposed to red maple, Chinese tallow and sweetgum treatments had the highest frequency of air-gulping behavior; while tadpoles exposed to loblolly pine and water oak leaf litter made the fewest trips to the water's surface. While some species of native plants leaf litter had little effect on water chemistry, other species caused drastic changes in dissolved oxygen which impacted tadpole behavior. More work needs to be done to understand the impact native and invasive plants have on water quality and their effects on wildlife.

MEDITERRANEAN HOUSE GECKOS EXPLOIT NOVEL RESOURCES IN A RECIPIENT LIZARD ASSEMBLAGE.

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The ecological niche of a species represents the environmental conditions needed for an individual to replace itself and is comprised of multiple resource axes including use of habitat, food, and time. The Mediterranean House Gecko (*Hemidactylus turcicus*) has been introduced in urban areas across Texas, yet little is known about their resource use relative to the native recipient lizard assemblages. We examined patterns of resource use of *H. turcicus* and native lizards (Green Anole [*Anolis carolinensis*], Little Brown Skink [*Scincella lateralis*], and Five-lined Skink [*Plestiodon fasciatus*]) across their habitat, dietary, and isotopic niche dimensions as well as their functional traits. We captured lizards during surveys across Stephen F. Austin State University's campus in Summer 2019. Each time a lizard was captured, we measured microhabitat variables. To quantify their dietary and isotopic niches, we conducted stomach content and stable isotope analysis. Compared to the native lizards, we found that *H. turcicus* utilized higher perches with lower temperature and relative humidity. The dietary niche breadth of *H. turcicus* was broader and exhibited low overlap with native lizards. The isotopic niche of *H. turcicus* not only encompassed the native lizards, but occupied niche space not used by the native species. It appears that the establishment of *H. turcicus* was facilitated by their ability to exploit novel resources in areas where they have been introduced. While it suggests that native lizards and *H. turcicus* are not sharing the same resources, the ecological consequences on the broader community is not known and should be investigated.

CHIGGER INFESTED AMPHIBIANS IN THE SOUTHEASTERN PARC REGION.

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Chiggers are the parasitic larval form of mites which infest all terrestrial vertebrates. In amphibians, chiggers burrow in and under the skin and have the potential of harming the infested animal through mechanical damage to skin and limbs, decreased reproductive success, or by introducing a pathogen. The two genera of chiggers that infect amphibians, *Hannemania* (an amphibian specialist) and *Eutrombicula* (a vertebrate generalist) are found in the United States,

Mexico, Central and South America. This review will concentrate on chigger infested amphibians in the Southeastern PARC region (AL, AR, FL, GA, LA, KY, MS, NC, SC, TN). Six species of chigger mite in two genera have infested 10 species in four genera and three families of anuran and 20 species in six genera and two families of caudate. All states in this region have at least one report of an infested amphibian except for KY, MS and SC. This is most likely due to underreporting of infestations rather than the absence of chiggers in those states. The most commonly reported species of chigger mite in the Southeastern PARC region is *Hannemania dumni*. In comparison, at least 10 species of chigger mite have infested 29 species in eight genera and four families of anuran and 26 species in six genera and two families of caudate across 20 of the 50 states. We join others in encouraging the study of chigger mites and the amphibians they infest to determine the risk they pose to the survival of species and populations.

CAN NATURAL HISTORY COLLECTIONS HELP ANSWER CONSERVATION QUESTIONS? A CASE STUDY IN GOPHER FROGS (*LITHOBATES CAPITO*).

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In the 1990s, a collection of two putative Gopher Frogs was made at Arnold Air Force Base (AFB) in Tullahoma, Tennessee. Morphological examination strongly suggests these are Gopher Frogs (*Lithobates capito*) of a relict population, but the closely related Crawfish Frog (*Lithobates areolatus*) is an alternative taxonomic possibility. Recent progress has been made in recovering useful genetic information from wet collections using high-throughput sequencing methods. With the presence of formalin-fixed specimens as well as comparative material from across the range of the two species, we designed a three-step method to determine the identity of this unusual population. We first demonstrate a test in formalin-fixed species of *Lithobates clamitans* and *L. sphenocephalus* for quantity and quality of DNA. We then present the results of DNA extraction from formalin- and ethanol-fixed specimens of Gopher Frog. Finally, we present the methods by which the identity of the Arnold AFB frogs will be determined, using Ultra-Conserved Elements (UCEs).

USING EDNA TO TEST WHETHER CUBAN TREE FROGS (*OSTEOPILUS SEPTENTRIONALIS*) CAN AMPLIFY THE AMPHIBIAN PATHOGEN PERKINSEA.

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While most of the disease-related declines in amphibians involve chytridiomycosis and Ranavirus, another pathogen, amphibian Perkinsea, has been documented causing mortality and die-offs in larval amphibians in the southeastern United States. Amphibian Perkinsea has been relatively understudied and how this pathogen replicates within different host species is largely unknown. One of the known hosts, the invasive Cuban tree frog species *Osteopilus septentrionalis*, can be infected with Perkinsea without exhibiting mortality or an observable decrease in fitness. Thus, we aimed to test whether *O. septentrionalis* may be serving as an

amplification host for Perkinsea, where the presence of a host species increases the overall abundance of a pathogen in a community. We collected water samples from containers housing Perkinsea-infected and uninfected animals and extracted environmental DNA. We then used an established qPCR protocol to test for Perkinsea occurrence and intensity. All samples were run three times alongside negative and positive controls, and if samples amplified two or more times, they were considered positive. No water samples collected from infected individuals were found to contain Perkinsea. However, one water sample, water collected from a container housing a control animal that had been inadvertently infected, was positive. Additionally, the intensity detected in the lone positive sample was lower than the intensity detected in the tail tissue. Based on this data, spores could not be consistently detected using eDNA despite sampling from containers housing known positive animals, thus, it appears unlikely that *O. septentrionalis* is serving as an amplification host for amphibian Perkinsea.

CAN SOUTHERN TOADS (*ANAXYRUS TERRESTRIS*) SERVE AS INTERMEDIATE HOSTS FOR THE INVASIVE PENTASTOME PARASITE *RAILLIETIELLA ORIENTALIS*?

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The exotic Asian parasite *Raillietiella orientalis* has been discovered in several snake species native to Florida. Snakes become infected with *R. orientalis* parasites when they ingest infected prey, however these intermediate hosts are currently unidentified. Knowing what species act as intermediate hosts will allow conservation efforts to target snake species likely to be affected given their diets. We dusted cricket fat bodies with pentastome eggs, collected from infected pygmy rattlesnakes' feces; we fed them to 16 toads (*Anaxyrus terrestris*), with eight toads serving as unexposed controls. Toad mass and length were measured at exposure and date of euthanization to determine if infection status affected fitness components. Thus far, nine toads have been euthanized after exposure periods ranging from 32 to 119 days. We dissected these toads using a stereomicroscope and recorded the number of parasites and their anatomical locations. Southern toads appear to be competent intermediate hosts for *R. orientalis* with 60% of exposed toads infected. Parasites were found associated with viscera in the abdominal cavity and later-stage nymphs were found embedded in thigh musculature. Currently, toad survival and growth rate have not been significantly affected by pentastome infection. Bufonid toads are abundant and widespread in North and South America and as intermediate hosts they could greatly extend the geographic range of *R. orientalis*. We have already found pentastome nymphs in wild-caught southern toads and these findings suggest that currently imperiled snake species, including *Heterodon spp.*, may face an additional conservation threat.

AN EVALUATION OF WESTERN CHICKEN TURTLE (*DEIROCHELYS RETICULARIA MIARIA*) SURVEY AND CAPTURE METHODS.

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The behaviors and activity season of the western chicken turtle (WCT; *Deirochelys reticularia miaria*) are poorly understood in Texas. Though its distribution within the state is widespread, turtle assemblage studies conducted within the WCT's Texas range have seldom documented presence. There is a lack of formal protection for the subspecies and its habitat, and past research suggests that its remaining habitat within the state is under threat from increasing urbanization. For these reasons, the U.S. Fish and Wildlife Service issued a 90-day finding that states listing the subspecies as threatened or endangered may be warranted. Here, we review species-wide capture techniques from the literature, recommend a survey season for the WCT in Texas, and evaluate the efficacy and potential biases in capture methods implemented during field studies in the state. Field studies compared road surveys, dipnet surveys, seine surveys, night wading surveys, and two types of unbaited fyke net traps. The effectiveness of baited traps in prior studies was regionally inconsistent. Fyke nets were effective in every study that deployed them, and captured WCTs in Texas at a rate of 0.25 captures per trap night. In Texas, aquatic trap success rates were highest during May. Dipnet surveys had the highest capture rate among active survey methods, but body size biases between methods were apparent. Best survey method selection will vary depending on research questions, budget, and time constraints. Utilizing proper survey protocols and understanding the activity season are crucial for performing effective studies on this species.

USING ECOLOGICAL NICHE MODELING TO DIRECT SURVEY EFFORTS FOR CRAWFISH FROGS (*LITHOBATES AREOLATUS*) IN LOUISIANA.

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One-third of amphibian species globally are experiencing population declines due to habitat loss and alteration, environmental contaminants, disease, introduced species, exploitation, and climate change. The crawfish frog has disappeared throughout much of its historic range primarily due to conversion of its habitat to agriculture. Most Louisiana records are from prior to the 1970s, and more recently the species has been documented at just one location in the extreme northwestern part of the state. In spring 2019, we conducted nighttime call surveys along roads near historic crawfish frog locations in Louisiana and in areas where potentially suitable habitat remains. Despite considerable effort (34 survey nights), we encountered no crawfish frogs. To confirm that our survey routes are in suitable areas for crawfish frogs and to identify other suitable areas for future surveys, we generated a distribution model using locality information from 1990–present from Louisiana, Texas, and Oklahoma, and 12 bioclimatic variables related to temperature and precipitation from the WorldClim database. Our model indicated that marginally suitable conditions for crawfish frogs exist in parts of northern Louisiana, especially the northwestern portion of the state. Perhaps most promising, the model predicted the best conditions occur in a small area that includes a unit of the Red River National Wildlife Refuge where there were unconfirmed reports of calling crawfish frogs in 2008. Future work will

incorporate additional environmental data on land cover, soil, and hydrology, and our final distribution model will guide call survey efforts for crawfish frogs across Louisiana in 2020.

THERMALLY MEDIATED REFUGE-SITE SELECTION BY GILA MONSTERS (*HELODERMA SUSPECTUM*).

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Refuge selection is an important topic in desert ectotherms as subterranean shelters are thought to be chosen based on appropriate structural and thermal properties that allow individuals to escape potentially lethal above-ground temperatures. We assessed the structural and thermal properties of shelters used by Gila monsters in southwestern New Mexico and compared attributes of shelters that could be potentially used for refuge to control shelters that appeared structurally similar but were not observed to be used as refugia. Mean temperatures between actual and potential refuge sites did not differ, but our analyses suggest that sites that were actually used (chosen by Gila monsters) have daily thermal regimes that allow increased opportunities for thermoregulation within the preferred body temperature range.

EVALUATING THE SUITABILITY OF CONFISCATED BOX TURTLES FOR RELEASE INTO THE WILD.

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Turtle populations in the southeastern United States are becoming more vulnerable due to the illegal wildlife trade. Poaching has had a negative impact on the native populations of both aquatic and terrestrial turtles, including the eastern box turtle (*Terrapene carolina carolina*). Even when illegally collected turtles are seized by law enforcement, often they are not returned to the wild either because their locality is unknown or due to disease concerns. However, as illegal collection increases in frequency, and adult turtles are targeted, there is an increasing need to determine whether and under what circumstances seized turtles can be returned to the wild. In collaboration with SCDNR and USFS-Savannah River, SREL is conducting a soft release of 200+ eastern box turtles seized by law enforcement and then translocated to a protected area on the Savannah River Site, where they now reside in a 2.5-acre soft release pen within a mature hardwood forest habitat. We collected extensive health data and samples for potential genetic analysis prior to release. For monitoring purposes, each individual is PIT tagged, 20 have i-buttons fixed to their carapace for collecting temperature data, and 30 animals will be fitted with a transmitter to track their movements post hibernation. Our project's purpose is to monitor dispersal, survivorship, and the behavior of the translocated eastern box turtles, examining the suitability for their release into the wild. We hope this project will help guide decisions regarding future confiscations of illegally collected turtles and how they can contribute to species conservation efforts.

MOVEMENTS AND SURVIVORSHIP OF TRANSLOCATED AMERICAN CROCODILES (*CROCODYLUS ACUTUS*).

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The American crocodile (*Crocodylus acutus*) is one of only two native crocodylians in Florida and is federally listed as threatened. Crocodile and human populations in Florida have increased since the 1970s, particularly over the last 15 years. As a result, human-crocodile encounters have increased. To address these conflicts, the Florida Fish and Wildlife Conservation Commission (FWC) implemented the American Crocodile-Human Interaction Response Plan that allows for the translocation of crocodiles in some situations. However, the effectiveness of translocations has been questioned because some crocodiles return to their original capture site, requiring additional translocations. The fate of translocated crocodiles that do not return is often not known. Managers want to know whether translocation is an effective means of managing human/crocodile conflicts and whether it exposes crocodiles to factors that increase mortality. This study is designed to use satellite/GPS telemetry to compare the movements and survival of translocated crocodiles to free-ranging wild crocodiles. To determine the effects of translocation on movement and behavior, we compared habitat use, daily movements, and long-term movements between translocated animals and non-translocated reference crocodiles. Information gathered from this study will be used by managers to evaluate and develop management strategies for mitigating human/crocodile conflicts in Florida.

CONSULTING FOR CONSERVATION.

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Biologists in the environmental consulting field frequently encounter unique reptile and amphibian conservation opportunities. While working within the framework of individual development projects including natural gas and transmission rights-of-way, dam removals, roads, bridge replacements, and other remediation projects, consultants gain access to private land, funds, and temporal scales potentially unavailable to traditional academic researchers. Collaboration with state and federal agencies provides data and a bridge for building partnerships with private landowners. Projects sometimes require, or project proponents authorize, monitoring during active construction, providing consultants a rare opportunity to inform and educate construction personnel. Construction workers and landowners are afforded firsthand observations of conservation science in action, and in some cases, participate in conservation activities. Such collaborations connect working scientists with individuals and professionals not formally trained in wildlife conservation biology. Recent projects in OH, PA, WV, VA, NC, and AL involving habitat assessments, presence/probable-absence surveys, and construction monitoring afforded Environmental Solutions & Innovations, Inc. (ESI) access to significant herpetological records and valuable data. Studies focused on Cheat Mountain Salamander (*Plethodon nettingi*), Timber Rattlesnake (*Crotalus horridus*), Eastern Tiger Salamander (*Ambystoma tigrinum*), Eastern Hellbender (*Cryptobranchus allegheniensis*), Neuse

River Waterdog (*Necturus lewisi*), Gopher Tortoise (*Gopherus polyphemus*), and Eastern Spadefoot Toad (*Scaphiopus holbrookii*).

HERPETOFAUNAL COMMUNITY SPECIES REORDERING TEMPORALLY DECAYS IN A TROPICAL CLOUD FOREST.

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Succession history is often a determinant of ecosystem complexity and, consequently, community composition and structure. This is especially true in structurally complex tropical forests. Specifically, the community composition of a secondary succession tropical forest is expected to differ, perhaps substantially, from that of an old growth or primary succession forest. Recent studies suggest that species reorderings (or shifts in rank abundance) are more common than absolute reductions in species richness in response to disturbance. Therefore, communities whose forested landscapes are distinguished only by differing succession histories should differ primarily based on species rank abundances. This assumption was investigated in a 6-month herpetofaunal community assessment at Nectandra Cloud Forest Reserve, where three regions of forest with distinct succession histories were systematically sampled. While species abundances of Nectandra old growth and secondary forests were similar, considerable species reordering was observed in a third, more recently disturbed forest. The finding of a near-identical herpetofaunal community composition between old growth and secondary forest sites suggests that, while tropical forest succession may dramatically restructure communities, this signal likely decays with time. Additionally, the influence of temporal change within each community's sampling period will be discussed.

WINTER IS COMING: TEMPERATURE DEPENDENT VIRULENCE OF BATRACHOCHYTRIUM SALAMANDRIVORANS.

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Batrachochytrium salamandrivorans (*Bsal*) is an emergent fungal pathogen that has caused population declines of European fire salamanders (*Salamandra salamandra*). Risk analyses suggest temperature will be an important factor driving *Bsal* invasion in the United States. We exposed adult and eft stage eastern newts (*Notophthalmus viridescens*) to one of four *Bsal* zoospore doses (5×10^3 - 6) at one of three ambient temperatures, 6°C, 14°C or 22°C. At 14°C, all adult newts at the three highest exposure doses became infected and died within one-month post-exposure. No adult newts became infected or experienced mortality at 22 C. Median survival durations at 14°C for adults were 26, 20, 14 and 7 days for 5×10^3 , 5×10^4 , 5×10^5 and

5x10⁶ exposed animals respectively. Adults exposed at 6°C lived 2 times longer than those exposed at 14°C. Efts exposed at 6 and 14°C lived 1.4 times longer than adults challenged with the same *Bsal* doses. Adult newts that died from chytridiomycosis at 6°C had lower zoospore loads than at 14°C, suggesting lower infection tolerance at lower temperature. Our results suggest that *Bsal* invasion in the United States may be geographically narrower than indicated in previous risk models, with greatest likelihood of emergence occurring at mid- to northern latitudes and higher elevations. Seasonal fluctuations in disease emergence also are expected, with autumn and winter months experiencing greatest mortality. Differences in infection tolerance observed between adult and eft life-stages suggest the pathogen may influence population demographics.

PLETHODON DORSALIS (COMPLEX): MICROHABITAT ANALYSIS.

