



# JMIH 2023

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## ABSTRACT BOOK



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## **Abstracts**

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# Abstract Book

## The Value of Long-Term Time-Series for Fishes: The Naturalist's Revenge

Kenneth Able

We lack a basic understanding of the natural history of fishes even for those that are ecologically and economically important. This has come about because there is a trend for a reduced focus on natural history in lieu of the more glamorous technologies in recent decades. One natural history approach that addresses this need is for long-term studies of the early life history. As an example, I review the advantages of a long-term larval fish survey inside and estuarine inlet in southern New Jersey. This 33 year sampling program has occurred weekly on night flood tides to measure the species composition, abundance, and length of resident and ingressing larval fishes. To date, we have used this fixed protocol to describe the early life history of several species, examine changes in abundance of commercially and recreationally important species relative to fisheries management, and, most recently, the effect of climate change on the larval fish fauna. Of these, climate change clearly has a profound effect on many species and is resulting in a change in the fish fauna of the study estuary with a decrease in the number and abundance of northern species and an increase for southern species. This and other patterns have been confirmed from other larval fish surveys elsewhere along the East Coast of the US and help support the critical value of these long-term natural history studies.

## Comparing Computer Assisted Pattern Recognition Software using Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) Photographs

Carissa Adams, Emily Gray, Sarah Ebert, Matthew Gacheny, John Holloway, Shane Welch, Jayme Waldron

Computer assisted pattern recognition software benefits herpetofaunal monitoring programs by increasing the speed at which photographs used to identify individuals can be analyzed. We compared the utility of two open-sourced computer assisted pattern recognition software packages, Wild-ID and HotSpotter, using dorsal head photographs of Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*; EDB). These photographs were collected by AHDriFT camera arrays (N=20) armed with two Reconyx Hyperfire 2 trail cameras located on a long-term EDB monitoring site in South Carolina. Since 2008 passive integrative transponder (PIT) tags and scale cauterization have been used to mark individual EDBs and starting in 2021 we began creating an all-size class photographic database of captured EDBs head scale patterns (N=314). To date, we have processed 37 AHDriFT photographs, and have identified six individual EDBs (five adults and one neonate). The results of this study will demonstrate the potential utility of AHDriFT camera monitoring in individual-based herpetofaunal monitoring programs and help identify software packages that are best suited to analyzing rattlesnake photographs. Establishing a better understanding of the nuances of camera traps and pattern recognition software will improve our ability to estimate demographic parameters while benefiting species monitoring programs.

## Is eDNA an Effective Method for Detecting Alligator Snapping Turtles

Cory Adams, Daniel Saenz, Christopher Schalk, David Rosenbaum, Joseph Apodaca

The alligator snapping turtle (*Macrochelys temminckii*) is being proposed for federal listing as threatened under the Endangered Species Act. Current information on the status, distribution, and abundance of alligator snapping turtles is lacking, which makes development of a comprehensive conservation plan difficult. Development of these management actions is dependent on reliable methodologies that can quantify the presence of alligator snapping turtles. Traditional survey methods (hoop traps) have been proven to successfully capture alligator snapping turtles; however, they can pose a threat to target and non-target species. We sought to evaluate the efficacy of using environmental DNA (eDNA) to determine the presence of alligator snapping turtles

as an alternative to traditional survey techniques. To test this, we surveyed 38 sites by collecting 3L of water per site to test for eDNA. Each site was concurrently surveyed using traditional hoop traps. At 21 sites (55%), we had congruence in detection outcomes of alligator snapping turtles with both eDNA and trapping efforts. Of the 28 sites where we captured turtles in traps, we only detected turtle eDNA in 12 of those sites (42%). At only one site did we fail to capture a turtle but had a positive result for eDNA. We successfully used eDNA techniques to detect alligator snapping turtles, however understanding the variability in detection outcomes in the context of habitat covariates would further refine this technique to be used more broadly by natural resource managers.

## Interactive Energetic Costs of an Immune Response in Ectotherms: Thermal and Metabolic Consequences of Apparent Ophidiomycosis in a Winter-active Snake

Joseph Agugliaro, Craig Lind, Jason Ortega, Terence Farrell

Ophidiomycosis (snake fungal disease), caused by *Ophidiomyces ophidicol* (Oo), afflicts wild snakes across a large geographic range in North America and beyond. A common correlate of ophidiomycosis is emaciation, possibly linked to energetic costs of the immune response to Oo. Pygmy Rattlesnakes (*Sistrurus miliarius*) from a winter-active population in central Florida have coexisted with Oo for decades, and demonstrate increased ophidiomycosis prevalence and clinical sign severity in winter. Previously, we documented increased resting metabolic rate (RMR) associated with ophidiomycosis across a range of biologically relevant temperatures in winter *S. miliarius*. Furthermore, we documented an increased frequency of basking behavior consistent with a febrile response and modeled possible effects of simulated fever scenarios on energy expenditure. In this study, we estimated the thermal and metabolic costs of ophidiomycosis by sampling body temperature (Tb) over two winters. Snakes were held in outdoor enclosures and implanted intracoelomically with thermal dataloggers as a function of ophidiomycosis status (based on clinical signs and qPCR) to directly assess the existence of a febrile response to Oo infection. During the second winter, we also measured the thermal sensitivity of RMR (CO<sub>2</sub> production rate across 10-35°C) in Tb-monitored snakes. Our data reveal significantly increased Tb in snakes with apparent ophidiomycosis during midday hours. Additionally, our individual-based thermal reaction norm model relating RMR to Tb demonstrates a positive relationship between total energy expenditure and clinical sign severity. We present results of modeling efforts illustrating how different Tb sampling approaches may bias energetic cost estimates of fever in ectotherms.

## Phylogeography of the Guiana Shield Clade of *Trichomycterus* Pencil Catfishes

Daniel Akin, Courtney Weyand, Matt Buehler, Jonathan Armbruster

Trichomycterid catfishes offer an opportunity to study vicariance by river capture, as they are rheophilic headwater specialists distributed throughout most of South America, which provides a model for biogeographical reconstructions of South American river drainages. In this study, we analyzed the phylogenetic relationships of the *Trichomycterus guianensis* clade and other members of the *Eremophilus* lineage using five loci and generated a time-calibrated phylogeny. Using our data, we find additional support for a hypothesized Grand Pakaraima River, which formerly would have connected the modern day upper Ireng, Potaro, and Mazaruni Rivers into one river flowing north into the present day Caroní River. Additionally, we suggest a likely dispersal route to the Guiana Shield for the *T. guianensis* clade via the Proto-Berbice River. Furthermore, we estimate the period of diversification in the *Eremophilus* lineage to coincide with the uplift of the Andes roughly 10 Mya. Using this estimation, we put forward a novel hypothesis that the ancestors to this group were lowland forms that diversified as the Andes lifted and were eventually isolated into multiple populations. Given the incomplete taxonomy and gaps in our understanding of the natural histories of many species within the Trichomycterinae and *Trichomycterus*, we echo the sentiment of other experts in suggesting that thorough phylogenetic studies of geographically

distinct clades and precise species descriptions must be completed before beginning the herculean effort of deeper taxonomic revision for both the Trichomycterinae and Trichomycterus.

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## Understanding the Drivers and Consequences of Live Birth in Fishes

*Ayomikun Akinrinade, Sarah Friedman, Christopher Martinez*

Live birth, or viviparity, is an adaptation that has independently evolved numerous times across the tree of life. One common explanation for the relative frequency with which viviparity has arisen is the “cold-climate hypothesis”. It posits that in cold climates, organisms experience greater pressure to become livebearers, allowing organisms to behaviorally modulate the temperature of their offspring and speed up development time. Although this hypothesis has been corroborated by macroevolutionary studies across squamates, it remains untested whether these same principles apply to other ectothermic organisms. Here, we leverage modern phylogenetic comparative methods to test if the cold-climate hypothesis is supported in perciform fishes and if the evolution of viviparity has consequences on body shape evolution. We found that the evolution of viviparity was not correlated with current colder climates, but instead, viviparity evolved during the warmest time periods on Earth—a striking contrast to the cold-climate hypothesis. Compared to oviparous fishes, viviparous species exhibit limited body shape variation and converge on slimmer and deeper body shapes. Overall, this study demonstrates that the cold-climate hypothesis is not a universally explanatory hypothesis for ectotherms and may impart strong selective pressure on body shape evolution.