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Understudied, high biomass animals such as the Northern Zigzag salamander (*Plethodon dorsalis*) are known contributors to essential nutrient cycles within their respective habitats. Knowing that these sensitive creatures serve as bioindicators for the overall health and quality of many ecosystems; we should aim to comprehend their habitat requirements. Doing so could ultimately allow conservationists to set more accurate baselines for various mitigation and land management practices. Due to the location within the southeast amphibian biodiversity hot spot sampling of *P. dorsalis* was conducted in the Tennessee River Gorge (TRG). Three 2,000 m² sites were selected based on presence of target species and a GIS processed land cover dataset in an attempt to best represent the diverse landscape of select slopes within the 27,000 acre TRG. Specimens were located from October 25, 2018 through July 8, 2019 by using a time based natural cover object survey method. Microhabitat was analyzed within one square meter of each location containing a new *P. dorsalis* specimen along an elevation gradient (250-300 m, 300-350 m, 350-400 m). For each animal-present location, a randomly selected animal-absent plot was analyzed. This assessment attempts to identify differences in microhabitat preference based on selected versus available habitat using predictive geospatial models using AICc values. The results suggest that different factors influence the distribution of *P. dorsalis* with respect to microhabitat selection.

DISTRIBUTION, ABUNDANCE, AND CONSERVATION STATUS OF TWO GENETICALLY DISTINCT LINEAGES OF GREEN SALAMANDER (*AENEIDES AENEUS*) IN TENNESSEE.

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The distribution and ecology of the Green Salamander (*Aneides aeneus*) in Tennessee has not been well-studied compared to other states. We sought to determine the status of historical populations and locate new populations within the suspected distribution of the species in Tennessee. We conducted over 120 surveys in suitable habitat throughout 27 counties in eastern Tennessee from October 2017 to November 2019. Surveys included both historical sites and sites with suitable habitat but no previous records of Green Salamanders. A total of 320 Green Salamanders were documented at 50 sites during 68 of the surveys, including 16 historical and 34 new sites. All but three of our observations occurred on sandstone or limestone rock outcrops or large boulders, typically in well-shaded, forested gorges and ravines. Including the results of the current study, we compiled a list of 160 georeferenced sites from several sources. Population genetic and phylogenetic approaches strongly support recognition of two lineages that have largely parapatric distributions. The southern lineage includes populations from the southern Highland Rim and Cumberland Plateau of northeastern Alabama, south-central Tennessee, Mississippi, and northwestern Georgia. The northern lineage includes populations from the Appalachian Plateau and Valley and Ridge ranging from Pennsylvania and Ohio southwestward through the Virginias and eastern Kentucky into north-central Tennessee. We evaluated the conservation status of the southern lineage as S3 (Vulnerable) and the northern lineage as S4 (Apparently Secure) based on NatureServe criteria.

RELATIONSHIP BETWEEN A REPRODUCTIVE POLYMORPHISM IN THE SPOTTED SALAMANDER AND THEIR UNICELLULAR ALGAL SYMBIONT.

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The spotted salamander, *Ambystoma maculatum*, exhibits a unique reproductive polymorphism wherein some females lay clear egg masses while others lay opaque masses. *A. maculatum* engages in a symbiosis with a unicellular algae, *Oophilia amblystomatis*, that lives intracellularly within the egg envelopes of *A. maculatum* and provides embryos with supplemental oxygen via photosynthesis. Clear and opaque egg masses often co-occur in breeding ponds, but proportions of egg mass types vary spatially. Our previous work shows that predators preferentially consume clear egg masses so it is perplexing that they persist. Clear jelly may be an adaptation to low dissolved oxygen environments, possibly by allowing for higher light transmittance and thus increased photosynthesis by *O. amblystomatis*. Here, we investigate the relationship between jelly polymorphism, algae symbiosis, and dissolved oxygen through both observational and experimental designs. We sampled *A. maculatum* breeding sites for egg mass proportions, predators, and water physicochemicals. Additionally, we raised clear and opaque egg masses under two different light treatments, sampled embryonic respiration at two developmental stages, and quantified algal growth. Our study elucidates some heretofore unknown differences between the two mass types, such as differential algal growth, clutch sizes, and hatchling viability. Finally, our data suggest that dissolved oxygen may be one selective pressure responsible for maintaining this polymorphism.

CREATED EPHEMERAL WETLANDS AS HABITAT FOR AMPHIBIAN POPULATIONS IN WESTERN KENTUCKY, USA.

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Loss of wetlands worldwide has necessitated the creation of wetlands. Yet, the physical attributes and community composition of created wetlands often differ compared to natural wetlands. We surveyed three types of created wetland [managed open canopy (MOC), unmanaged open canopy (UMOC), and unmanaged closed canopy (UMCC)] in western Kentucky to estimate amphibian richness and occupancy among wetlands, and estimated abundance of three common species: Southern Leopard Frog (*Lithobates sphenoccephalus*), Spotted Salamander (*Ambystoma maculatum*), and Crawfish Frog (*L. areolatus*). In addition, we quantified variability in physical characteristics and water quality parameters among wetland types. MOC wetlands had a greater percent of submergent vegetation than both UMCC and UMOC wetlands, shallower depth at 1.0 m from the wetted wetland edge than UMOC wetlands, and larger wetland surface area than UMCC wetlands; water quality values were highest at UMCC wetlands. Mean predicted amphibian species richness and occupancy were highest at larger wetlands and detection probability between May and July was highest in July. Occupancy of the three common species was not influenced by wetland management type and varied little among species. Estimated abundance of *L. areolatus* was higher at MOC wetlands, and conversely, *Am. maculatum* abundance was highest at UMCC wetlands. Wetlands with greater surface area had higher estimated abundances of *L. areolatus* and *L. sphenoccephalus*. Our results suggest creating large, open canopy managed wetlands as tools for bolstering amphibian richness and abundance.

FLORIDA SCRUB LIZARD REINTRODUCTION INTO PALM BEACH COUNTY.

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In the past 30 years, the range of the Florida scrub lizard (*Sceloporus woodi*), which has been petitioned for federal listing, has contracted 77 km northward along southeastern Florida's Atlantic coast. In February–March 2019, we experimentally reintroduced 50 male and 50 female lizards to Hypoluxo Scrub Natural Area in central Palm Beach County, 37 km south of the species' current distribution. The donor sites were two state parks with robust populations located 51 and 62 km away in Martin County. Lizards were released on the day of capture after taking measurements and genetic samples. Despite county-owned Hypoluxo Scrub containing 24 ha of suitable scrub habitat, the species disappeared from here ca. 2005, possibly because of feral cat predation. The population will be monitored via pedestrian surveys at least every other month for 2 years, at which time complete turnover of the population should have occurred. Genetic samples will then be collected and possibly analyzed for genetic diversity and relatedness to determine if population augmentation is warranted. The first hatchlings were detected on 12

June, and surveys detected a low of 15 adults in April and 31 adults and 23 juveniles in September. The population has dispersed slightly from the release area, which contained the most optimal habitat. The public was informed of the reintroduction via a flyer posted at Hypoluxo Scrub and a video that reached over 128,000 people by the end of June.

TURTLES ALL THE WAY DOWN: GLOBAL CONSERVATION PRIORITIES AT THE SUB-BASIN LEVEL.

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Turtles are a highly imperiled group of vertebrates with nearly 60% of species threatened with extinction. In order to effectively conserve turtle species, conservation biologists often employ a variety of decision-making tools. One such approach identifies and prioritizes spatial units based on their biodiversity and imperilment metrics, thus focusing conservation resources to areas of need. Prioritization schemes for determining global priority areas for turtles have largely focused on regional scales and not at the sub-basin level. Here, we conduct the first global prioritization at a sub-basin level using several biodiversity and imperilment metrics and compare our results to findings that are already available at larger spatial scales. We used bivariate maps and local indicator of spatial association to characterize spatial patterns and prioritize sub-basins as high, intermediate, and low priority. Most high-priority sub-basins were in lowlands and along coastlines in tropical and subtropical regions and commonly found in previously recognized turtle hotspots and turtle regions. For example, we identified high-priority sub-basins within 10 of the 16 original turtle hotspots, and these sub-basin scale results now focus conservationists in on highest priority areas in each of those hotspots or regions. Yet, our analyses identified a new area, southwest India, with global significance for turtle conservation. Our results support the well-known *Asian Turtle Crisis* because most of our high-priority sub-basins were in the Indomalayan Realm. The Southeastern United States consisted largely of intermediate-priority sub-basins but had two high-priority sub-basins in coastal Louisiana due to high species and endemic richness and threat of sea level rise. Conservation action for turtles is warranted in all biogeographical realms.

MONITORING STRATEGIES FOR REPATRIATED EASTERN INDIGO SNAKES IN SOUTHERN ALABAMA.

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The Eastern Indigo Snake (*Drymarchon couperi*) was extirpated from Alabama in the 1950s and was declared threatened throughout its range under the Endangered Species Act in 1978. In 2010, a repatriation program began in Conecuh National Forest, and now that ten years have passed since the inception of the program, monitoring of this population is crucial. Previous

projects in CNF involved radio tracking indigo snakes after release and looking at release methods, home range, and habitat selection. However, since 2014, monitoring has been restricted in temporal scope and sampling methods. Monitoring of a population after reintroduction is crucial in determining population size, survival rates, reproduction and the success of the reintroduction effort. We are evaluating five different monitoring methods: the previous radio tracking data, intensive pedestrian surveys, camera trapping, remote RFID readers and passive box traps. Our objectives are to evaluate multiple monitoring methods with respect to the value of information gathered and monitoring costs. In 2019, increased pedestrian surveys and box traps successfully yielded an increased magnitude of encounters and captures of released snakes. In 2020 camera traps recorded indigo snakes at multiple gopher tortoise burrows, however individual IDs could not be made. We will deploy remote RFID PIT tag readers by placing antennas around tortoise burrows in 2020 and 2021. This will allow for identification of all PIT- tagged individuals using a burrow, when, and how often. These five methods all show potential for success, but have different costs and quality of gathered information.

SWABS AS A NONLETHAL ALTERNATIVE FOR DETECTION OF THE PROTISTAN PATHOGEN PERKINSEA IN ANURANS.

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Amphibians are declining faster than any other vertebrate group and disease is a significant factor in these declines. In the United States, the protist, *Perkinsea* is responsible for several mass mortality events and may be linked to population declines in some species. Current techniques to detect *Perkinsea* in anurans rely on tissue collection for histology or qPCR analysis. This often requires the euthanasia of individual anurans or toe/tail clipping. Skin swabs have been used to non-destructively sample for other amphibian pathogens for over a decade and may serve as a reliable alternative to tissue collection. We aimed to determine the reliability of swabs to detect *Perkinsea*. We sampled 94 frogs from the Archbold Biological Station in Florida, USA, collecting swabs and a variety of tissues (toe/tail, mouth, liver, and intestines) from all individuals to compare detection capability for *Perkinsea*. We then quantified the pathogen load in these samples using a *Perkinsea*- specific qPCR assay and compared the swab and tissue results. There was a significant decrease in detection for swabs compared to toe/tail tissue ($p = 0.006$). When comparing all tissue types, *Perkinsea* was significantly less likely to be detected in intestinal samples ($p < 0.0001$). Additionally, liver samples had a significantly higher intensity compared to all other tissue types ($F = 6.149$, $P = 0.002$). While less reliable than toe/tail tissue, swabs are a viable method to nondestructively detect *Perkinsea* and better understand its impacts on frog populations.

MULTISTATE WETLANDS ECOLOGICAL RESTORATION PROJECT: FLORIDA & GEORGIA.

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Geographically isolated wetland habitats are identified as priorities for conservation action in the State Wildlife Action Plans of Florida and Georgia and are home to 73 wildlife species identified as Species of Greatest Conservation Need. Despite their importance for wildlife, isolated wetlands throughout the Southeast are overgrown and hydrologically altered due to fire exclusion and incompatible fire regimes, greatly reducing their ability to support native wildlife. As part of a multistate wetlands ecological restoration project aimed to address these concerns, we will restore a minimum of 45 wetlands across the Florida panhandle and southern Georgia using prescribed fire and a variety of shrub and tree removal methods. The wetland vegetation response and the adult and larval occupancy of five species of rare and imperiled amphibians will be monitored at restoration sites pre- and post-treatment to determine effectiveness. In Florida, we will complete a study comparing the amphibian and plant response to light and heavy mechanical wetland restoration techniques to guide future restoration efforts.

PARAPHYLY, HOMOLOGOUS STRUCTURE, AND SHARED PHENOTYPES OBFUSCATE IDENTIFICATION OF SIREN SPECIES.

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The family Sirenidae comprises two genera, *Siren* (Linnaeus 1766) and *Pseudobranchius* (Gray 1825). All extant members of Sirenidae are completely aquatic, eel-like, and have large fimbriate external gills and only front limbs. Despite sirens having distinctive morphology for a salamander and often abundant populations relatively little research has been conducted on their natural history and phylogeny. We collected Sirenids from most drainages in Florida and specimens from type localities not in FL, sequenced several mitochondrial and nuclear loci for each specimen, photographed live specimens, and collected morphological data standard for salamanders (linear measurements and costal groove counts). We found that most *Siren* are indistinguishable from each with linear measurements, many biogeographic regions contain a unique mitochondrial clades for *P. cf. striatus* and *S. cf. intermedia*, and that next generation sequencing will be required to have enough informative loci to determine whether *S. cf. intermedia* mtDNA clades warrant taxonomic recognition. We also found that non-sister species would share color and pattern phenotypes and that some animals would shift color and expression intensity of black spotting during time in captivity. Despite these confusing factors, we found that every species of *Siren* in drainages east of Mobile Bay has a distinct range for costal groove counts that does not overlap with any other species native to the same drainage, thus, combining costal groove counts and geography allows one to identify the species.

APPLICATION OF DNA METABARCODING TO CHARACTERIZE AMPHIBIAN DIETS FROM NON-INVASIVE FECAL SAMPLES.

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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 current project, an effort to refine and apply these methods to study competition among sympatric dusky salamanders (*Desmognathus*). Over the course of our current research project, we hope to evaluate and refine our methodology and prove the utility of a versatile tool with which amphibian diets can be characterized and ecological questions can be investigated.

***GEOEMYDA SPENGLERI*: UNRAVELLING THE MYSTERIES OF THE ELUSIVE YIN-YANG TURTLE THROUGH INTERNATIONAL COLLABORATIONS.**

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The Asian turtle crisis has decimated wild populations of many Asian turtle species due to high levels of collection for the illegal wildlife trade. The Black-breasted Leaf Turtle (*Geoemyda spengleri*), is no exception as it has been collected heavily from the wild for 40+ years to fill the demand of the international pet trade. While we have learned a great deal about certain characteristics of *G. spengleri* from captive individuals, we still know very little about their natural ecology and life history. In 2015 collaborations were established to begin research on the trade and ecology of *G. spengleri* throughout its range. Village and field surveys have been ongoing in Vietnam, Guangdong, Guangxi and Hainan. These surveys have resulted in finding wild individuals in Vietnam, Guangdong and Hainan. Village surveys revealed that a majority of populations in historical locations have been severely decimated or even extirpated. In addition to surveys, ecological data on canopy cover, ground cover, temperature, and UV intensity have been collected. The ultimate goal of this research is to assist in the conservation and persistence of this species in both captivity and the wild.

RANAVIRUS AND PERKINSEA INFECTION DYNAMICS VARY WITH HOST CHARACTERISTICS AMONG INVASIVE CUBAN TREE FROGS IN LOUISIANA, USA.

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Invasive species can negatively impact ecosystems in numerous ways, including vectoring and amplifying pathogenic organisms, some of which can contribute to anuran mass-die offs in native species. The Cuban tree frog (*Osteopilus septentrionalis*) is invasive to the southeastern USA and may serve as a reservoir host for the fungus *Batrachochytrium dendrobatidis* (Bd), the iridovirus *Ranavirus* (Rv), and the protist *Perkinsea* (Pr). To characterize pathogen dynamics in invasive Cuban tree frogs, we sampled 82 adult and larval individuals from two recently invaded areas in New Orleans, Louisiana, USA. We used established quantitative PCR protocols to assess prevalence and intensity of Pr, Bd, and Rv from intestinal, mouthparts (tadpoles only) and toe/tail tissues of all sampled individuals. We then compared these disease metrics against across host characteristics, including Fulton's body condition (K), sex, and life stage. We found 45% of individuals were infected with Pr, 60% with Rv, and none were infected with Bd. Body condition did not differ significantly based on Rv infection status, but individuals infected with Pr had significantly lower body condition. Males were more likely to be infected with Pr, and tadpoles were more likely to be infected with Rv. We also found that 28% of individuals were co-infected across both study sites with Pr and Rv, but co-infection did not significantly affect the prevalence intensity of either Pr or Rv. This study establishes that Cuban tree frogs in the invasion range in New Orleans are infected with two major pathogens of concern for anurans, and that Pr infections impose sub-lethal fitness costs to the invasive host.

LONG-TERM ANURAN OCCUPANCY DYNAMICS IN THE ATCHAFALAYA RIVER BASIN OF LOUISIANA.

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The Atchafalaya River Basin in southern Louisiana is the largest contiguous tract of river bottomland remaining in North America. The U.S. Geological Survey monitored anurans in the Atchafalaya for the years 2002–2016. Monitoring from 2002–2006 involved vocalization surveys conducted on rainy nights at 40 sites. Surveys were suspended in 2007 and from 2008–2016 monitoring involved both visual encounter and vocalization surveys. All but six of the 40 original sites were surveyed from 2008–2016, and an additional 30 sites were added from 2009–2013 that varied significantly in hydrology from the original sites. This long-term dataset provides an excellent opportunity to examine the occupancy dynamics of the anuran species found in this area. Most species were found to decline in site occupancy during the period 2002–2006 when only vocalization surveys were employed. However, visual encounter data from surveys from 2008–2016 showed annual variation, but relatively stable occupancy throughout the study. Pronounced differences in occupancy were observed between the original sites and the 30 sites added from 2009–2013. Our study demonstrates the value of capture data in estimating occupancy and detecting a trend relative to vocalization surveys alone. Anurans call for specific

purposes and calling is influenced by environmental conditions. Therefore, it is difficult to account for all causes of heterogeneity in detection probability using occupancy analysis based solely on vocalization surveys. Combining visual and vocal detections provides a dataset that is more reliable for determining true occupancy dynamics with increased power to detect trends.