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## Molecular Evolution of Lens Crystallin Genes in Frogs

*Ron Alesker, Rayna Bell, Matthew Fujita, David Gower, Jeffrey Streicher, Kate Thomas, Ryan Schott*

The ocular lens, a structure that refracts incoming light onto the retina, plays an important role in the initial steps of vertebrate vision. The refractive index of the lens is influenced by its constituent crystallin proteins, meaning that differences in crystallin gene sequences, and the proteins they encode, may help species meet the refractive requirements of their unique visual environments (dependent on lifestyle and habitat). However, to pinpoint the potential adaptive importance of ocular lenses within the broader context of visual evolution, the molecular evolution of lens crystallin genes must be studied. Using a dataset of 80 eye transcriptomes and 40 genomes, we looked for 26 crystallin genes that are potentially expressed in frog eyes. Maximum likelihood gene trees were inferred with PhyML and codon-based likelihood models (PAML) were used to infer variation in selective patterns (i.e. positive, neutral, and negative selection) across genes and species. We further tested for long-term shifts in selection patterns associated with differences in species ecology (e.g., adult habitat, activity pattern) to determine the degree to which environmental factors correlate with lens crystallin gene evolution. We found that certain lens crystallin genes evolved through positive selection and that there is a strong linkage between frog ecology and crystallin gene evolution, providing an important basis for future research examining the adaptive role of the lens in frog vision.

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## Documenting darter diversity: systematic evaluation of *Etheostoma duryi*, Blackside Snubnose Darter, and *Etheostoma flavum*, Saffron Darter (Percidae)

*Hannah Alloway, Benjamin Keck, Thomas Near, Rebecca Blanton*

*Etheostoma duryi* is found in streams across much of the Tennessee River system. Its sister species, *E. flavum*, is largely restricted to the Cumberland River system, but occupies several locations in the Tennessee River, where it may co-occur with *E. duryi*. Whether *E. duryi* and *E. flavum* are reciprocally monophyletic across their ranges has not been tested and co-occurrence may provide opportunities for hybridization. Geographic variation in nuptial male pigmentation in *E. duryi* and isolation of *E. flavum* across the Cumberland-Tennessee River drainage divide suggest possible unrecognized diversity in both species. We sequenced the Cytochrome b (Cyt b) gene for *E. duryi* and *E. flavum* to evaluate their monophyly and phylogeographic relationship. We

also assessed geographic variation in morphology of *E. duryi* by collecting and analyzing scale and fin-element counts. The preliminary Cyt b phylogeny recovered each species as clade. Five geographically definable clades were observed within *E. duryi*: (1) Duck River + Shoal Creek, (2) Elk River, (3) Paint Rock River, (4) Sequatchie River + Emory River, and (5) all other Tennessee River systems; two clades were recovered for *E. flavum*: (1) Cumberland River and (2) Tennessee River. Scale and fin-element counts showed low levels of variation overall, but some shifts in mean values among clades of *E. duryi* were noted. Future work will examine lineage diversification using ddRAD sequencing to better define phylogeographic relationships, delimit undescribed diversity, and explore the potential for hybridization in zones of known or possible sympatry.

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## First report of Reptarenavirus in the invasive Red-tail boa (*Boa constrictor*) from Puerto Rico

*Aslin Almeda Castro, Alberto Puente Rolón, Alondra Díaz Lameiro*

Reptarenavirus is an arenavirus that has only been detected in captive snakes, and it is known to be the etiological agent for Boid Inclusion Body Disease (BIBD), which is deadly to members of Boidae and Pythonidae. The red-tail boa (*Boa constrictor*) was introduced to Puerto Rico during the 90's. We conducted the first survey for Reptarenavirus in Puerto Rico, sampling introduced *Boa constrictor* from the western part of the Island. The establishment of a monitoring program for this disease is essential because *Boa constrictor* could potentially act as vectors and can spread the virus to local endemic and endangered snakes in Puerto Rico, especially the Puerto Rican Boa (*Chilabothrus inornatus*). To quantify the incidence of the pathogen in wild environments, Reptarenavirus RNA was extracted and amplified from 34 liver samples. Currently, we have found at least one positive result, a female *Boa constrictor* captured in Hormigueros municipality. Two attempts were made to confirm the positive results, however, these were negatives, probably due to RNA degradation. Considering this, we cannot definitively conclude that the virus is present in Puerto Rico. However, we will continue to add samples, in order to keep gathering data that will help determine the frequency of the virus, if present, and to develop strategies to manage this potentially dangerous environmental pathogen.

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## Using Reproductive Hormones Extracted from Archived Muscle Tissue to Assess Maturity and Reproductive Status in Porbeagles *Lamna nasus*

*Brooke Anderson, Juliana Kalocz, Courtney Holden, Amanda Einig, Linda Donaldson, Hunter Malone, Michelle Passerotti, Lisa Natanson, Heather Bowlby, James Sulikowski*

While lethal sampling can be the most effective technique to collect critical reproductive data for elasmobranchs, non-lethal techniques need to be validated for future use. Concentrations of reproductive hormones in plasma and muscle have been found to correlate to sexual maturity and/or reproductive cycles in oviparous as well as yolk-sac, placental, and histotrophic viviparous elasmobranchs, offering a potentially non-lethal technique to study reproduction. However, reproductive hormone analysis is scant for oophagous sharks. This study utilized muscle tissues from porbeagles *Lamna nasus* that were dissected for other life history studies and were stored frozen for up to 42 years to quantify testosterone (T) and estradiol (E2) concentrations in relation to previously-known maturity and reproductive state. A total of 208 samples (93 males, 115 females) from porbeagles ranging in size from 80 to 256.5 cm fork length were analyzed. Muscle T and E2 concentrations were related to maturity and reproductive state in porbeagles, with the highest T concentrations found in reproductively active mature males and the highest E2 concentrations found in gravid females. These results suggest muscle hormone concentrations have the potential to serve as a non-lethal proxy of reproductive status in oophagous sharks. This study also demonstrates the value of specimen sharing and the potential for continued use of stored vertebral muscle tissue for reproductive hormone analysis in order to optimize the amount of data gained from biological samples. Future use of these methods would be particularly valuable for threatened or endangered species for which lethal sampling is restricted.



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## The Population Genetics of Speciation by Cascade Reinforcement

Carlie Anderson, Oscar Ospina, Peter Beerli, Alan Lemmon, Sarah Banker, Alyssa Hassinger, Mysia Dye, Michelle Kortyna, Emily Moriarty Lemmon

Species interactions drive diverse evolutionary outcomes. Speciation by cascade reinforcement occurs when the divergence of mating traits in response to selection against interspecific hybridization incidentally leads to reproductive isolation among populations of the same species. Here, we investigated the population genetic outcomes of cascade reinforcement in North American chorus frogs (Hylidae: Pseudacris). Specifically, we estimated the frequency of hybridization among three taxa, assessed genetic structure within the focal species, *P. feriarum*, and ascertained the directionality of gene flow within *P. feriarum* across replicated contact zones via coalescent modeling. We found that hybridization occurs among *P. feriarum* and two conspecifics at a low rate in multiple contact zones, and that gene flow within the former species is unidirectional from allopatry into sympatry with these other species in three of four contact zones studied. We found evidence of substantial genetic structuring within *P. feriarum* including a divergent western allopatric cluster, a behaviorally-distinct sympatric South Carolina cluster, and several genetically-overlapping clusters from the remainder of the distribution. Furthermore, we found sub-structuring between reinforced and nonreinforced populations in the two most intensely-sampled contact zones. Our field observations and literature review indicated that *P. feriarum* hybridizes with at least five heterospecifics at the periphery of its range providing a mechanism for further intraspecific diversification. This work strengthens the evidence for cascade reinforcement in this clade, revealing the geographic and genetic landscape upon which this process can contribute to the proliferation of species.

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## Horizontal and Vertical Movement Patterns of Female Scalloped Hammerhead Sharks (*Sphyrna lewini*) in Hawaii

James Anderson, Patrick Rex

Scalloped hammerhead sharks are considered globally threatened or endangered across their range, with the exception of the central Pacific distinct population segment (DPS). This Federal assessment is largely based on genetic data from juveniles sampled in one nursery location. To date, there are no published data regarding movement patterns, core-use areas and critical habitat requirements for adults of the species around the Hawaiian Islands, nor on the geographical extent of their movements. Thus, conservation and management strategies for this DPS are hampered by data deficiency. Although a telemetry study focusing on males tagged within the same nursery location is currently in review, Scalloped Hammerheads are known to follow sexually divergent habitat use patterns. Thus, there remains a paucity of knowledge regarding this DPS.