SERUM-BASED INHIBITION OF PITVIPER VENOM BY EASTERN INDIGO SNAKES.

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When organisms possess chemical defenses, their predators may eventually evolve resistance to their toxins. Eastern Indigo Snakes (*Drymarchon couperi*; EIS) subdue and consume a variety of pitviper species and it has been suggested EIS possess a physiological resistance to their venom. In this study, we formally investigated this hypothesis by using microassays that measured the ability of EIS blood sera to inhibit A) hemolytic and B) snake venom metalloproteinase (SVMP) activity of Copperhead (*Agkistrodon contortrix*) venom. To serve as controls, we also tested the inhibitory ability of sera from inbred House Mice (*Mus musculus*) and from a snake that does not feed on pitvipers, the Checkered Gartersnake (*Thamnophis marcianus*). As expected, mouse sera exhibited little effect on the activity of either class of toxins tested. However, sera from both EIS and gartersnakes inhibited over 60% of SVMP activity. EIS sera also inhibited 78% of venom hemolytic activity, while gartersnake sera failed to inhibit these toxins. Our results demonstrate that EIS serum is indeed capable of inhibiting two of the primary classes of toxins found in Copperhead venom, suggesting that EIS may possess physiological resistance to venom upon injection. Because we documented resistance to hemolytic components of pitviper venom within EIS but not gartersnakes, we speculate this resistance may be driven by antagonistic interaction while resistance to SVMP may be relatively widespread among snakes and not necessarily relate to the diet and ecology of extant species.

DETECTABILITY OF EASTERN BOX TURTLES: IMPLICATIONS FOR REPTILE DISEASE DETECTION AND SURVEILLANCE.

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The ability to estimate population abundance or survey populations for disease is heavily dependent on estimating the detection of available animals during intentional surveillance efforts or through opportunistic encounters. For reptiles and amphibians, the likelihood of detecting individuals is low due to their small sizes, cryptic behavior, and ability to camouflage naturally. We examined the detectability of the Eastern Box Turtle (*Terrapene carolina carolina*) using distance to line sampling of 3-dimensional printed models along a 1 km forest transect. Each model was situated along the transect using a stratified random design of distance from the transect and whether the model was fully visible, partially covered, or fully covered. We included surveyor self-reported level of experience sampling for wildlife, reptiles, and box turtles

as additional covariates. Detection of box turtles was highest directly on the transect but was not perfect and declined dramatically more than 1 meter from the transect. Whether the model was partially or fully covered by leaf litter also significantly reduced detection. Detection was not different among individuals who self-reported as “professional”, “professional in training”, “amateur”, or “novice”. Our results demonstrate that detection of box turtles during deliberate surveys is generally poor and not related to observer experience, suggesting that effective detection of animals for population studies or disease surveillance will be challenging.

ADDRESSING EROSION PROBLEMS IN WETLANDS: THREE CASE STUDIES OF RESTORATION IN BOG TURTLE HABITAT.

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The bog turtle (*Glyptemys muhlenbergii*) is a federally protected species occupying spring-fed wetlands in portions of the eastern United States. One of the bog turtle’s primary threats is habitat loss from draining, ditching, and ponding for agriculture, land conversion for development, and through vegetative succession. Therefore, it is imperative for this species’ success that wildlife managers maintain the habitat that still exists. Future climate change could make this task increasingly difficult. Extreme precipitation events are increasing in frequency and intensity in the Southeast, and as a result, soil erosion is also predicted to increase. Thus, habitat managers will likely have to increasingly deal with erosion-related threats to wetland habitat in the future. We present three case studies of successful restoration projects to resolve erosion-related issues at bog turtle wetlands in North Carolina. When restoration is needed, managers must balance the risk of habitat loss from erosion with the direct threat from heavy machinery often used during these projects, so we provide lessons learned and recommendations for maximizing the positive outcome.

DISPERSAL AND GENETIC CONNECTIVITY OF AMPHIBIAN POPULATIONS IN AN ALTERED LANDSCAPE.

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Movement between habitat patches shapes the genetics, demography, and persistence of populations. However, dispersal in fragmented, multi-habitat networks is poorly understood. The Central Appalachians represents a global hotspot for salamander diversity; however, mountaintop removal mining (MTR) and valley filling (VF) is ubiquitous in the region. Previous research has shown that salamander occupancy, colonization rates, persistence rates, and species richness are reduced in streams affected by MTR/VF. For example, Price et al. 2018 observed a 47%, 59%, and 32% reduction in occupancy rate, colonization rate, and persistence rate for *Desmognathus fuscus* adults in MTR/VF streams compared to reference streams. Specifically, reduced colonization in a fragmented landscape strongly implicates inhibited dispersal. For my proposed project, we plan on combining demographic and genetic measures through capture

mark recapture (CMR) and next generation sequencing techniques to elucidate the patterns of dispersal and genetic connectivity within altered landscapes. Specifically, we will evaluate the dispersal probabilities of each species and life-stage of salamander within and across first-order streams, the heterozygosity and genetic differentiation across different hierarchical scales, and gene flow through demographic inference modeling. We predict that overland and upstream dispersal will be reduced in MTR/VF sites, which will result in decreased heterozygosity, increased genetic differentiation, and reduced gene flow. If our predictions are supported, the persistence and stability of stream salamander populations are indeed declining as a result of disrupted dispersal and increases in local extinction rates of subpopulations may destabilize metapopulation connectivity throughout the Appalachian landscape.

THE TURTLE COMMUNITY OF THE NORTH LOUISIANA REFUGES COMPLEX.

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The southeastern United States is an area of high species richness for freshwater turtles, and several of these species are of conservation concern. Documenting the assemblage and habitat preferences of the turtle communities in this region is fundamental to their conservation. Over the course of 2018 and 2019, we deployed traps for turtles at 23 different sites across the four national wildlife refuges that comprise the North Louisiana Refuges Complex, totaling 1,342 trap-nights. Trapping was conducted across a broad range of ecotypes in the Mississippi alluvial plain, from cypress-tupelo swamps to an anthropogenically restored bayou channel. In addition, basking surveys were performed at 5 sites across 3 of the refuges. Through this combination of surveys, we confirmed the presence of 11 turtle species, including 3 classified as state and/or federal species of concern. Five hundred seventy-two total turtles were caught in traps, giving a total catch-per-unit-effort (CPUE) of 0.5648 turtles/trap-night. Red-eared sliders (*Trachemys scripta elegans*) were the most common turtle caught, with a total of 572 individuals and a CPUE of 0.4262, followed by common musk turtles (*Sternotherus odoratus*) with 49 captures and a CPUE of 0.0365. The other species caught in traps were, in order of abundance: Mississippi mud turtles (*Kinosternon subrubrum hippocrepsis*), alligator snapping turtles (*Macrochelys temminckii*), southern painted turtles (*Chrysemys dorsalis*), common snapping turtles (*Chelydra serpentina*), razorback musk turtles (*S. carinatus*), spiny softshells (*Apalone spinifera*), eastern river cooters (*Pseudemys concinna concinna*), and a Mississippi map turtle (*G. pseudogeographica kohnii*). One additional species, the Ouachita map turtle (*G. ouachitensis*), was observed through basking surveys. Three-toed box turtles (*Terrapene carolina triunguis*) were observed at two of the refuges through incidental encounters.

CHANGING PERCEPTIONS OF WILDLIFE: HOW COLLABORATION CAN PROTECT GOPHER TORTOISES (*GOPHERUS POLYPHEMUS*) WHILE MAINTAINING INDUSTRIAL PROGRESS.

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Often an uneasy topic for wildlife advocates is how we protect wildlife while, at the same time, supporting beneficial industries. Through strategic planning and key partnerships, ecologists and industry can come to an agreement on clashing goals: protecting wildlife amidst advancing industry progress. Initiated in 2015, the University of Georgia Applied Wildlife Conservation Lab has maintained a positive collaboration with Southern Ionics Minerals, a subsidiary of Chemours, to protect wildlife, focusing mainly on gopher tortoises (*Gopherus polyphemus*), at heavy mineral sand mines in South Georgia. Collectively, we maintain a multifaceted approach to protect gopher tortoises and other wildlife from mining activities through on-the-ground wildlife impact mitigation, ecological research, and educating mine personnel. This collaboration has supported multiple research projects that study the ecology, reproduction and health of relocated gopher tortoises from the mineral mines. Through the relocation and headstarting of gopher tortoises, we have contributed to augmenting minimal viable populations of gopher tortoises on Georgia Department of Natural Resources Wildlife Management Areas. Our critical and challenging balance is avoiding local extirpation of gopher tortoises from habitats surrounding the mines while accommodating industrial activities that are vital to resources used commonly in our society. These efforts have been made possible by proactively maintaining open communication between miners, wildlife professionals, and natural resource agencies. Here, we present some of the challenges and successes we have faced throughout this collaboration and the ways that gopher tortoises have benefited as a result.

ECOLOGY OF UPLAND SNAKE COMMUNITIES IN MANAGED MONTANE LONGLEAF PINE HABITATS OF GEORGIA.

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Longleaf pine ecosystem decline in the Southeast United States has led to intensive land management implementation with the goal to benefit both the ecosystem and at-risk species. Addressing at-risk snake populations in these longleaf pine ecosystems, for instance, requires understanding both community and species level ecology of snakes in these managed forests. Data for snakes in the montane (mountain) longleaf pine habitats remains unclear since management practice implementation. Currently, intensive restoration of montane longleaf pine habitats is taking place within two Wildlife Management Areas (WMA) in the Raccoon Creek Watershed of Northwest Georgia, Sheffield and Paulding Forest. These areas differ in both historic forest management and intensity of restoration for longleaf pine habitats. To survey these areas for snake diversity and abundance, we used drift fence trap arrays at six locations within the two WMAs, yielding a total of 85 captures representing nine species, including the five most frequently trapped species: Black racers (*Coluber constrictor*), copperheads (*Agkistrodon contortrix*), corn snakes (*Pantherophis guttatus*), Eastern hognose (*Heterodon platirhinos*), and timber rattlesnakes (*Crotalus horridus*). Northern pine snake (*Pituophis melanoleucus*

melanoleucus), a taxon of concern in Georgia, was detected within both WMAs, along with evidence of recruitment of new individuals. Montane longleaf pine habitats in Sheffield WMA were found to support a significantly greater diversity of upland snake species than similar habitats in Paulding Forest. This study collected baseline data for the upland snake communities in this ecosystem and will inform restoration of this ecosystem.

EXPLORING WATER CHEMISTRY VARIABILITY IN MESOCOSMS AND A NATURAL WETLAND: HOW DO ABIOTIC CUES STACK UP FOR AMPHIBIANS?

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Mesocosms, or artificial ponds, are constructed bodies of water used to simulate natural aquatic systems in ecological experiments. In Spring 2019, we conducted a mesocosm experiment to study the effects of increased water temperatures, increased drying rates, and the combination of both on the larvae of wood frogs, *Lithobates sylvaticus*, and spring peepers, *Pseudacris crucifer*. We examined how key abiotic cues to tadpole growth, including specific conductivity (SPC), pH, dissolved oxygen (DO), and temperature (°C), varied between and within treatments. Water chemistry data were collected from mesocosms and two natural wetlands from which amphibian eggs were collected to determine the degree of differentiation and variation among and within mesocosms and the natural wetland. DO and temperature did not differ among mesocosms. However, we did find significant differences in pH (wood frogs (WF): $p=0.001$; spring peepers (SP): $p=0.005$) and SPC (WF: $p=0.001$; SP: $p<0.001$) among treatments for both experiments. In the wood frog experiment, wetland pH was lower and SPC was higher than the control mesocosms (no warming or drying treatment). In the spring peeper experiment, wetland pH did not differ significantly from any mesocosms, but SPC of the wetland was significantly lower than warming and drying + warming mesocosms. These results indicate that increased temperatures and drying have an effect on pH and SPC in the mesocosms. Analyzing these effects will provide a detailed understanding of the tadpoles' abiotic environment and how this ultimately affects their growth and survival.

PRESENCE AND PREVALENCE OF *OPHIDIOMYCES OPHIODIICOLA* IN TENNESSEE SNAKE POPULATIONS: AN INITIAL ASSESSMENT.

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In recent times it has been well documented that disease has played a major role in population collapse of certain taxa. In just the past few years a new pathogen, *Ophidiomyces ophiodiicola* which causes the manifestation of snake fungal disease (SFD), has emerged and has been proven to cause death and declines in North American-endemic snakes. Snakes are extremely important for ecosystem function and play key roles in complex predator-prey relationships. To proactively engage this issue in Tennessee we have sampled a variety of suitable serpentes habitat across the state. Non-destructive systematic sampling has been utilized to locate free-ranging snakes throughout a variety of landscapes. Habitat types that have been monitored include wetland, forest, riverine and UTC biological field stations. Utilizing visual encounter surveys, randomized

walks and road cruising to locate animals. Once an individual is located it is photographed, assessed for symptoms of SFD and is then subjected to a thorough body swab (using lab grade aseptic cotton swabs) for a period of 45 seconds. Once a sample is collected the animal is released and the sample is placed on ice packs until it can be transferred to a -80- degree Celsius freezer. The goal of this initial assessment was to determine where SFD occurs in TN and what species can be considered at risk. Utilizing qPCR analysis we have determined SFD is present in TN snake populations. This project will now lead to more specialized research into a potential high magnitude conservation issue.

AN INVESTIGATION OF MICROHABITAT PARTITIONING BETWEEN TWO SPECIES OF TROGLOXENIC SALAMANDERS, *EURYCEA LUCIFUGA* AND *PLETHODON GLUTINOSUS*, AT SEQUATCHIE CAVE.

Rachel Head*, Team Salamander, Dr. Thomas Wilson
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Many species of non-adapted salamanders facultatively use the entrance and twilight zones of caves as habitat during some portion of the year. The Appalachian region is recognized for its substantial diversity of caudates and high density of karst features. In habitats reliant on allochthonous energy inputs, salamanders are frequently the most dominant vertebrate and have the ability to exert direct and indirect biological controls on energy flow between surface and subterranean environments. Despite their potential to influence local food webs, habitat use by non-adapted salamander species has repeatedly been ignored in ecological investigations of cave fauna. Community assemblages from underground ecosystems are frequently unique, due to the isolation and lack of available nutrients that supports epigeal populations. In particular, the behaviors and microhabitat preferences of two widespread, regularly sympatric, cave-adjacent salamander species, *Eurycea lucifuga* (Cave salamander) and *Plethodon glutinosus* (Northern slimy salamander), have been under-reported. This research project has two goals: 1) to observe and record current population demographics of all salamander species at Sequatchie Cave to provide baseline data for future ecological investigations, and 2) to collect data on behaviors, activity periods, and microhabitat preferences and/or partitioning between the two most abundant species, *Eurycea lucifuga* and *Plethodon glutinosus*. Here we report on data collected from our summer and fall sampling periods (June and December 2019). Weekly to biweekly investigations of salamander demographics and microhabitat measurements, including substrate temperature, substrate type, cover presence/type, placement on/above ground, response to light, and moisture level, are being undertaken.

A MIXED-METHODS STUDY REVEALS STRONG PUBLIC ENGAGEMENT CULTURE IN HERPETOLOGY.

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AAAS defines public engagement with science (PES) as “intentional, meaningful interactions that provide opportunities for mutual learning between scientists and members of the public.” In past studies, PES activities have improved attitudes and behavior of audiences towards

uncharismatic wildlife, including reptiles and amphibians. To understand and improve PES in herpetology, we conducted a mixed-methods research study to assess American-based herpetologists' experiences, involvement, and needs related to PES, and investigated factors associated with their participation. We conducted 60-minute semi-structured qualitative interviews with 15 herpetologists who regularly participate in PES. Transcripts were coded and analyzed using thematic analysis with expected and emergent codes. In the quantitative phase, we developed a closed-ended question survey, which was distributed via the listservs of four American herpetological organizations. Despite most sampled herpetologists having limited formal training, time, resources, and institutional support, many participated in a variety of PES activities, often utilizing partnerships and their own resources. Herpetologists held strong outcome beliefs related to their involvement in PES activities which served as a major driver in their intent to participate. Survey participants also cited an expectation to participate in PES activities and that many of their colleagues in the field were also involved in PES. Sampled herpetologists rarely evaluated their PES activities or considered publishing them, and some expressed unease with the idea of message framing. We found that sampled herpetologists were interested in evaluation training, and that providing accessible opportunities and grant funds were the most likely interventions to increase herpetologists' participation in PES.

GRAY AREA: WHERE TO DRAW THE LINE BETWEEN SPECIES OF GRAY-CHEEKED SALAMANDERS?