Here, we present results of analyses of vertical and horizontal movement patterns of eight adult female scalloped hammerhead sharks outfitted with pop-off archival satellite tags off the leeward coast of Hawaii Island over a period of three years. Tag retention periods ranged between ~11 to 240 days. Tagged sharks showed a seasonal affinity to insular shelf areas during winter and early spring, punctuated by regular likely excursions to nearby deeper waters and seamount habitat. Daily Maximum Likelihood Estimates suggest tagged sharks spent more time offshore or away from the insular shelf as time progressed. Generally, tagged sharks remained in waters proximate to the Main Hawaiian Islands, with one shark making a long-distance (>300 nm) journey south of the Islands before returning. Vertical movement profiles were characterized by use of shallower depths (<150 m) by day, with repeated (average 5 to 12) excursions to meso and bathypelagic depths by night. The number of deep dives per night showed an increasing trend as time progressed, likely reflecting gradually shifting habitat use.

Together these data show that mature female scalloped hammerhead sharks in Hawaii utilize a broad range of habitats, but appear to have affinity to specific coastal and offshore habitat areas. Importantly, these data show that the current assessments of the potential impacts and threats to this DPS likely fall significantly short, and warrant re-examination.

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## Life history traits of ringed and marbled salamanders in response to hydroperiod variation

Thomas Anderson, Mariah Mack, Jessica Sandoval

Wetland hydroperiod affects the ecology and evolution of numerous freshwater organisms. Pond-breeding amphibians are particularly affected by hydroperiod, with their life histories and distributional patterns often resulting from how long ponds remain inundated. Substantial variability exists among species in their responsiveness to fluctuations in hydroperiod, making taxon-specific investigations needed to understand its general impacts. We tested whether two fall-breeding salamanders, *Ambystoma annulatum* and *A. opacum*, each of which have long (> 6 month) larval period durations, respond to variation in hydroperiod length. We manipulated hydroperiod in outdoor experimental mesocosms under three scenarios (short hydroperiod, medium hydroperiod and constant water levels), focusing specifically on how hydroperiod affected their life history traits (survival, size and time to metamorphosis). We found that in the shortened hydroperiod treatments, few individuals of either species completed metamorphosis and would have died, whereas nearly all surviving individuals underwent metamorphosis under longer hydroperiod treatments. After correcting for differences in survival, body sizes and larval periods were on average longer in constant hydroperiods. The relationship between size and time to metamorphosis was positive for both *A. annulatum* and *A. opacum*, with limited differences between hydroperiod treatments in this relationship for either species. Overall, these results indicate these fall-breeding species are indeed affected by hydroperiod variation, but in different ways, depending on the life history trait. These diverse responses highlight the complexities of building generalized responses of amphibians to a shared stressor like hydroperiod variation, as taxa and trait-specific responses appear to be common. Future work should consider other aspects of hydroperiod as a structuring abiotic force (e.g., onset of drying), or the life history traits of amphibians (e.g. their breeding phenology), to better understand the ecology and evolution of pond-breeding amphibians, as well as provide insights into management actions for their conservation.

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## Variation in emigration phenology and climate affect juvenile salamander demography