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The Southern Appalachian Mountains harbor the highest known salamander biodiversity including many endemic species. These endemic montane salamanders have limited ranges and are vulnerable to anthropogenically induced habitat shifts. *Plethodon amplus* (the Blue Ridge Gray-Cheeked Salamander) is a North Carolina endemic salamander whose current published range is likely inaccurate due to data deficiencies. *Plethodon amplus* are visually indistinguishable from other Gray-Cheeked Salamanders which occupy adjacent mountain ranges, making it difficult to locate exact boundary lines between species. To re-delineate the range of *P. amplus*, we collected tissue samples from Gray-Cheeked Salamanders from sites surrounding and within *P. amplus*' known range. We extracted DNA from each tissue sample then amplified and sequenced mtDNA using primers for three protein-coding regions. The results did not indicate clear species boundaries and suggested that there was likely genetic exchange between species in their recent evolutionary history. This study also found evidence to support that current published boundary lines for two of the Gray-Cheeked Salamander species (*P. amplus* and *P. meridianus*) are inaccurate. Future studies using additional mtDNA genes and nuclear DNA genes or next-generation sequencing techniques will be necessary to draw accurate boundary lines between species of Gray-Cheeked Salamanders.

THE VENOM GLAND TRANSCRIPTOME OF A MONOTYPIC SNAKE: HALLBERG'S CLOUD FOREST SNAKE.

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Hallberg's Cloud Forest Snake (*Cryophis hallbergi*) is a monotypic rear-fanged species in the family Dipsadidae. Since its first description in 1963, little is understood about *C. hallbergi*'s life history other than its small endemic range in Mexico's montane forest. Transcriptome analysis will inform our understanding of the venom characteristics of this snake, which can give indication of diet and other aspects of life history. We used RNAseq to sequence, identify, and determine relative expression of all known venom toxins of the specimen. The venom of *C. hallbergi* is primarily composed of the third type of snake venom metalloproteinases, or SVMPIIIs. SVMPIIIs have shown differing coagulation methods among prey items; SVMPs in rear-fanged snakes may even exhibit taxon specificity. Cysteine-rich secretory proteins, or CRISPs, also make up a substantial proportion of toxin expression. Despite their prevalence in many reptile venoms, particularly in other rear-fanged snakes, little is known about CRISPs' function. The venom composition and geographic distribution of *C. hallbergi* likely indicates a high degree of ecological specialization, potentially making this species far more susceptible to disturbance. Future studies could further investigate the relationship between ecological niche and venom protein composition by comparing the venom composition of *C. hallbergi* to that of other monotypic snake species and/or species inhabiting a similar ecological space.

HEADSTARTING THE FROSTED FLATWOODS SALAMANDER (*AMBYSTOMA CINGULATUM*) IN THE APALACHICOLA NATIONAL FOREST, FL.

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The Apalachicola National Forest (ANF) is one of only two remaining population strongholds for the federally threatened frosted flatwoods salamander (*Ambystoma cingulatum*). Recent larval surveys have indicated precipitous declines and extirpations, mostly attributed to the ecological succession of wetlands due to incompatible prescribed fire practices. Wetland restoration is underway, but populations are unlikely to recover without additional conservation measures. Headstarting has been identified by stakeholders as a potentially effective tool for boosting recruitment and survival while habitats recover. Here we report on the implementation of this strategy in the ANF from 2016-2019. Nearly 3500 eggs were collected from the wild, most of which would have failed to hatch due to recurring winter drought. Rescued eggs were either hatched into aquatic "cattle tank" mesocosms or donated to a captive assurance colony. Larvae were raised to large sizes or metamorphosis before re-release in their natal ponds. Our methods have produced high larval survival rates (90%) and the release of 1735 headstarts back to the wild. Headstarting is paired with a mark-recapture study at two focal populations, which aims to determine the fate of headstarted populations over time. Return rates of headstarts have been low, and focal populations have declined to critical levels. Furthermore, efforts have been complicated by extreme winter rainfall patterns in all 4 years of this study. Continued monitoring and methodological adjustments will be necessary to determine if headstarting is a viable conservation strategy.

THE EFFECTS OF SILVICULTURAL TREATMENTS AND CLIMATE ON *PLETHODON CINEREUS*.

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Woodland salamanders (genus *Plethodon*) are fully terrestrial and lungless, depending on cool and moist environments to exchange gasses across their skin. With the removal of canopy trees, terrestrial salamanders can be exposed to conditions that can lead to warmer and drier microhabitats, which may compromise their foraging abilities and opportunities. Using data collected from 1994-2018, we determined how climate and seven different silviculture treatments affect the reproductive condition in the red-back salamander (*P. cinereus*). Nocturnal sampling was done after rainfall. We did not find a relationship between the reproductive condition and silvicultural practices but we did find that larger body size and higher precipitation during the previous year led to larger clutch sizes and a greater proportion of gravid females in the following year. Our research suggests that even after timber harvest, salamanders are able to acquire the resources necessary to reproduce at similar rates compared to forests that have not been harvested. Future work will examine how reproductive conditions varies over time after canopy trees have been removed, and at the effects on recruitment of juveniles into the population, to compare with analyses conducted shortly after the removal of the trees.

AQUATIC TURTLE COMMUNITIES IN THE MINED LANDS WILDLIFE AREA OF SOUTHEASTERN KANSAS.

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Mined Lands Wildlife Area (MLWA) occupies approximately 5,900 ha in southeastern Kansas that was the site of extensive strip pit coal mining from the 1920s to 1970s. These pits are now inundated, and account for most of the 1,000+ lakes and ponds present in MLWA, which range in size from 0.1 to 24.3 ha in surface area and from <1 to 18 m deep. Most management at MLWA has focused on enhancing fish habitat to serve anglers. To determine whether MLWA waters also provide suitable habitat for diverse turtle communities, we conducted trapping surveys at 6 MLWA pits and 5 natural lakes in the surrounding area for comparison. We documented 7 species of aquatic turtle, 6 of which occurred in both pits and oxbows. At all sites in both types of wetland, the community was dominated by Red-eared Slider Turtles (*Trachemys scripta elegans*). Common Snapping Turtles (*Chelydra serpentina*) were notably uncommon in strip pits and were captured at significantly higher rates in natural lakes. All other species occurred in pits in numbers at least comparable to those in natural lakes. Painted Turtles (*Chrysemys picta*) and Eastern Musk Turtles (*Sternotherus odoratus*) occurred in considerable numbers in some pits, but overall differences in their capture rates between the two wetland types were not significant. Our results indicate that strip pits support turtle communities that are at least as robust as those in surrounding bodies of water, and though largely unused by Common Snapping Turtles, may in some cases be particularly suitable for other species.

DISPERSAL AND SURVIVAL OF CAPTIVELY-REARED JUVENILE GOPHER FROGS (*RANA [LITHOBATES] CAPITO*) AT SANDHILLS WILDLIFE MANAGEMENT AREA.

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Conserving sensitive species on small fragments of managed lands is challenging, especially for vagile species. Juvenile Gopher Frogs (*Rana [Lithobates] capito*) can disperse more than 600 m from natal wetlands, potentially being lost by emigrating from managed properties and into unsuitable habitat. We radio-tracked 64 captive-reared juvenile Gopher Frogs in 2018 and 2019 on the Sandhills Wildlife Management Area (SWMA) in Taylor County, Georgia, where industrial solar farms have been constructed along much of the boundary. We estimated survival and, using a random walk model parameterized by observed movements, estimated the likelihood that individuals emigrate onto privately owned lands or into solar farms. Within a week, frogs moved a maximum total distance of 602 m and a maximum net distance of 441 m from the wetland edge. One frog dispersed onto private property, but no frogs were tracked into the solar farms. We estimated the probability a frog would disperse beyond SWMA boundaries or into the solar farms was 19.6% and less than 0.02%, respectively. These results suggest that a relatively small proportion of juvenile Gopher Frogs are likely to disperse into current solar farms and likely represent a limited direct threat to Gopher Frog recruitment at SWMA. Survival through the tracking period was 14% to 20%, with most mortality due to predation by snakes and birds. We hypothesize that high predation rates may reflect a concentration of predators in the study area as the surrounding landscape was converted into unsuitable habitat.

THE DISCOVERY AND ORIGIN OF A LIKELY INTRODUCED POPULATION OF BLACK-BELLIED SALAMANDERS (*DESMOGNATHUS QUADRAMACULATUS*) IN THE UPPER PIEDMONT OF GEORGIA.

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Black-bellied salamanders (*D. quadramaculatus*) are large-bodied and abundant plethodontids found in rocky mountain streams in the southern Appalachians. In early 2019, we discovered numerous *D. quadramaculatus* in a small Piedmont stream in the Oconee River drainage, approximately 7.5 km north of Athens in Clarke County, Georgia. This disjunct population is >25 km SW of the nearest documented populations in the Broad River drainage. We sought to evaluate whether this population mostly likely represented a natural range extension or the result of human introduction. We assembled a complete mitochondrial genome for one adult *D. quadramaculatus* from this disjunct population and used publicly available DNA sequence data to estimate maximum-likelihood phylogenies. Our results suggest a close relationship between salamanders in this disjunct population and those in the Blue Ridge of northern Georgia, rather than those in the nearer Broad River drainage. This likely points to bait introduction via recreational anglers as the origin of the Athens population—a hypothesis supported by the

historical prevalence of *Desmognathus* in bait shops in the region. Here, we summarize other records of putatively introduced populations of *Desmognathus* and describe opportunities for future research in this system.

MODELING INDIVIDUAL GROWTH IN TWO NATRICINE SNAKE SPECIES.

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Growth can provide significant knowledge regarding the ecology, life history, and population dynamics of an organism since body size and growth often determine factors such as diet, age of sexual maturity, and number of offspring. We used a modified von Bertalanfy growth function, which takes into account measurement error, to model growth and size at maturity of the Queensnake (*Regina septemvittata*) and the Common Watersnake (*Nerodia sipedon*). The inclusion of measurement error in this model, as a latent parameter, is vital given the propensity of snakes to seemingly shrink between measurements. The model was run using a long-term dataset (2013-present) collected during surveys of seven riparian sites in central Kentucky. These surveys were conducted using a combination of capture mark recapture and passive integrated transponder (PIT) tag telemetry methods. Once captured, Common Watersnakes and Queensnakes were weighed, measured (SVL and TL), and PIT tagged. This provided us with the morphometric data required to model growth and allowed for easier recapture using a PIT tag scanner. These efforts provided us with morphological data from 489 unique individuals that were captured between 2 and 11 times. With these data, we modeled (SVL) growth within these two species respectively. The growth model employed in this project provides us with a better understanding of individual growth within these two species, and by extension may inform our understanding of their respective ecology, life history, and population dynamics.

DISCORD AND HARMONY: THE REGULATION OF AMPHIBIANS AND REPTILES ACROSS SOUTHEASTERN STATES.

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The ability to properly conserve amphibians and reptiles, specifically across the Southeastern United States, is directly tied to readily available species status reports. However, there are vast discrepancies across state governing bodies regarding legal regulation of amphibians and reptiles. We assert that the discord of regulatory practices among the regional states lead to inconsistencies in understanding the conservation status of species. Lack of centralized, easily accessible and comprehensive comparisons of the region's regulations are not easily available to the general public. This reality, coupled with the limited enforcement mechanisms in place for the existing regulations, perpetuates the uncertainty of the effectiveness of regional regulations. In this analysis, we showcase Tennessee, Georgia, and Florida as representative governances to highlight the discord by which native and non-native species are regulated. While we acknowledge the need for laws to be case and location specific, these regulations should be consistent at the macro level, allowing for harmonious conservation efforts regarding possession,

take, transport and trade of amphibians and reptiles. Because politically species status is the foundation for legal standing, this position is of the utmost importance. Our comparisons indicate that the discord between bordering states suggests discrepancies among regulation of amphibians and reptiles, thus compounding the system. Proposed suggestions regarding how to close these information gaps will be discussed at length in this poster. Overall, our objective is to provide a more streamlined basis to analyze the effectiveness of these regulations, in efforts to ensure consistent, harmonious conservation for the region's amphibian and reptiles.

ON THE DIVERSITY OF EROSION CONTROL PRODUCTS: IMPLICATIONS FOR SNAKE ENTANGLEMENT.

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The negative impacts of roads on biodiversity are well known. Roads fragment habitat and cause mortality via wildlife-vehicle conflicts. The construction and maintenance of roadways is often followed by the placement of the erosion control products (ECPs [e.g., erosion control blankets, spray-on mulch]) on the landscape. The Texas Department of Transportation's (TXDOT) approved products list (APL) contains 141 different ECPs that a contractor can install on a construction site. Only two criteria must be met for an ECP to be listed on the APL: 1) the ECP must promote vegetation growth, and 2) the product must adequately prevent soil loss. There are no criteria that consider the impacts ECPs on wildlife. Recent studies have found that snakes are vulnerable to entanglement in ECPs with certain traits (e.g., plastic netting with fused corners). We reviewed and quantified the diversity of traits of the 141 ECPs (i.e. material type, aperture size, mesh type). In addition to identifying the ECPs that would put snakes at a high risk to entanglement, we also identified those ECPs that would likely be the most snake-friendly based on their traits (e.g., woven mesh with a large aperture size). These results can be used to inform and mitigate against the entanglement of snakes and other wildlife in ECPs and limit an additional source of mortality.

MODELING HABITAT SELECTION OF A TRUE GENERALIST; THE WESTERN COTTONMOUTH (*AGKISTRODON PISCIVORUS*).

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Wetlands are typically transitional habitats that represent a dynamic hydrologic gradient between terrestrial and deep-water aquatic systems. As hydrologic fluctuations within a wetland can cause the habitat to become periodically unsuitable and resource-limited, species with traits that can adapt to these environmental changes will be favored. We investigated habitat selection of cottonmouths inhabiting an isolated riverine slough, from the Cumberland River, adjacent to mixed lowland forest and upland limestone bluff habitats. Field collection was by visual-encounter surveys, and individuals were measured and sexed. Environmental variables were recorded for each observed snake location (N = 149) and paired with a random location to assess

available habitats and microhabitats within the study area. Random Forest modeling was used to elucidate trends in habitat selection, and results suggest that cottonmouths generally select habitats that are within or near a water source during the active season (April – October). However, cottonmouths were predicted, from Random Forest modeling, to utilize a variety of microhabitats within the wetland and adjacent edge habitats. We suggest that cottonmouth preference to wetland habitats is because they provide quick escape routes from potential predators, optimal hunting grounds, and are habitats with thermal heterogeneity to facilitate thermoregulation.

USING GEOSPATIAL ANALYSIS TO MODEL FOR THE CRYPTIC SALAMANDER *HEMIDACTYLIUM SCUTATUM* IN TENNESSEE.

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The Southeastern United States retains some of the highest salamander diversity on Earth, with a high degree of endemism. However, this region has also experienced consistent declines across recent decades. The seasonality and complex life-history of salamander populations leads to data gaps and detection difficulties. As a consequence, researchers have begun using citizen-science and museum data as a means of projecting current distributions. Habitat specialists like, *Hemidactylum scutatum* (Four-toed Salamander), have experienced habitat loss and subsequent populations declines. The Tennessee Wildlife Resources Agency has listed it as a species of “Greatest Conservation Need”. To mitigate data gaps, geospatial models are generated using various existing data sources. Landscape metrics that are used to populate the model include land cover type, elevation, slope, soil type, and hydrology. Using geospatial tools, we will be better able to elucidate the current habitat suitability for this cryptic species and these results will be useful to resource managers who require tested models using occupancy and habitat data. Models created in ArcGIS will be cross-validated using data collected in the field from predicted occupancy. The anticipated sampling for this project will begin in March 2020 and this poster presents preliminary models to be field tested.

THE EFFECTS OF PRESCRIBED FIRE ON THE DEVELOPMENT OF LARVAL CRAWFISH FROGS (*LITHOBATES AREOLATUS*).

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Prescribed fire is a common technique used in the restoration and management of a variety of habitats (e.g. grasslands, pine savannahs, glades) to reduce the abundance of non-native vegetation and prevent succession. Seasonal prairie wetlands can be affected by prescribed fire through a reduction in vegetative biomass, which serves as an important nutrient base, or through the deposition of ash from surrounding terrestrial habitat. While it has been well-documented that terrestrial amphibians can experience mortality when prescribed fire occurs, few studies have assessed the effects of fire on larval amphibians. We used a mesocosm approach to investigate the effects of prescribed fire on larval amphibian development. We had treatments

representing three scenarios: (1) Control – unburned prairie vegetation, (2) Ash Addition – unburned prairie vegetation, with added ash to simulate deposition from surrounding terrestrial habitat, and (3) Vegetation Reduction – 50% of vegetation burned, with ash retained. We compared average time to metamorphosis, snout-vent-length, mass, and survival of larval *Lithobates areolatus* among treatments. Larval survival was lowest in the Vegetation Reduction treatment, but other developmental metrics were similar among treatments. Additionally, overall biomass of successful metamorphs in the Vegetation Reduction treatment was significantly lower than the control treatment. Our results suggest that prescribed fire has negligible effects on the development of aquatic amphibian larvae, but might result in reduced survival and metamorph biomass if burning occurs prior to wetlands filling.

MHC DIVERSITY IN *RANA YAVAPAIENSIS*: A TWELVE-YEAR COMPARISON OF IMMUNOGENETIC VARIATION IN THE PRESENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* (Bd).

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Batrachochytrium dendrobatidis (Bd) is a fungal pathogen that is capable of infecting hundreds of amphibian species and has caused massive population declines and extinction events worldwide. However, some amphibian populations have demonstrated an adaptive immune response to Bd. In vertebrates, adaptive immunity is controlled by the major histocompatibility complex (MHC) genes. Experimental infections of the lowland leopard frog (*Rana yavapaiensis*), a species with a variable immune response to Bd, have shown that heterozygosity or the presence of an allele dubbed allele Q at the conserved MHC class II β exon 2 locus correlates with decreased susceptibility to Bd. However, these patterns are not always reflected in natural populations. To better understand how Bd drives MHC evolution in natural populations we collected tissue samples from 126 *R. yavapaiensis* individuals from 7 populations across their native Arizona range between 2017-2018. We compared MHC diversity of these individuals with 184 *R. yavapaiensis* individuals from the same 7 populations collected previously between 2006-2008. We sequenced the MHC class II β exon 2 of all 310 individuals using a unique barcoding PCR on the Illumina MiSeq 2x250 platform. Our sequencing results showed previously undetected MHC diversity in certain populations of *R. yavapaiensis*, with some individuals expressing multiple gene copies of MHC class II. This study demonstrates the power of using massive parallel sequencing to assist in conservation efforts.

ABIOTIC FACTORS THAT INFLUENCE CAVE USE BY SALAMANDERS.

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Lungless salamanders (family Plethodontidae) rely on cutaneous and buccopharyngeal gas exchange, which strongly influences temperature and moisture regimes that can be inhabited.

While many plethodontid salamanders have been documented to use caves for at least part of their life cycles, few studies have examined the factors that affect the abundance and diversity of salamanders in caves. In particular, no such studies have been conducted on salamander communities in caves of northern Alabama. This study aims to determine a relationship between salamander diversity and abundance among northeastern Alabama caves and environmental variables. Surveys are being conducted in 15 caves every season from July 2018 through June 2020, documenting salamanders along transects from the entrance to 160 m into the dark zone. Environmental variables, such as air temperature, humidity, and wind speed are being characterized along study transects, and climatological and landscape data around cave locations, such as rainfall, surface temperature, slope, and land cover, are being recorded to determine relationships between these variables and salamanders diversity and abundance among seasons. We will be presenting the preliminary data on the use of caves by salamanders, displaying seasonal preference, species abundance, and spatial preferences.

BRIDGE PROGRAM FOR AMPHIBIAN CONSERVATION RESEARCH: A NEW ROUTE INTO THE FIELD.

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While higher education faces declining enrollments in large parts of the country, many young adults are dropping out, transferring or changing majors as they struggle with academic, intellectual, and social challenges of traditional university settings. People who are into amphibian and reptile conservation often have options limited by academic biology programs that may not offer conservation or organismal courses. While some students get opportunities to conduct research as part of their studies, most students can't get these opportunities as part of formal secondary education. Many realize later in life that they may not have studied the things in college that they cared about most and want to dedicate their lives to. Adults wishing to get experience in conservation research often struggle to find opportunities that will nourish their interests as well as their intellectual and social needs. Bridge programs are tools to help young adults get into a new field. These programs can help students fill gaps in their experience, skills and education stemming from many potential sources in the leaky academic pipeline. We propose our new bridge program as a model of a new tool to train conservation biologists. Our amphibian conservation research bridge program promises to integrate more diversity into the field of conservation by creating a route for people to work with imperiled species in the southeastern United States without first going through the traditional academic path of undergraduate and graduate school.

LAND USE AND ANATOMICAL LOCATION DETERMINE COMPOSITION OF FROG SKIN MICROBIOME AND OCCURRENCE OF THE PATHOGENIC FUNGUS *BATRACHOCHYTRIUM DENDROBATIDIS*

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Habitat loss and pathogens, particularly *Batrachochytrium dendrobatidis* (*Bd*), have been identified as primary factors contributing to amphibian decline. These factors may cause decline independently or they may interact with one another. For example, habitat features that affect microclimate such as forest canopy density, natural vegetation and water temperature are good predictors of the presence of *Bd*. However, recent evidence suggests that forest habitat loss is negatively associated with occurrence, prevalence and infection intensity of the fungal pathogen. Anurans are known to possess innate defenses against pathogens in the form of antimicrobial secretions produced in glands on skin of the frogs. In addition, recent evidence suggests that the probiotic role of symbiotic bacteria may be important in the defense against pathogens, specifically *Bd*. In this study we investigated how habitat, urbanization, and microbiome interplay in the context of *Bd* occurrence and pathogen load. We found that urban frogs had lower incidence and load of *Bd* while forested frogs had lower species richness and diversity of symbiotic bacteria. Symbiotic bacterial communities did not differ between *Bd*⁺ and *Bd*⁻ frogs within habitat type suggesting that deforestation, not bacterial community, was the main driver of the fungal pathogen's occurrence in our focal anuran populations with low-level *Bd* infections.

JUVENILE COTTONMOUTH DISTRIBUTION IN RELATION TO HIBERNACULA SITES.

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The Cottonmouth (*Agkistrodon piscivorus*) is a semi-aquatic pit viper found throughout the southeastern United States. Previous studies have indicated a relationship between Cottonmouth habitat selection and location of their hibernaculum, particularly for neonates and juveniles. We hypothesize that neonates choose habitats close to hibernacula sites as a result of mother's preference to give birth near those sites. We predict that neonates will be found in habitats closer to hibernaculum sites than juveniles. We captured 48 snakes using visual encounter survey and recorded their locations using a GPS. Of the 48 individuals captured, 19 were neonates (≤ 290 mm in SVL) and 29 were juveniles (> 290 , but < 500 mm in SVL). Each individual's location was used to calculate their distance to previous known hibernacula sites. A one-way ANOVA comparing distances between the two groups suggested that neonates were found closer to hibernacula sites than juveniles. Distance from hibernacula was positively correlated with snout-to-vent length. Our study suggests that neonates limit their dispersion from the upland hibernaculum due to their familiarity with those sites and that juveniles disperse farther from hibernacula than neonates, likely in search of aquatic foraging areas.

A LONG-TERM RESEARCH PROGRAM IN COTTONMOUTH ECOLOGY HIGHLIGHTING RECENT INVESTIGATIONS ON THE BEHAVIORAL CONTEXT OF BRAIN LATERALIZATION.

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We present past and current research related to a long-term research program using the Northern Cottonmouth (*Agkistrodon piscivorous*) as an investigative model. Since 2000, we have worked with an east Texas population of cottonmouths that use the riparian zone of Harmon Creek within the Trinity River watershed. Our research program has focused on questions related to spatial ecology, physiology, behavior, and more recently population biology, genetics, neurobiology, and the monitoring of snake fungal disease. In outlining this long-term research program, we will present current collaborations and focus on highlighting current undergraduate and graduate research investigating the behavioral context of brain lateralization. Little is known about the cognitive processes in snakes and potential differences in responses to stimuli between right or left visual-fields may suggest that snakes likely exhibit asymmetrical cognitive processing and brain lateralization. By investigating how lateralization of cognitive processes modify behavior, we may gain some insight for understanding reptile cognition and the comparative evolutionary implications of behavioral responses to stimuli processed by a particular visual field. These data will further help elucidate how snake perceive their spatial environment and potentially use visual-field stimuli in site selection and behavioral interaction.

TEMPERATURE DEPENDENT SEX DETERMINATION OF CAPTIVE-REARED GOPHER FROGS.

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Environmental sex determination is common among reptiles but is poorly documented among amphibians. Amphibians generally have genetically-determined sex; however, recent studies show a number of environmental factors can also affect sex determination including water temperature. A recent study demonstrates that warmer water temperatures increase the probability that Wood Frog tadpoles (*Rana sylvatica*) develop into males. If this is a general pattern, it has husbandry implications for captive-rearing programs. We raised replicate groups of Gopher Frog tadpoles (*R. capito*) among four temperatures. At the coolest temperature, Gopher Frog tadpoles failed to develop or sexually differentiate. Among the three warmer treatments and consistent with prior studies, we found that the probability that a Gopher frog tadpole developed into a female declined with increasing water temperature from a probability of ~0.9 at 22 degrees C to a probability of 0.25 at 32 degrees C. The relationship between water temperature and probability a tadpole became female was nearly identical in our study to the pattern documented in Wood Frogs. An additional novel finding was that cooler water temperatures resulted in sexual differentiation occurring at later Gosner stages. Our results demonstrate that - if reared at constant warmer temperatures - Gopher frog tadpoles are more likely to develop quickly to metamorphosis but are also significantly more likely to be male, which would reduce the effectiveness of captive rearing for augmenting or reintroducing Gopher frog populations. These results likely apply to other species of Ranidae.

EFFECTS OF TREE LEAF LITTER SPECIES ON TREEFROG OVIPOSITION SITE SELECTION.

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Larval amphibian performance can be greatly affected by the species of leaf input to ponds. Some species of leaves are toxic, like invasive Chinese tallow, due to the concentrations and types of plant secondary compounds (e.g., tannins). Female frogs generally choose to oviposit in pools with environmental characteristics likely to maximize their offspring's survival and growth. As such, we would expect females to avoid ponds with toxic plant litter containing high tannin concentrations. To test this, we placed 45 wading pools near a forest edge in Louisiana in 2018. Each pool contained leaf litter from one of 15 different tree species. We included 13 native species and 2 nonnative (Chinese tallow and Bradford pear). We measured leaf chemical traits (C, N, P, tannins) prior to and water quality (conductivity, pH, oxygen, tannins, water depth) throughout the experiment. We checked pools three days following rain and counted Cope's gray treefrog eggs in each pool for 60 days from early May to July. We used zero-inflated models and AIC_c to determine the importance of leaf chemistry, water chemistry, and leaf species on the number of eggs laid. We found that water depth best predicted whether eggs were laid in a pool, but tree species best predicted the number of eggs. The most eggs by far were laid in pools containing post oak, and pools containing nonnative species were not avoided more than average. No leaf or water chemistry variable explained these patterns, showing no avoidance of high tannin concentrations.

AN EVALUATION OF CANE TOAD TADPOLE SALINITY TOLERANCE AND INVESTIGATING VARIATION BETWEEN COASTAL AND INLAND POPULATIONS.

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A worldwide pest and ecological homewrecker, *Rhinella marina* (South American Cane Toad) is understood to have multiple advantages over other anuran species. They are larger, toxic to potential predators, and extremely fecund. While most anurans cannot tolerate salt, the cane toad can. Adults can tolerate a salt concentration of 14-15 ppt, and in brief situations higher than that. What is not as well understood is how well embryos and tadpoles of *R. marina* withstand salt. Previous studies have looked at inland populations and have found them able to reach metamorphosis in 5-8 ppt. However, salt tolerance is plastic in many anurans and a comparison of inland and coastal offspring is needed to evaluate genetic variation of the trait. This study aims to understand the full potential of *R. marina* tadpole salinity tolerance and understand another advantage they have over other anuran species. Understanding the advantages cane toads have may help aid in conservation of more specialized and sensitive anuran species.

PRELIMINARY MORPHOLOGICAL RESULTS OF EASTERN DIAMONDBACK RATTLESNAKES (*CROTALUS ADAMANTEUS*) IN SOUTHWEST FLORIDA.

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The Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) is a large pit viper endemic to the Southeastern United States. The species is typically associated with upland xeric habitats and gopher tortoise (*Gopherus polyphemus*) burrows. However, populations in south Florida may differ significantly from those north of Florida's frost line due to climate and hydrologic variations. There is little research on the species as a whole, particularly in the southern portion of their distribution. Eastern Diamondback Rattlesnakes have experienced declines in their population throughout their range; however, they have not been granted protective status at the federal or state levels. Our lab has conducted a comprehensive study looking at this species in Southwest Florida. The research presented in this poster represents ongoing morphological data collection since 2015. Herein, we provide preliminary results for *C. adamanteus* morphology including snout vent length, total length, diamond counts, ontogenic shifts, and sexual differences. This data provides important information on life history traits for this understudied species. Understanding population dynamics of *C. adamanteus* will increase attention to conservation and management needs of this species.

GOPHER TORTOISE BURROW ABUNDANCE IN A PINE PRODUCTION FOREST.

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Pine (*Pinus sp.*) production forests are a widespread component of the southeastern United States, comprising approximately 25% of southeastern-forested land. It is well documented that gopher tortoises (*Gopherus polyphemus*) persist within these production forests; however, much of the research regarding gopher tortoise ecology within these landscapes does not reflect recent changes in forest management (e.g. lower planting densities, longer rotations, etc.). Furthermore, published burrow survey studies within pine production forests rarely report stand level structural conditions and the influence of these parameters on gopher tortoise ecology. Therefore, we assessed the influence of stand level structural conditions on gopher tortoise burrow abundance within a loblolly (*Pinus taeda*) pine production forest located in south central Georgia. We surveyed for gopher tortoise burrows using line transect distance sampling and collected stand level vegetation measurements (e.g. visual obstruction, percent canopy cover) during the summer of 2019. We estimated burrow abundance using multi-species distance sampling model built in a Bayesian framework. Our results reduce the uncertainty regarding the relationship between modern pine production forests and gopher tortoise burrow abundance, improving our understanding of gopher tortoise ecology within these systems.

COMPARATIVE CALLING PHENOLOGY OF ANURANS ACROSS ECOSYSTEMS.

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As ectotherms, annual activities vital to the life cycle of amphibians are greatly dependent upon abiotic factors within their environment. Phenologies not only vary interspecifically but may also vary for widespread species that occur across different habitats and climates. By pinpointing the period when an amphibian is active, as well as the abiotic correlates associated with that activity, researchers can begin to make predictions about how species may respond in the context of shifting climates. We examined calling phenologies of four native species that breed in both southern pine forests and coastal prairieland ecosystems (*Acris blanchardi*, *Incilius nebulifer*, *Pseudacris fouqueti*, *Hyla cinerea*). We hypothesized that anurans occupying the prairie ecosystem would call earlier and longer than those in forested ecosystems due to higher annual mean temperatures along the coast. To evaluate how calling phenologies are associated with habitat types, Song Meters were deployed at breeding ponds within the two ecosystems. Despite our predictions, we discovered that phenologies between habitat types exhibited considerable overlap. Calling tended to begin sooner in the coastal prairie but peaked and ended at approximately the same time as in the piney woods. Calling activity and intensity appeared to be driven by day length duration, daily temperature, and precipitation. The similarity in calling phenology of these species across ecosystems suggests that species are responding to the same environmental cues, and may exhibit consistent responses in reproductive phenology, regardless of habitat type. Implications of this study can benefit further studies of how shifting climates may impact anuran reproduction.

INVESTEGGATOR PILOT STUDY: RECOMMENDATIONS AND FEASIBILITY FOR COSTA RICA.

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Despite their protected status, a major threat to sea turtles is egg poaching for local consumption and commercial trade. In Latin America alone, unprotected beaches are currently experiencing poaching rates as high as 90%. To help address sea turtle egg smuggling, we worked with Paso Pacífico who developed the InvestEGGator – an artificial 3D printed egg containing a GPS tracker which resembles a real sea turtle egg and can be placed inside a turtle nest. We aimed to understand whether conservation nonprofits could utilize the InvestEGGator to track poaching routes via a pilot study of the physical deployment of the device. Semi-structured interviews guided the development of the study and a set of key recommendations for both the deployment of the decoy egg and future selection of project partners. Over the course of two field visits in the summer of 2019, we discovered that NGOs followed different monitoring protocols; different sea turtle species can overlap during the nesting seasons; cell tower signal strength and availability was limited; and poaching activity varied across five NGO partner locations. We found that these site differences had major implications for the deployment of the InvestEGGator. Two partner NGOs successfully deployed 15 decoy eggs in total between October and December 2019 and only two were poached. We were unable to retrieve positional data for the stolen decoy eggs. These findings show that the InvestEGGators are capable of deceiving poachers based on appearance but require improvements in their internal hardware to render them reliable for future deployment.

MULTI-SCALE OVERWINTER HABITAT AND REFUGIA SELECTION OF THE EASTERN DIAMONDBACK RATTLESNAKE (*CROTALUS ADAMANTEUS*) IN THE LONGLEAF PINE ECOSYSTEM.

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Across much of its range, the Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) requires underground refugia during the winter months. Although use of Gopher Tortoise (*Gopherus polyphemus*) burrows as a refugia by these snakes has been well documented, other refugia including stump holes may be important as well. We tracked 14 Eastern Diamondback Rattlesnakes over the winter from October 2018 – February 2019 and identified the refugia they used. We then investigated the influence of availability and habitat characteristics on refugia selection. I documented use of six unique habitat features as refugia and assessed refugia selection by investigating proportional use with permutation-based combination of sign tests. Within home ranges, snakes used Gopher Tortoise, Nine-banded Armadillo (*Dasypus novemcinctus*), and Eastern Woodrat (*Neotoma floridana*) burrows and stump holes in proportion to availability and avoided large down woody debris and tip ups. Snakes used all habitats in proportion to availability except closed-canopy and non-forested habitats (agricultural fields and wetlands) which were avoided. Poorly drained soils were avoided but all other soil drainage classifications were used in proportion to availability. Management to provide overwintering habitat for Eastern Diamondback Rattlesnakes should focus on maintaining open-canopy upland forests on well-drained soils. If Gopher Tortoise or other animal burrows are not present, retention or limits of harvest of pine stumps as refugia for Eastern Diamondbacks should be considered.

CHYTRID GROWTH INHIBITION IN TWO FULLY AQUATIC SALAMANDERS.

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Chytrid fungal pathogens, *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) are causing amphibian die-offs worldwide. Unlike *Bd*, *Bsal* has not yet been detected in North America but poses an imminent threat to native salamander biodiversity. While susceptibility to *Bsal* varies among salamander species, the factors underlying such variation are poorly understood. Because anuran skin secretions are known to contain antimicrobial peptides (AMPs) shown to kill *Bd in-vitro*, AMPs are hypothesized to be important for limiting *Bd* infections, thereby protecting some anuran species from disease. The objective of this study was to test whether fully aquatic salamander, *Siren intermedia* (Lesser Siren) secretes peptide mixtures that inhibit the growth of chytrid pathogens using *in-vitro* growth inhibition assays. Skin secretions were collected from 10 wild *S. intermedia*. Skin secretions were purified, enriched for peptides, pooled, and combined with either *Bsal* or *Bd* zoospores in a 96-well plate. Optical density was read daily for 14 days to assess changes in fungal growth. We found that even at low concentrations, the skin peptides of *S. intermedia* inhibited the growth of *Bsal* and *Bd*. These results suggest *S. intermedia* secrete AMPs which may protect this species from *Bsal* infections

and disease. We are in the process of repeating this experiment using the skin secretions of *Necturus beyeri* (Gulf Coast Waterdog) and *Necturus maculosus* (Mudpuppy). We predict that results will be similar to those observed with *S. intermedia*.