Thomas Anderson, Jacob Burkhart, Kenzi Stemp, Arianne Messerman, Jon Davenport

Changes in the timing of life history events (phenology) are occurring for numerous taxa, primarily because of shifts in weather patterns associated with climate change. For pond-breeding amphibians, the main way in which phenological changes have been observed is through altered breeding phenology patterns, with some populations exhibiting shifts to earlier or later, depending on the taxa. However, breeding is not the only critical phenological event that is driven by weather patterns: the timing of metamorphosis onto the terrestrial landscape is also influenced by these patterns. Yet, few investigations have examined how altered metamorphosis phenology could impact amphibian populations. Here, we manipulated variation in emigration phenology of ringed (*Ambystoma annulatum*) and marbled (*A. opacum*) salamanders in outdoor terrestrial enclosures. We released captive-reared PIT-tagged juveniles of each species either all at once (i.e., metamorphosis synchrony) or spread across three temporally separate emigration dates (i.e., metamorphosis asynchrony). We monitored individuals in enclosures using capture-mark-recapture surveys for one year post-metamorphosis, and then analyzed survival and recapture rate differences among treatments using Cormack-Jolly-Seber models. We found that metamorphosis phenology treatment did not substantially affect either recapture or survival probabilities. Other factors, including body size at metamorphosis and temperature, did affect survival and recapture probabilities, but were species-specific in their effects. Overall, this suggests that differences in emigration phenology (synchronous vs asynchronous) may have limited impacts on juvenile demography, and that other factors likely have greater impacts on the vital rates of terrestrial stages of ambystomatids, with potential consequences for population persistence.

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## Identifying species hypoxic limits at the intersection of physiology and long-term species monitoring

Alyssa Andres

Low oxygen environments are becoming more pervasive and severe in coastal habitats around the globe. As low oxygen can compromise the ability of organisms to derive energy necessary for vital life functions, it is imperative to understand how coastal shark species may respond to such changes. Our previous physiological studies quantified how hypoxia limits energetic scope and performance for neonate and young-of-the-year bull sharks, (*Carcharhinus leucas*), and blacktip sharks, (*Carcharhinus limbatus*). We found that once ambient oxygen could no longer support locomotion for ram ventilation, survival was time limited, indicating a lowest limit to viable habitat for these species. Long-term fisheries independent shark surveys can further identify trends in species distribution, abundance, and habitat use, and help distinguish potential drivers of such trends. The current research ties historical shark survey data to our metabolic research, and demonstrates the value of long-term species monitoring in ground truthing physiological predictors of population limits. We compared the hypoxic thresholds derived in the lab to bull shark and blacktip shark presence and abundance, in relation to DO, within Florida's coastal waters. Here we present preliminary results of our comparisons. These results are part of a larger collaborative effort to use ecophysiology to model and verify viable habitat for coastal shark species under climate change.

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## Sexually Dimorphic Biofluorescence of the Postcloacal Gland in the Terrestrial Salamander, *Plethodon cinereus*

Carl Anthony, Cari-Ann Hickerson, Teah Evers, Kelsey Garner, Ryan Mayer

Recent research has documented widespread biofluorescence across amphibians. Among caudates in particular, representative species from 8 of the 10 families fluoresce under blue light excitation. Although fluorescence has been reported on the ventral surface of the tail in Eastern Red-backed Salamanders, *Plethodon cinereus*, nothing is known about the source, or the function of the fluorescence. This fully terrestrial salamander species has a broad geographic range, complex mating behavior involving chemosensory and visual behaviors, defends territories from conspecific and heterospecific intruders, and is a model organism for studying many aspects of sociobiology. Our goal was to learn more about the source of fluorescence in *P. cinereus* and to explore demographic patterns of fluorescence. Additionally, we wanted to establish whether there is seasonal and geographic variation in fluorescence in this widespread salamander species. Through our examination of the histological literature and close examination of photos of the ventral surface of the tails under both white and UV light we conclude that the S1 glands, which comprise the postcloacal gland (PCG) in *P. cinereus*, are the source of fluorescence on the ventral portion of the tail. We found this trait to be highly sexually dimorphic with males having significantly more fluorescent S1 glands both across seasons, and localities compared to females. Additionally, we found that fluorescence only occurs in adult salamanders. Further we hypothesize that male fluorescence of the PCG may function in the context of both mate choice and territoriality.

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## Working through Redhorse (Catostomidae: *Moxostoma*)

Jonathan Armbruster

The Redhorse and Jumprock of the genus *Moxostoma* are common species in streams in eastern North America with a few species jumping the divide in Mexico to the Pacific drainage. *Moxostoma* are among the most common native fishes found in boat-shocking surveys and contain among the greatest biomass collected. In addition, many species are imperiled, with one species, the Harelip Sucker (*M. lacerum*) extinct. Currently, there are four well-known undescribed species, but many of the species with large distributions need to be examined. In 2022, Dr. Bob Jenkins decided to pass a lot of his sucker work and data on to me to administer. These files contain a lot of information on the morphology, taxonomy, and life history of suckers. The purpose of this talk will be to detail the information that is available and to gauge interest in the community to work collaboratively on it.