HERPING FOR ENGAGEMENT.

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Conservation and education outreach programs often highlight charismatic species or species of economic or ecological importance. However, without appreciable connections to nature, the foundation necessary to empathize with these programs is insufficient. While many students are familiar with charismatic herps, such as sea turtles, there are countless other species of conservation concern that many students are entirely unaware of, for example Green Salamanders (*Aneides aeneus*). As species loss is expedited, it is imperative to counter biodiversity blindness, particularly since public education and awareness are central to successful conservation efforts. The most impactful education experiences foster direct engagement with biodiversity by students. We highlight the use of project-based learning to nurture student exposure and understanding of amphibian and reptile diversity. Specifically, we address the use of iNaturalist in Herpetology courses at The University of Alabama in Huntsville and The University of Illinois Champaign-Urbana. These courses are taught at the undergraduate and graduate levels, and most students enrolled in these courses are novice herpers. Throughout the course, students were required to document observations from class field experiences as well as personal explorations. These projects transformed the learning of herp diversity from passive in nature to a meaningful and active process allowing students to connect with species observed while concurrently participating in citizen science initiatives. We summarize the documented observations from these courses and emphasize species of conservation concern. We also share recommendations for successful implementation of iNaturalist projects by educators based on our experiences.

MICROHABITAT SUITABILITY IN GREEN SALAMANDERS.

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Species occurrence and abundance are both partially explained by the presence of suitable habitat. In many cases, ecologists describe habitat at the site or population scale; however, measures of within-site features (microhabitat) may also identify predictors of species' abundance. Such assessments can help determine habitat suitability for sites with unknown occupancy status. Rock outcrops are embedded components of mountainous forest landscapes and are critical habitats for terrestrial salamanders such as the green salamander (*Aneides aeneus*). Most habitat suitability studies for the green salamander have been conducted on a macrohabitat (outcrop) level, neglecting the immediate microhabitat (rock crevices) these individuals interact with and the importance of within-habitat connectivity. Size and physiology

limit green salamander movement capabilities, affecting foraging, predation evasion, and searching for mates. As a result of these constraints, I predicted crevices with features directly related to within-habitat connectivity would be more likely to be selected by a green salamander. I evaluated microhabitat features that contribute to both microclimate and within-habitat connectivity, including crevice width (cm), length (cm), depth (cm), temperature (°C), humidity (%), crevice density (1/m²), nearest crevice (cm), and nearest tree (m). We surveyed 424 crevices across 9 sites. 310 crevices did not have salamanders and were interpreted as available but unused microhabitat; 116 of the crevices were occupied. A global logistic regression model identified crevice width, canopy cover, and crevice density as significant features in predicting salamander presence. Current continuations of this work include estimating salamander abundance and survivability, as well as assessing genetic connectivity between subpopulations.

COMPARING THE EFFECTIVENESS OF DIURNAL AND NOCTURNAL SURVEYS FOR EASTERN HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*).

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Hellbender studies have traditionally used diurnal rock-turning surveys that alter microhabitat conditions and may result in injuries to hellbenders and surveyors. Additionally rock-turning surveys are marginally effective in streams dominated by large boulder or bedrock substrates. Between June and August 2019, we compared the catch per unit effort (CPUE) of nocturnal snorkel surveys and diurnal rock-turning surveys at 11 sites in western North Carolina. A second round of nocturnal surveys was conducted during the breeding season (late August-September) to assess whether seasonal changes in water temperature and activity influence nocturnal detection rates. Kruskal-Wallis tests revealed no significant differences in CPUE ($H = 2.75$, $df = 2$, $P = 0.254$) across all survey periods and nocturnal CPUEs were higher than diurnal CPUEs at 82% of sites sampled during the summer and nocturnal survey CPUE increased at 88% of sites sampled later in the Fall. Although no hellbenders were found sheltering beneath bedrock during diurnal surveys, 28% of nocturnal detections were from bedrock crevices. These results suggest that nocturnal surveys may yield more reliable population size and site-occupancy estimates compared with rock-turning surveys in streams that have a high proportion of immovable boulders or bedrock outcroppings. Nocturnal surveys can also help extend monitoring into the breeding season when disturbance of spawning animals becomes a management concern. Although more work is needed to refine this approach, nocturnal surveys provide a rapid, if somewhat more logistically challenging alternative to daytime, rock-turning surveys that may enhance monitoring efforts while reducing disturbance to hellbender microhabitats.

EXPERIMENTAL EVALUATION OF BROWN ANOLES (*ANOLIS SAGREI*) AS INTERMEDIATE HOSTS FOR THE INVASIVE PENTASTOME PARASITE *RAILLIETIELLA ORIENTALIS*.

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An Asian parasite (*R. orientalis*) has been found in several species of snakes native to Florida. Snakes become infected with *R. orientalis* when they eat infected prey, but these intermediate hosts are currently unknown. Understanding which species can act as intermediate hosts of *R. orientalis* will enable biologists to determine which species of snakes are likely to be negatively impacted based on their diet and to predict the parasite's potential dispersal. We fed pentastome eggs, harvested from infected pygmy rattlesnake feces, to 10 brown anoles. We compared them to 10 unexposed control anoles to determine if the lizards could be infected with *R. orientalis* and if infection affected lizard fitness components. The lizards were weighed at capture and again when euthanized. We euthanized both pentastome-exposed and unexposed anoles at several intervals ranging from 5-168 days post treatment. The lizards were dissected using a stereomicroscope and the number and location of parasites were recorded. *Anolis sagrei* did serve as a competent intermediate host, with 70% of exposed anoles hosting multiple pentastome nymphs. The pentastomes were found in the body cavity associated with the surface of the gut, liver, and heart, and in late infections in retroperitoneal areas. The pentastome treatment did not significantly impact survival or growth rate. *Raillietiella orientalis*' use of *A. sagrei*, an abundant and easily transported species, as an intermediate host might facilitate its further rapid geographic spread. This study is the first documentation of any potential intermediate host for *R. orientalis* in North America.

PRELIMINARY SPATIAL AND THERMAL ECOLOGY OF STERNOTHERUS ODORATUS (EASTERN MUSK TURTLES) IN A SUBURBAN ENVIRONMENT.

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Today, most habitat fragmentation results from direct anthropogenic modifications to the landscape. Besides modification, urban expansion has resulted in a decline of natural habitat for native biota. Turtle populations across North America have decreased at an alarming rate, with a major cause being habitat destruction. Urbanization impacts on herpetofauna is limited and inference on whether urban environments provide suitable habitat for turtle populations is novel. The objective of this study is to describe movement patterns, thermoregulatory behavior, and population demographics of *Sternotherus odoratus* within a fragmented suburban environment. Nine individuals were collected in a man-made canal in Kenner, Louisiana and fitted with radio transmitters and iButton temperature loggers. Telemetry locations were obtained from spring of 2019 to winter of 2020. Opportunistic captures of additional musk turtles were reserved for mark-recapture to understand population demographics. Trapping took place from June to winter of 2020. Preliminary surveys have indicated a dense population and a female-biased population. Data collected over the past several months will be presented and discussed.

SPATIAL ECOLOGY OF GILA MONSTERS IN A SUBSIDIZED RESOURCE ENVIRONMENT.

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Animal movements and space use are often conceptualized using the home range concept. Home ranges are determined by temporal, spatial, and individual-level processes. Within the environment, one of the key factors influencing space use is the distribution of resources. Alterations to resource distribution and availability can have profound consequences on spatial habitat use. We analyzed spatial data collected from Gila Monsters in a subsidized resource environment (golf course) and a non-subsidized (natural) environment. We calculated kernel density polygons and minimum convex polygons for estimates of space use. After adjusting area estimates for sex, number of fixes, and year, males in the subsidized environment had an average overall area of 13.6 ha while the females had an area of 8.3 ha. In the unsubsidized environment males had an average overall area of 43.2 ha while females had an area of 23.6 ha. Gila Monsters between the two environments also exhibited seasonal differences, primarily in the dry and monsoon seasons. This suggests that Gila monster home ranges may be smaller in subsidized resource environments than those of un-subsidized environments due to increases in available resources.

SOUTHEASTERN ANURAN CALL IDENTIFICATION USING ATTENTION BASED NEURAL NETWORKS.

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Initiatives such as FrogWatch and the North American Amphibian Monitoring Program (NAAMP) organize, recruit, and train volunteers for large scale Anuran population monitoring. Without the efforts of these citizen scientists, Anuran conservation efforts would be restricted by the availability of qualified field biologists. After qualifying for capacity to identify regional calls, volunteers sample Anuran populations by documenting the presence and intensity of calls at select habitats. Automating call identification would reduce both the qualifications necessary to contribute in these projects, and the potential subjectivity associated with determinations made by different researchers. Here we present the results of using an attention based convolutional neural network to automate call identification. The network was trained to differentiate 13 southeastern Anuran calls using field recordings available through iDigBio. Attention modules are utilized within this network to retain sequential context when evaluating the diverse and relatively noisy field recordings. Although expanding this method to recognize additional taxa will be necessary for distributed services, we demonstrate the method's potential to expand Anuran monitoring in the face of species decline throughout the southeast.

UNEARTHING A NATURAL HISTORY TREASURE: MODERNIZING AND DIGITIZING THE UNIVERSITY OF ALABAMA HERPETOLOGICAL COLLECTION.

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Data from natural history collections have generated much of what biologists understand about the biodiversity and ecology of herpetofauna. In addition, museum specimens represent historical biogeography, population size, and demography which are critical to contemporary assessments of conservation priorities. The University of Alabama Herpetological Collection (UAHC) was created in 1947 by Dr. Ralph L. Chermock. Many collections by Chermock and associates represent some of the earliest wide-ranging records of Alabama's herpetofauna. Additionally, collections from the 1960s and 1970s include numerous stream amphibian and reptile collections from Alabama and neighboring states as well as international trips to Costa Rica by Chermock and colleagues. Like most natural history collections, the UAHC needs modernization (i.e., updating taxonomy, containers, tags, and labels) so that the specimens and their data are protected. Additionally, the UAHC requires further digitization so that data are easy to search and manipulate. Although there have been attempts to modernize and digitize the UAHC in the past few decades, obscure or mistaken localities, misidentifications, and an outdated numbering system are still common problems in the UAHC. Here, progress on modernizing and digitizing the UAHC is reported with a discussion on how the improvements on this collection can be lessons to those interested in curating or starting their own collections. The UAHC is not the only important herpetological collection in need of modernization and digitization and as many herpetologists move away from collections-based research, it is more important than ever to preserve and protect these invaluable records of the natural world.

THE EFFECT OF HABITAT RESTORATION ON TURTLE POPULATIONS IN A GEORGIA LAKE AND WETLAND.

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Ideally, habitat restoration will provide only benefits to species living in the restored area. However, sometimes the process of restoration involves significant habitat disturbance which may, at least temporarily, negatively affect some species. I will present results from an 8-year (2012-2019) study of turtle populations in a wetland and lake in Lakeshore Park in Dalton, GA. In 2017, these habitats underwent restoration including the draining of much of the water in the wetland and lake and the reconfiguration, laying back, and revegetation of the banks. The purpose of the study was to investigate the effects of the restoration on the turtle populations by comparing them before, during, and after restoration. Each summer, turtles were collected over a 2-week period using baited hoop traps placed along the edges. Captured turtles were identified, measured, weighed, marked, and released. Six species of turtles have been collected in this study. During the year of restoration, when the water levels were very low, the highest catch per unit effort was recorded. This was most likely due to the concentration of turtles in the wetland area where there was sufficient water depth. The catch per unit effort for 2018 and 2019, the two post-restoration years, fell in within the range of the 5 pre-restoration years. The relative

abundances of most species post-restoration appear similar to those before restoration, however, painted turtles (*C.picta*) have been found in much lower numbers.

METABOLIC AND STRESS PHYSIOLOGY OF COTTONMOUTHS.

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The analysis of glucocorticoid hormones has become an increasingly popular technique for measuring stress levels exhibited by animals facing environmental challenges. Glucocorticoids (GCs) are stress hormones released by the hypothalamus–pituitary–adrenal axis (commonly referred to as the stress response system) when an environmental stressor starts a chain reaction in the body signaling the release GCs to cope with the stressful situation. Responses to the increase of GCs have included increased locomotor performance, decreased immunity levels, and increased metabolism to provide immediate energy to overcome challenges. The relationship between metabolism and GCs has shown mixed relationships in mammals and birds with little research on reptiles, despite it being an important physiological response. The objective of this study was to gain an understanding of the relationship between metabolism and corticosterone (CORT; primary GC in reptiles) levels by comparing stress-induced changes in CORT with standard metabolic rates in cottonmouth snakes. Baseline blood samples were collected upon initial contact followed by a 60-minute confinement and a second blood sample. Plasma samples were analyzed using ELISA corticosterone assay kits. Snakes successfully blood-sampled in the field underwent metabolic testing via an open-flow respirometry system to determine rates of standard metabolism. This provides additional insight into the relationship between metabolism and stress levels in reptiles and the impacts of a stressful environment on a physiological response.

DETERMINING THE EFFECT OF CHINESE PRIVET (*LIGUSTRUM SINENSE*) INVASION ON THE OCCURRENCE OF GROUND-DWELLING HERPETOFAUNA SPECIES.

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In bottomland forests of the southeastern United States, Chinese privet (*Ligustrum sinense*) is a common and highly invasive shrub species that can substantially change native communities. Research has examined its impact on certain taxa, however virtually no studies have evaluated if it changes the habitat for smaller, forest ground-dwelling species such as amphibians (e.g., salamanders, toads, tree frogs) and small reptiles (litter-dwelling snakes and lizards). To address this knowledge gap, a study was initiated in 2019 on a large property (>7000 ac) with extensive bottomland forests on the Black Warrior River in Moundville, AL. Using this property, 40 long-term forest monitoring plots (circular plots, 10-m radius) were established across the property: 20 dominated by mature Chinese privet and 20 with little to no privet present. Over 9 months in 2019-2020, data will be repeatedly collected on key habitat variables related to ground-dwelling species including: coarse woody debris, leaf litter, soil moisture, and temperature. These data

will be compared to corresponding herpetofaunal occupancy data surveyed at the same plots. Herpetofaunal surveys will consist of constrained searches (conducted seasonally over one-quarter of the plot) and cover-board data (collected monthly). By gathering microhabitat and herpetofaunal data, associations between important habitat variables and ground-dwelling species will be determined. These results will provide a much better assessment of the relative risk that Chinese privet invasion has on these fauna.

MARINE LEECHES ARE ASSOCIATED WITH THE TUMOR DISEASE FIBROPAPILLOMATOSIS IN GREEN SEA TURTLES BUT NOT IN LOGGERHEAD SEA TURTLES.

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Fibropapillomatosis (FP) is a tumorous disease affecting all species of sea turtles, and is associated with the pathogen Chelonid herpesvirus 5 (ChHV5). Suggested ChHV5 vectors include the marine leeches *Ozobranchus branchiatus* and *Ozobranchus margoii*, but data on their associations with FP and ChHV5 are minimal. To establish relationships between leech infections, turtle hosts, and FP, we compared *O. margoii* and *O. branchiatus* in terms of (1) host preference, (2) ChHV5 viral load, (3) association with FP, and (4) seasonal variation in presence. Using DNA barcoding, we identified 77 leeches collected from turtles sampled in FL, USA and found strong host specificity. Out of the 77 sequenced leeches, ten *O. branchiatus* and five *O. margoii* were infected with ChHV5. Excluding two outlier leeches with >20,000 viral copies, the average viral load among ChHV5-positive leeches was 647 (± 179 SE) and did not differ among species. Using 12 years of turtle capture data from the Indian River Lagoon, FL, we found that leech parasitism was significantly correlated with FP and capture month in green turtles (*Chelonia mydas*), but not in loggerhead turtles (*Caretta caretta*). These results suggest that *O. margoii* and *O. branchiatus* may differ in their ability to detect FP or to transmit the causative agent of FP. This study is the first to show statistical evidence of: 1) a significant association between leech presence and FP in *C. mydas*, 2) no association between leech presence and FP in *C. caretta*, and 3) seasonal fluctuations in leech presence on *C. mydas*.

USING GEOMETRIC MORPHOMETRICS TO EVALUATE THE RELATIONSHIPS WITHIN THE GENUS *SIREN*.

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Sirens are enigmatic in their biology and behavior. These eel-like salamanders are unique morphologically, with slender bodies and absent lower extremities. Species in the genus *Siren* all maintain this body plan, with some slight variation in phenotype and body length. Additionally, these species sometimes have sympatric distributions. These attributes can present a challenge in differentiating among these species. Molecular data have allowed for construction of phylogenetic trees, inferring the evolutionary relationships among siren populations. This study will employ a novel approach in drawing comparisons between sirens found in different regions

of the southeastern United States. Using geometric morphometric analyses, I will quantify variation in morphology between different species within the genus *Siren*, as well as different populations within the same species, i.e. subspecies of *Siren intermedia*. Analyses will be specifically conducted using external head anatomy and the cranial skeleton. Shape data acquired through geometric morphometric analyses will be compared with the currently-recognized phylogenetic tree of the genus *Siren*. Reevaluating species within this genus may have conservation implications moving forward.

EXPERIMENTAL INFECTION WITH SNAKE FUNGAL DISEASE: THE MICROBIOME, DISEASE STATE, AND ANIMAL HEALTH THROUGH CLINICAL TIME.

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Snake Fungal Disease (SFD), caused by *Ophidiomyces ophiodiicola*, is an emerging infectious disease which affects snakes. SFD is correlated with alterations to the wild snake microbiome. This experiment will elucidate the impact of SFD and shedding on the host microbiome by examining these processes as ecological disturbances. We inoculated 22 field-collected Northern Watersnakes (*Nerodia sipedon*) with *O. ophiodiicola* to assess the effects of infection with SFD on body condition, fungal load, and host microbial assemblages. Snakes were maintained in mesocosms, containing a homogenous mixture of soils taken from snake capture locations, for 82 days. Snakes were weighed and measured weekly. Swabs were taken of each snake/mesocosm weekly and during shedding/mortality events. Swabs were utilized for quantitative PCR of *O. ophiodiicola* (fungal load) and high-throughput sequencing of the host microbiome to characterize microbial assemblages. A significant relationship was found between experimental time and fungal load in the inoculated population (GLMM, $X^2(1) = 28.59$, $p < 0.001$). No relationship between experimental time and fungal load was found in the control population (GLMM, $X^2(1) = 3.0159$, $p > 0.05$). A significant relationship was found between body condition and fungal load (GLMM, $X^2(1) = 5.4113$, $p < 0.05$). It is expected that SFD will alter the host microbiome and that shedding will briefly reduce microbial abundance and diversity of skin assemblages. Measuring changes in the snake microbiome throughout infection will improve our ability to predict the effects of SFD on snake populations and further understanding of host-microbiome-pathogen interactions.

DISTRIBUTION, OCCUPANCY, AND MERCURY LOAD IN TEXAS ALLIGATOR SNAPPING TURTLES.

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The alligator snapping turtle (*Macrochelys temminckii*) is declining throughout its range. In Texas, it is officially considered a Species of Greatest Conservation Need. Among the threats contributing to the decline of *Macrochelys* are habitat alteration and illegal harvest; therefore, understanding the species' distribution in relation to habitat variables and risks pertaining to

consumption are of value to conservation efforts. Over the next two years, we will replicate the methodology of a 1999-2001 survey of this species in Texas in order to attain contemporary comparative data. We will survey the locations utilized during the prior study, in addition to sites where the species has not been documented historically but could plausibly inhabit, to understand its statewide distribution. Morphometric data and biopsy samples will be collected from all trapped *Macrochelys*. We will analyze biopsies for concentrations of mercury and build models to examine mercury bioaccumulation across ontogeny. Because this species occupies a high trophic position and is long-lived, adult tissues likely contain high mercury concentrations. Contingent on results, these data may be useful for discouraging illegal harvest. Furthermore, we will correlate microhabitat and landscape-level factors with *Macrochelys* occupancy and distribution. Anthropogenic influences including dams, channelization, agriculture, urbanization, as well as direct human pressures such as trotline prevalence, are all factors hypothesized to inversely correlate with *Macrochelys* habitat occupancy. We will synthesize the microhabitat, watershed, and landscape factors to build a predictive occupancy model, and determine what relations, if any, there are between these variables and mercury concentrations, to inform future conservation efforts.

DISTRIBUTION, OCCUPANCY, AND HABITAT ASSOCIATIONS OF GRASSLAND-ASSOCIATED REPTILE AND AMPHIBIAN SPECIES OF GREATEST CONSERVATION NEED IN NORTHWEST ARKANSAS.

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Grasslands historically covered much of inland North America, and many species have adapted to the unique conditions found in grassland ecosystems, including fourteen reptile and amphibian species of conservation concern in Arkansas. Less than 1% of grassland habitat remains in Arkansas, with much historic grassland having been converted for urban and agricultural development, resulting in steep population declines for many grassland-associated species. Many reptile and amphibian species are difficult to detect, resulting in sparse, unreliable distribution and abundance estimates for many understudied species. Thus, we assessed the state of herpetofaunal communities in intact grassland habitat, as well in degraded and developed historic grassland habitat throughout Northwest Arkansas. We performed repeated, low intensity herpetofauna surveys, vegetation surveys, and quantified landscape characteristics to determine the influence of land use, hydrology, and vegetative communities on herpetofaunal community composition using hierarchical Bayesian occupancy modeling. We selected 34 sites ranging from fire-maintained intact grassland to active agricultural areas and performed repeated visual encounter surveys at each site over two field seasons. Preliminary results suggest intact grassland and restored grassland habitats can support diverse herpetofaunal communities, including grassland obligate species and species of conservation concern, while degraded and active agricultural historic grassland sites primarily supported less diverse communities composed of generalist species. Herpetofaunal community composition appears to be dictated largely by a few key landscape and habitat variables affected by land use. Our results provide insight into the effects of habitat degradation and restoration on grassland-associated herpetofauna and will help guide management planning.

DETERMINING PERCEPTIONS AND OPERATIONAL RESTRICTIONS ON GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) HABITAT MANAGEMENT.

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Over the last century, gopher tortoise populations have experienced an 80% decline, primarily as a result of habitat loss and degradation. Gopher tortoises are federally listed as threatened in west Alabama to southeastern Louisiana and are a candidate for protection throughout the remainder of their range. Life history traits, such as delayed sexual maturity and low offspring survival, make the gopher tortoise vulnerable to chronic demographic perturbations and impede population recovery efforts (e.g., population augmentation). Although these efforts have proved to be relatively successful, habitat management remains a necessary cornerstone for ensuring continued persistence of gopher tortoises at currently occupied sites. Gopher tortoises can persist in a variety of open upland habitats; however, habitat management at many sites does not currently promote those conditions associated with the optimal tortoise habitat necessary for supporting viable populations, even on areas purportedly designated for gopher tortoise conservation. Many management and conservation agencies are investing considerable resources in gopher tortoise conservation, but it is unclear how management decisions for tortoise habitat are being made and what factors influence the specific management techniques that are being implemented. This research proposes to use semi-structured interviews to determine land managers' perceptions regarding habitat management needs of gopher tortoises and to identify operational restrictions to implementing management actions that promote ideal tortoise habitat conditions in Georgia.

SEARCHING FOR A NEEDLE IN A HAYSTACK: USING COMPUTER ALGORITHMS TO DETECT REINTRODUCED LOUISIANA PINESNAKES CAPTURED WITH CAMERA TRAPS.

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Rare and secretive snake species with low occupancy and detection rates are expensive to monitor and study using traditional box traps. Advancements in camera trap technology have provided wildlife researchers with a more efficient technique to monitor such species, like the federally listed Louisiana Pinesnake (*Pituophis ruthveni*) which can be difficult to detect due to both rarity and a life history with secretive behaviors. However, the task of converting camera images to snake detections from extremely large image collections using these techniques is soul-crushing. Can computer algorithms help streamline the process? Here, we report the results of an eight-month camera trapping study using time-lapse triggered camera traps to detect snakes, in particular reintroduced *P. ruthveni*, in a Louisiana upland forest. We scored all camera images manually and then compared the manual snake detections to those generated using

computer algorithms. We report the false positive and false negative rates from our computer algorithms for all snake species detected with camera traps. Future research will focus on refining species-specific detection protocols using this technique and computer algorithm.

PARTNERSHIP DRIVEN CONSERVATION OF THE RETICULATED FLATWOODS SALAMANDER ON ESCRIBANO POINT WILDLIFE MANAGEMENT AREA, FL.

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The Reticulated Flatwoods Salamander (*Ambystoma bishopi*), a federally endangered species, has suffered significant population declines in recent decades, largely due to loss of suitable habitat. The species has been reduced to two known areas with multiple breeding wetlands in the Florida Panhandle- Eglin Air Force Base and Escribano Point Wildlife Management Area (EPWMA). A few additional sites with single occupied wetlands remain throughout the historic range. Through the U.S. Department of Defense's Legacy Management Program and the National Fish and Wildlife Foundation, Flatwoods Salamander conservation has been greatly accelerated on EPWMA with the addition of a five-year, large-scale recovery effort through the Readiness and Environmental Protection Integration (REPI) program. The Longleaf Alliance (LLA), Virginia Tech, Florida Fish and Wildlife Conservation Commission, and Eglin AFB have dedicated significant resources and provided invaluable assistance in working towards recovery goals. Methods include large-scale habitat restoration through prescribed burns, manual and chemical treatments to breeding habitat, headstarting of larval salamanders, and monitoring through larval sampling and tissue collection for an ongoing genetic study. Mark-recapture efforts are underway through drift fence monitoring to determine success of head-started animals. As of year two, 1,034 acres of prescribed burns have been completed on EPWMA and LLA staff have begun mechanical restoration in two historical breeding wetlands. In year one, 246 larvae were successfully headstarted and released back into natal ponds. Currently, there are 268 larvae in cattle tank mesocosms nearing metamorphosis. Through ongoing collaboration with partners, LLA will continue working toward conservation goals for the Reticulated Flatwoods Salamander.

SUMMER ACTIVITY PATTERNS OF SNAKES IN SHORTLEAF PINE FORESTS.

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Snake activity patterns are influenced by a number of factors including both abiotic (e.g., temperature) and biotic factors (e.g., seasonal peaks coinciding with mate searching behavior). However, the consistency of snake activity patterns across space and time as well as species are not well understood. We examined the daily activity patterns of five snake species in shortleaf pine forests in East Texas: Copperhead (*Agkistrodon contortrix*), Coachwhip (*Coluber flagellum*), Racer (*Coluber constrictor*), Western Ratsnake (*Patherophys obsoletus*), and Western Ribbonsnake (*Thamnophis proximus*). From mid-May to mid-July across two years (2018 and 2019), we deployed boxtraps in two shortleaf pine forests experiencing different management

regimes: 1) South Boggy Slough Conservation Area (subjected to frequent thinning and burning), and 2) Stephen F. Austin Experimental Forest (subjected to infrequent thinning and burning). Traps were checked daily and we examined daily patterns of activity across the trapping period. Copperheads generally exhibited the broadest and most consistent spatiotemporal activity across both the trapping periods and sites types, while Coachwhips were trapped the most infrequently across space and time. Western Ribbonsnakes and Copperheads were trapped more frequently at the Stephen F. Austin Experimental Forest compared to South Boggy Slough Conservation Area. These results suggest that activity patterns exhibit spatial variability across both space and time. The variation in activity patterns across each forest type may be attributed to microclimate differences in their abiotic factors. Future efforts will explore species-specific relationships to abiotic factors (temperature, rainfall) across each site and year.

ASSESSING THE EFFECTS OF WARMING AND DRYING ON AMPHIBIAN LARVAE IN AN ARTIFICIAL POND EXPERIMENT.

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Anthropogenically driven shifts in climate put multiple pressures on many ecosystems. An important knowledge gap exists in our understanding of how changes in temperature and precipitation interact, and whether these interactions result in sub-lethal effects on sensitive species, such as amphibians. The primary goal of this study was to examine the developmental responses of two species of amphibian larvae to the individual and interactive effects of warming temperatures and increased drying rate of larval habitat - both plausible consequences of climate change in the eastern United States. We used replicated mesocosms to rear two anuran species: wood frogs, *Lithobates sylvaticus*; and spring peepers, *Pseudacris crucifer*. We evaluated survivorship, body size, and time to metamorphosis in response to experimentally manipulated temperature and drying over 12 weeks. Our manipulations created warming treatments that were on average 2 ± 4 °C higher than controls. Wood frogs had significant increases in body mass and shorter time to metamorphosis for warmed treatments. Spring peepers had significant increases in body mass for warmed treatments; however, we did not see significant differences in time to metamorphosis between treatments. We also saw a strong relationship between treatment and survivorship in wood frogs that was not seen for spring peepers. Temperature had the greatest effect on growth and development time in larval amphibians, but the combined effects of temperature and drying were also significant. This research demonstrates how amphibians may be affected by multiple, potentially interacting, climate pressures which are expected to occur more frequently with climate change.

BATRACHOCHYRTIUM SALAMANDRIVORANS IN THE CUBAN TREEFROG (OSTEOPILUS SEPTENTRIONALIS).

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Batrachochytrium salamandrivorans (*Bsal*) is a fungus that infects amphibians, causing skin lesions and eventually death. It has been thought that *Bsal* leads to asymptomatic carrier states in frogs and does not cause disease as it does in susceptible salamanders. Therefore, we hypothesized that anurans would not develop disease when challenged with concentrations of *Bsal* that result in disease in salamanders. To test this hypothesis, we exposed Cuban treefrogs (*Osteopilus septentrionalis*) to varying concentrations of *Bsal* zoospores. Frogs exposed to 5×10^6 zoospores tested positive for *Bsal* with qPCR starting at four days post-exposure and began showing signs consistent with chytridiomycosis two weeks post-exposure. Post-mortem histopathology was performed on frogs from this group, confirming *Bsal* as the causal agent. *Bsal* was also isolated from the infected frogs and successfully used to infect Eastern Newts (*Notophthalmus viridescens*), fulfilling Koch's postulates. This experiment represents the first documentation of *Bsal* chytridiomycosis in an anuran species.

CLINICAL PATHOLOGY OF *BSAL* CHYTRIDIOMYCOSIS: HEMATOLOGICAL, BIOCHEMICAL, AND SERUM PROTEIN ANALYSES OF INFECTED *TARICHA GRANULOSA*.

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Batrachochytrium salamandrivorans (*Bsal*) is a recently discovered pathogen that is of global concern because of its potential to cause high mortality in amphibians, especially salamander species. Although little has been reported on the pathophysiological effects of *Bsal*, studies have made headway in understanding the pathogenesis of *B. dendrobatidis* (*Bd*), a similar fungus that has led to global amphibian population declines throughout the world. Studies found that electrolyte losses occur in amphibians infected with *Bd* from loss of osmoregulation through extensive skin pathology, and it is hypothesized that changes in electrolyte concentrations lead to paralysis and cardiac arrest. In our study, we hypothesize that species susceptible to *Bsal* chytridiomycosis would have similar losses in electrolytes, because like *Bd*, *Bsal* damages the epidermis and may affect skin osmoregulation. To test this hypothesis, we collected blood from the hearts of a larger susceptible species, *Taricha granulosa*, at necropsy using heparinized capillary tubes. A blood smear was prepared, and a portion of whole blood was added to Natt Herrick's solution for total blood cell counts. The remaining blood was centrifuged and plasma was collected for blood chemistry profiles and protein electrophoresis. Overall, the results suggested that *Bsal* may compromise osmoregulation and cutaneous respiration as well as lead to a systemic stress response in infected individuals. By understanding the pathogenesis of *Bsal*, we

aim to gain insight in developing treatment options and opportunities to mitigate and prevent spread of disease.

THREE'S COMPANY: OBSERVATIONS OF A NON-NATIVE MAP TURTLE (*GRAPTEMYS PSEUDOGEOGRAPHICA*) OCCURRING SYNTOPICALLY WITH TWO ENDEMIC *GRAPTEMYS* IN THE PEARL RIVER, MISSISSIPPI.

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Introductions of non-indigenous species (NIS) can have numerous effects on native wildlife, and the rate of NIS introductions is increasing. Humans commonly move turtles and tortoises across geopolitical and geographic boundaries through food markets and the pet trade. One group commonly encountered in the pet trade is the genus *Graptemys*. In the late 1980s, *Graptemys pseudogeographica* (False Map turtle) was documented outside of its native range in the Pearl River near Jackson, Mississippi. Through replicated visual surveys during the summer of 2017 and 2018, we sought to determine if the species still occurs in the Pearl River, and if so, document their distribution and density. We also wanted to evaluate the historical avenues for introduction via natural means or through human means. We found that *G. pseudogeographica* persists in the Pearl River near Jackson, but densities were much lower (0.22/river km; 0.5% of all observations) compared to the two native *Graptemys* species. Based on topographic profiles and historical hydrologic records, we strongly refute a prior hypothesis that *G. pseudogeographica* naturally immigrated into the Pearl River from a neighboring drainage during the Easter Flood of 1979. Rather, we suggest that introduction via the release of unwanted pets is a more parsimonious conclusion. Because the lineage is shallowly diverged on an evolutionary scale, it seems likely that hybridization is occurring between *G. pseudogeographica* and *G. oculifera*. Consequently, genetic introgression of the non-native *G. pseudogeographica* genome is likely occurring into *G. oculifera*, a federally threatened species. This is a conservation concern to be further evaluated.

ASSESSING THE CURRENT DISTRIBUTION OF THE SUWANNEE ALLIGATOR SNAPPING TURTLE (*MACROCHELYS SUWANNIENSIS*) IN GEORGIA.

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The Suwannee Alligator Snapping Turtle (*Macrochelys suwanniensis*) was recently described as a separate species, based on morphological and genetic distinctions from the Alligator Snapping Turtle (*Macrochelys temminckii*). The distribution of *M. suwanniensis* has not been thoroughly investigated in Georgia, as the majority of *Macrochelys* records in the state are of *M. temminckii*, so understanding their distribution of *M. suwanniensis* is vital for successful conservation. In 2018, we began surveying for *M. suwanniensis* throughout the Suwannee River drainage, by setting baited hoop traps in river mainstems, tributary creeks, and oxbow lakes. Areas that lacked previous survey efforts or would constitute range extensions were the primary focus. From 2018-2019 we captured 33 individuals (13 males, 9 females, 1 sex undetermined, and 10 juveniles)

during 37 capture events with a catch-per-unit effort (CPUE) of 0.08. Mean adult mass, mean adult SCL, and CPUE were all lower in the river mainstems (12.1 kg, 373 mm, 0.05) than in less navigable water (26.7 kg, 472 mm, 0.15), possibly due to historical overharvest on the large rivers. We documented turtles in six new Georgia counties and confirmed the presence of *M. suwanniensis* in Georgia's upper Suwannee drainage for the first time since 1985. The scarcity of records from the Okefenokee area and upper Suwannee River could be due to river characteristics or lasting impacts from historical overharvest, and while the size and demographics of this persisting population remains unknown, there is recent evidence of reproduction.

AMPHIBIAN AND REPTILE DISEASE SURVEILLANCE AND RESEARCH AT THE SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY.