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## Effects of Urbanized Roads on Northern Diamondback Terrapins (*Malaclemys terrapin terrapin*)

John Arnett, Neeta Connally, Michael Ravesi, Theodora Pinou

Roads are considered ecological traps for turtles therefore roads can compromise the demographic structure of turtle populations. To test this, we sampled nine populations of *M. terrapin* representing diverse roads along the Connecticut coast. We used a Probability of Occurrence Model to assign road mortality ranking, ranging from Rank 1 (high road mortality) to Rank 3 (Low to no road mortality). Baited hoop traps and intermittent seine net surveys were used for *M. terrapin* mark and recapture sampling in the Summer of 2022. We recorded location, sex, estimated age, and morphological data for 1073 *M. terrapin*. After one-year, preliminary data suggest that high road mortality sites have statistically smaller *M. terrapin* compared to moderate and low road mortality sites. The results of our study suggest that road mortality may be eliminating the large adult female *M. terrapin* from Connecticut populations, eventually impacting the reproductive success of the species in Connecticut.

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## The Impact of Temperature Induced Vertebral Anomalies on Fast Start Swimming Performance in *Astyanax mexicanus* (Teleostei: Characidae)

Kaleigh Arnold, Owen Howard, Windsor Aguirre

Freshwater communities in the Neotropical region have been facing threats brought on by climate change and anthropogenic factors, including predicted increases in temperature, changes in hydrological connectivity, agricultural and industrial runoff, and increased presence of plastic and heavy metal pollutants. Direct and indirect impacts on Neotropical freshwater fish (NFF) communities, including changes in species composition and biodiversity, have been observed in response to these challenges. Along with altered community dynamics, individuals have been experiencing disruptions in development, including skeletal anomalies that can occur due to altered environmental conditions. Vertebral anomalies within the axial skeleton can have impacts on an individual's fitness by affecting a fish's ability to swim, eat, and escape. Most research on skeletal anomalies currently focuses on temperate economically important fish including salmon and trout. Due to this, a model fish species, *Astyanax mexicanus*, was used as a Neotropical representative for the second largest order of NFF, Characiformes. To understand how vertebral anomalies may affect the performance of individuals, fish were raised in three different temperature treatments to induce the development of vertebral anomalies. To track the performance of each individual, fast start escape responses were recorded and head displacement distance, displacement velocity, and curvature coefficient data was collected and correlated to vertebral anomaly data. Individuals presenting anomalies were sorted into a severity scale, where the most severe fish were used for further 3D visualizing with a double stain clear and stain technique.

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## Mesopelagic fish community structure in an eastern Clarion-Clipperton Zone (CCZ) mining site, a region with a pronounced oxygen minimum zone

Victoria Assad, Jessica Perelman, Jesse van der Grient, Gina Selig, Tamara Frank, Jeffrey Drazen

Micronekton, including mesopelagic fishes, are a vital part of midwater food webs and have the potential to be impacted by deep-sea mining (DSM). DSM activities will have a variety of potential impacts on midwater ecosystems including sediment plumes and release of dissolved metals. As such, understudied eastern Clarion-Clipperton Zone (CCZ) micronekton communities need baseline community descriptions. Utilizing a 10m<sup>2</sup> Multiple Opening Closing Net and Environmental Sensing System (MOCNESS) at a control and impact site in the CCZ's NORI-D claim zone (Spring & Fall 2021) day and night fish samples were collected from 0-1500m. As this region has a very pronounced oxygen minimum zone (OMZ), nets were deployed based upon oxygen patterns. Seasonal differences showed higher springtime densities and biomasses of fishes, crustaceans, and cephalopods; springtime reflected juvenile recruitment, suggesting seasonal mining, particularly fall, may have the least impact. Observed site differences suggest the impact site may not be representative of the control site. The micronekton community in NORI-D is

clearly structured by oxygen; the fish community exhibits species with daytime abundance peaks in the OMZ core and strong vertical migration patterns. The fish community displayed high rates of endemism and OMZ specialization, suggesting this site is unique from other North Pacific regions. A 1200m discharge depth could not only affect the unique community in the 1000–1500m depth zone but additionally impact organisms adapted to extremely low oxygen levels from the input of oxygenated discharge water. Preliminary results on diversity, composition, migration patterns, and control site representation will be discussed.