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The Southeastern Cooperative Wildlife Disease Study (SCWDS) was established in 1957 at the University of Georgia to investigate wildlife diseases. As a state-federal cooperative supported by 18 state wildlife agencies and federal agency partners, SCWDS studies wildlife diseases and their impacts on wildlife health. SCWDS supports partner agencies through mortality investigations, surveillance, research, and training across multiple disease systems and taxonomic groups, including herpetofauna. Here, we give examples of SCWDS activities assisting managers with herpetofauna health in the Southeast to highlight this cooperative model. SCWDS regularly receives herpetofauna for necropsy and samples (e.g., biopsies, swabs) for diagnostic testing or pathogen surveillance. For example, investigation of a mortality event involving illegally trafficked Eastern box turtles (*Terrapene carolina carolina*) revealed coinfections with *Mycoplasma* sp., *Ranavirus*, and herpesvirus, along with bacterial pneumonia and aural abscesses, in multiple individuals. Also, testing for snake fungal disease (*Ophidiomyces ophiodiicola*) confirmed 31 cases from 102 snake submissions since 2015. Research is another integral component of SCWDS and recent examples of herpetological studies conducted with partnering agencies include 1) free-living and translocated gopher tortoise (*Gopherus polyphemus*) health in Georgia and Florida; 2) an epidemiological investigation of translocated gopher tortoises following an unexplained mortality event; 3) characterization of *Salmonella* spp. in aquatic turtles from Georgia; 4) *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, and *Ranavirus* surveillance in Costa Rican salamanders; and 5) the first detection/molecular characterization of *Ranavirus* in African amphibians. SCWDS looks forward to our existing and future partnerships focused on herpetofauna health in the Southeast.

DOES SPECIES LIFE HISTORY INFLUENCE VULNERABILITY OF SNAKE EGGS TO ANT PREDATION?

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The red imported fire ant (RIFA) is an aggressive non-native ant species that is expanding its range across the southeastern United States. RIFA are reported to predate nests of multiple reptile species in the U.S., including snakes, and have been cited as a threat to declining oviparous snake species in the Southeast. We are compiling data on field and captive observations of nesting and incubation to gain a better understanding of nesting ecology of southeastern snake species and determine whether traits relating to nesting and reproduction (i.e., clutch size, pip-to-hatch time, nest site selection, etc.) predict species declines. We hypothesize that snakes that spend longer in the egg after pipping or nest in particular microhabitats are more vulnerable to predation by RIFA. In a pilot study, we aimed to test whether RIFA predation was higher in a species that spends relatively longer in their eggs (*Lampropeltis getula*; N = 12) than one that emerges relatively quickly (*Coluber constrictor*; N = 2). Preliminary experimental results have been inconclusive, but we plan to repeat the field experiment with a larger sample size and conduct additional experimental studies to answer basic questions about vulnerability of snake eggs to RIFA predation.

MAKING INFORMED MANAGEMENT DECISIONS BASED ON PREVALENCE OF *CRYPTOSPORIDIUM SERPENTIS* IN THE WILD.

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Cryptosporidium serpentis (hereafter, "Crypto") is a highly contagious protozoan that effects the gastrointestinal pathway of snakes. The primary clinical sign is food regurgitation, and mortality is frequent due to complications from emaciation. There is no known cure for this pathogen. Crypto is primarily known from captive collections of snakes. However, little is known about the prevalence of this disease in naturally occurring snake populations. The only ongoing snake repatriation in Florida is for the Federally threatened eastern indigo snake (*Drymarchon couperi*), for which snakes are being bred and reared at Central Florida Zoo's Orianna Center for Indigo Conservation (OCIC). In 2015, OCIC first detected Crypto at their facility and followed all proper quarantine procedures to reduce transmission potential among animals at the facility. Because eastern indigo snakes may harbor Crypto while appearing asymptomatic, all repatriation animals are tested multiple times before release to assure they are not carriers of Crypto to wild populations. To make more informed management decisions, we measured the prevalence of Crypto among wild snakes at the repatriation site, The Nature Conservancy's Apalachicola Bluffs and Ravines Preserve in Bristol, Florida. In 2019, we captured and sampled 55 snakes (11 species) from the study site. All samples were processed by the same laboratory, using the same methodology as those at OCIC, and all animals were negative for Crypto. Because Crypto is not apparent on the landscape, repatriation of infected snakes may not be a valid management consideration, even if infections are subclinical. In addition to informing management decisions, this study adds to the scarce knowledge of naturally occurring Crypto among wild snakes.

ASSESSING THE DISTRIBUTION AND POPULATION STATUS OF THE NEUSE RIVER WATERDOG (*NECTURUS LEWISI*).

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The Neuse River Waterdog (*Necturus lewisi*) is an aquatic salamander endemic to the Neuse and Tar- Pamlico River basins of eastern North Carolina. As listed in the North Carolina State Wildlife Action Plan, *N. lewisi* is experiencing range contraction and decline in overall abundance, likely caused by habitat loss via upstream land use change. North Carolina Wildlife Commission surveys conducted 2011- 2015 prompted the United States Fish & Wildlife Service to issue a Species Status Assessment in 2017 and propose *N. lewisi* as Federally “Threatened” in 2019. The objective of this study is to provide baseline occupancy and abundance data to guide management efforts. Analyses will utilize multi-year capture-mark-recapture (CMR) data collected throughout the Neuse and Tar-Pamlico River basins. During the 2018-2019 field season, 65 locations were surveyed, yielding *N. lewisi* detections at 32 locations. Preliminary occupancy models using detection rates and capture success indicate stream habitat instability caused by agricultural land use and urban sprawl is the primary source of apparent population declines. Additional survey data collected in the 2019-2020 sampling season will improve predictive models of occupancy and abundance.

REPRODUCTIVE ENERGETICS OF MUSK TURTLES (*STERNOTHERUS ODORATUS*).

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In oviparous species, parental care is often limited to the energy allocated to embryos prior to oviposition. Energy used by reproducing females is allocated towards, nutrient intake, fertilization, vitellogenesis, number and size of eggs, calcified shells, oviductal egg retention (retaining eggs within the oviduct after fertilization), and nesting. Oviductal egg retention in turtles ranges from two weeks to half a year and permits flexibility in the timing of oviposition and therefore is a potentially pivotal stage in the reproductive phenology of turtles. The metabolic cost of oviductal egg retention in Eastern Musk Turtles, (*Sternotherus odoratus*), was investigated by measuring the metabolism of females prior to and following oviposition. Gravid female metabolic rates were adjusted for clutch metabolic rates and were significantly elevated from both males and from non-gravid females, indicating an associated energetic cost for egg retention. The metabolism of females did not change across the period of oviductal egg retention, however, a significant difference in metabolism was observed between pre-oviposition and post-oviposition (40% increase in pre-oviposition metabolism). Costs associated with egg retention were correlated with clutch mass, but not correlated with clutch size or female body mass. These results suggest there are costs associated with oviductal egg retention, however other components of reproduction need to be quantified to determine the relative energetic costs to females.

INCREASED MANAGEMENT FREQUENCY DECREASES LIZARD ABUNDANCE IN FOREST ECOSYSTEMS.

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East Texas forests have experienced dramatic changes over time due to widespread implementation of applied silviculture and anthropogenic variation in disturbance regimes (e.g., prescribed fires, silvicultural techniques). Small ground-dwelling Scincids, (*Scincella lateralis*), are generally found in high abundances in East Texas pine forests, and consequently skink abundance may be influenced by such anthropogenic disturbance variation. To assess the impact of forest management on skink abundance, we performed 40 time constrained surveys at sites of contrasting forest management frequencies: Stephen F. Austin Experimental Forest (low-frequency) and South Boggy Slough Conservation Area (high-frequency). Environmental variables correlated with skink occupancy were measured at each site to determine abiotic factor variation resulting from forest management regime frequencies. Skink abundance varied across these forest types, with 15 observed (0.0125 skinks per person minute) at SBSCA and 23 observed (0.0191 skinks per person minute) at SFAEF. Environmental factors also varied with frequency; SFAEF's canopy openness and leaf litter depth averaged 7.9348 percent and 14.1087 millimeters, respectively, while averaging 40.0833 percent and 4.6667 millimeters at SBSCA. A principal component analysis of the environmental variables revealed that 75.51 percent of the variation between the sites can be explained by leaf litter depth. A significant positive correlation of leaf litter depth and skink abundance was observed (Adj. $r^2 = 0.1678$, p -value = 0.04). Therefore, increased forest management frequency can have a substantial impact on the environmental parameters that affect habitat quality, subsequently contributing to the persistence and abundances of the taxa that inhabit these systems.

HELLBENDERS IN KENTUCKY: CURRENT STATUS AND RESEARCH EFFORTS.

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Although extensively monitored in some southeastern states, limited data are available for Hellbenders in Kentucky. In this presentation, we describe the current status and future research efforts for Hellbenders in Kentucky. Using Hellbender observations submitted to the Kentucky Department of Fish and Wildlife Resources, we found that Hellbenders were historically distributed across most of Kentucky from the Big Sandy River in Pike County westward to Kentucky Lake and the Tennessee River. Yet, current population size tends to be quite low and most populations are likely declining. Furthermore, recruitment, as indicated by the presence of eggs and juveniles, is only known from one stream in the state. Current research efforts include an extensive state-wide eDNA survey to determine their current distribution, as well as assess how seasonal variation in breeding behavior and distance sampled downstream of hellbender

habitat affect eDNA concentrations. Results from this study will facilitate future investigations on recruitment and population genetic structure in Kentucky waterways.

DENSITY-DEPENDENT MORTALITY OF EASTERN NEWTS EXPOSED TO *BATRACHOCHYTRIUM SALAMANDRIVORANS*.

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Batrachochytrium salamandrivorans (*Bsal*) is an emerging chytrid fungus that is spreading across Europe, causing rapid declines in salamander populations. *Bsal* has yet to be confirmed in North America; however, it is likely only a matter of time before it arrives. A first step to understanding the possible epidemiology of *Bsalis* investigating whether pathogen transmission and subsequent disease-induced mortality is density-dependent. We performed our experiment in a controlled research facility with eastern newts (*Notophthalmus viridescens*) housed in 20 1-m² tanks at 14°C with three initial *Bsal* infection prevalence (12.5, 25, 50%) and 11 host density (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 32) treatments. Infected individuals were exposed to a high dose of zoospores (2.56×10^6) for 24 hours to guarantee infection then placed into tanks with uninfected susceptible individuals. Newts were uniquely marked and swabbed every three days for 60 days to determine infection status. All susceptible individuals eventually became infected with *Bsal*, indicating transmission was 100% among all treatments. However, tanks with higher initial infection prevalence reached 100% infection faster than those with lower initial infection prevalence. Within a density, tanks with higher infection prevalence also experienced more rapid mortality than lower infection prevalence. Disease-induced mortality was strongly density-dependent at lower host densities (2 – 8 newts / m²) and reached asymptotic maximum at higher densities, suggesting a Holling's Type II functional response. Densities up to 8 newts / m² are common in the eastern United States. Although reducing newt density might not affect transmission of *Bsal*, it reduced overall mortality, hence be a viable disease management strategy.

CREATING A NON-NATIVE SPECIES PREDICTIVE MODEL BASED ON LAND-USE CATEGORY.

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Human development can promote invasibility in species that thrive within urbanized landscapes. Florida is particularly susceptible to non-native species' success because of characteristics that make these species more invasible. An abundance of natural habitat types and disturbed landscapes offer refugia for introduced species. The Brahminy blind snake (*Indotyphlops braminus*) is the most successfully spread snake species in the world and is found throughout Florida. Similarly, the Greenhouse Frog (*Eleutherodactylus planirostris*) is a well-established

non-native in Florida. There are several factors that aid in both species' successful invasion, including their small size, generalized diet, and efficient reproductive styles. Most sightings of both *I. braminus* and *E. planirostris* take place in areas with ornamental plants, such as nurseries and gardens. So, while they may be abundant statewide, their distribution into other habitats may be limited. Currently, it is unknown if these animals naturalized across novel and native landscapes or limited to their introduction. This study aims to create a model depicting population density of both *I. braminus* and *E. planirostris* across habitat types in Southwest Florida. Using population densities collected in representative habitats in Lee County and existing land use data, a choropleth map will be created showing species' densities over the county. These data will show whether these species are moving beyond introduction points along a gradient of habitat modification. In addition, these data will be used to estimate the overall invasiveness of the species.

A RE-EXAMINATION OF BODY SIZE REDUCTION IN *PLETHODON*.

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Climate change is rapidly altering Earth's natural systems. Some organisms, such as amphibians, are particularly vulnerable to these detrimental effects. One documented response to climate change by some taxa is to reduce body size. Recent research has found evidence for reduction in body sizes of several salamander species from the genus *Plethodon*, including one of the largest species, *Plethodon yonahlossee*. However, the sample size for *P. yonahlossee* was small (n=36) relative to the other *Plethodon* species examined. Therefore, we re-examined the possibility of body size reductions in *P. yonahlossee* populations from North Carolina over time. We measured and Snout-Vent Length (SVL) and head morphology of 189 specimens collected from 1979-2004 in North Carolina. We were also able to account for museum shrinkage when data was available. We found evidence for declines in SVL over time. We also found significant differences in head morphology; however, site rather than time was significant. We also found original SVL measurements made at or near the time of preservation was significantly larger than measurements made in 2019, indicating museum shrinkage had taken place. The significant decline in SVL over a 25-year period represents a rapid phenotypic change, adding further evidence to the literature of in body size reduction as a strategy of salamanders in response to climate change.

SEX DETERMINATION METHODS FOR *AMBYSTOMA MEXICANUM*.

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Axolotl (*Ambystoma mexicanum*) is a critically endangered, aquatic salamander species. Despite being endangered in the wild, laboratory colonies of axolotl are bred for research and are studied extensively for developmental and embryological biology. This species has a genetic sex determination system (ZZ/ZW system), but have also been shown to change functional sex in

differing environmental conditions implies that there are more determining factors to the sex of the species than genetics. For example, in many cold-blooded vertebrates and especially reptiles, sex determination and sex reversal are influenced by temperature, and understanding how and when sex is influenced by environmental conditions would prove useful especially for effective implementation of conservation breeding programs. In this study, we investigated sexual dimorphism and steroid hormone metabolites in three age classes of axolotl as methods for determination of sex in *A. mexicanum*. Determining sex at a younger age would benefit researchers and culturists especially when organisms take a long time to develop and become sexually mature. Our results indicate that adult males have significantly longer tails in relation to body size compared to females, which suggests sexual dimorphism as a possible sex determination method, however this is indeterminate in the younger age sizes. In addition, steroid hormone analysis of fecal and tissue samples revealed significantly higher testosterone to 17 β -estradiol concentration ratios in female axolotl than male axolotl. Both the sexual dimorphism and endocrinology findings provide a strong foundation for continued development of less invasive sex determination methods for endangered and vulnerable species.

ECOMORPHOLOGICAL VARIATION IN SHELL SHAPE OF STRIPE-NECKED MUSK TURTLES (*STERNOTHERUS PELTIFER*).

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With six species of musk turtles currently recognized, the genus *Sternotherus* composes a significant component of Alabama's turtle diversity. *Sternotherus* morphology varies substantially along a continuum of shell shape from flattened to domed. This body shape divergence is presumably a consequence of local adaptation; species from swift streams with ample bedrock likely evolved to be flatter (e.g., *S. depressus*) than species from slower, sandy or muddy streams (e.g., *S. carinatus*). However, as much is currently unknown about the ecology and evolution of the genus, adaptive significance of shell shape variation remains an unanswered question. The stripe-necked musk turtle, *S. peltifer*, occupies a wide variety of stream habitats in Alabama and demonstrates significant intraspecific morphological variability in the Cahaba River of central Alabama, including a flattened phenotype reminiscent of *S. depressus*. This variation may be associated with location relative to the Fall Line, a geological boundary demarcating a rapid change in river bottom from bedrock to sand. Similar clines in shell shape exist for several species of Emydid turtles, but whether this convergent shell phenotype has resulted from a similar adaptive process in *S. peltifer* is undetermined. We trapped *S. peltifer* throughout the Cahaba River drainage in summer 2019 to quantify shell morphology and conducted a PCA to assess variation in relative shell height. Multiple regression was used to assess relationships among relative shell height, sex, and location relative to the Fall Line. This study contributes to a growing body of knowledge regarding the ecology and evolution of bottom-walking turtles.

BORN INTO DARKNESS: THE NESTING AND DEVELOPMENT OF THE NORTHERN SLIMY SALAMANDER (*PLETHODON GLUTINOSUS*) IN A NORTH ALABAMA CAVE.

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Cave ecosystems are notoriously difficult to study. Consequently, there often are significant gaps in our knowledge related to the ecology and life history for many species who inhabit them. The Northern Slimy Salamander (*Plethodon glutinosus*) is a direct-developing plethodontid salamander common in mesic forests throughout the eastern North America. In karst regions, this species also occurs in caves. Despite their abundance, little life history information exists related to their use of caves, particularly with respect to reproduction. We conducted weekly visits (since September 2019) to a privately-owned cave in Marshall County, Alabama to study the reproductive ecology and parental care of *P. glutinosus*. We tracked 6 nests with attending females through hatching until the young left the nests to better understand nesting ecology, parental care, development, and reproductive success. Nests with 3 to 14 eggs were found in horizontal crevices in the cave walls 20-40 meters from the cave entrance. Three nests experienced 100% mortality before hatching, possibly due to predation. Based on staging of developing embryos, we estimate the incubation period ranges around 80 days. Young remained with females for up to 8 weeks; considerably longer than previous reports for the *P. glutinosus* complex in the literature. We now are implementing a mark-recapture study of all *P. glutinosus* in this cave system to study demography, growth, movements, and microhabitat preferences and plan to study reproductive ecology of this population gain next breeding season.