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## Experimental *Perkinsea* infections cause lethal and sub-lethal disease in three frog families

Matthew Atkinson, Erin Brosnan, Anna Savage

Amphibian *Perkinsea* (hereafter Pr) is a protist that infects numerous species of anurans globally. The taxonomic and geographic distributions of this pathogen are only just emerging, and little is known about the virulence of Pr under different environmental conditions. Here we sought to determine the lethal and sub-lethal consequences of Pr infection across multiple anuran families previously observed with Pr infections in wild populations. We infected tadpoles of three species (*Rana sphenocephala*, *Xenopus tropicalis*, and *Osteopilus septentrionalis*) with Pr spores using five inoculation dosages (sham-infected control, 225,000, 450,000, 675,000, 900,000 spores per individual). We monitored the tadpoles for 45 days (or until metamorphosis) and recorded weekly growth rate, and K (as a proxy for fitness). We then used qPCR to determine Pr presence and infection intensity for each individual. *Rana sphenocephala* ( $p < 0.001$ ) and *X. tropicalis* ( $p < 0.001$ ) inoculated with Pr had significantly higher mortality than sham-inoculated controls, but there was no significant difference in survival among spore inoculation dosages. In contrast, *O. septentrionalis* individuals had equivalent survival in controls relative to Pr-exposures, however, control individuals had significantly lower metamorphosis rates ( $p = 0.02$ ) compared to Pr-exposed tadpoles regardless of spore inoculation dosage. We found no significant differences across species, inoculation dosages, or infection status for weekly growth rate and K. This is the first study to definitely establish that Pr infections can result in mortality outside of Ranidae. Furthermore, this study demonstrates the need to broaden the focus of amphibian disease ecology beyond just Chytridiomycosis and Ranaviruses.

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## Collaborating with Museums to improve the Value and Management of the Association of Zoos and Aquariums Cuba Crocodile Population

Lauren Augustine

Cuban crocodiles, *Crocodylus rhombifer*, were exported to the United States prior to the widespread hybridization of the species with American crocodiles, *Crocodylus acutus*. The Association of Zoos and Aquariums (AZA) Crocodilian Advisory Group (CAG) began managing *C. rhombifer* populations in North America in 1993. Over the last decade, two collaborations between Natural History Museums and the AZA have filled gaps in knowledge about *C. rhombifer*. First, in 2018 when captive bred *C. rhombifer* and museum specimens were evaluated for scale irregularities. Phenotypic variation in scalation is unknown for many species of crocodilians and the scalation on the ventral portion of the tail posterior to the cloaca, or the subcaudal region, has been used to distinguish species and identify commercial crocodile skins. This study found that over 60% of the *C. rhombifer* evaluated had subcaudal scale inclusions. The second collaboration, in 2020, involved the genetic evaluation of the AZA *C. rhombifer* population for improved management. Samples were submitted to the Conservation Genomics Laboratory at the American Museum of Natural History because, along with Cuban colleagues, this lab has amassed the world's most comprehensive DNA database on both *C. acutus* and *C. rhombifer* from Cuba. All 45 samples were genetically identified as *C. rhombifer*, and six of these samples possessed rare alleles previously unknown to the *C. rhombifer* genome. These results indicate that the AZA population has preserved original genetic variation that was once found in *C. rhombifer* populations in the wild, but has since been extirpated.

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## Distribution and Phylogeography of Michigan's Lost Salamander - The Western Lesser Siren

Adam Austin, Tiffany Schriever

Lesser sirens (*Siren intermedia*) are a large aquatic salamander species last seen in Michigan in 1963, but a living individual had never been found there. In 2019, we discovered the first living siren population in Michigan and the only verified population in the Great Lakes Basin. Rediscovering Michigan's rarest amphibian species has generated interest in listing them as state 'endangered' and identifying additional conservation actions to ensure the viability of the population. However, sirens are rarely studied and almost nothing is known about the distribution or origins of Michigan's population. We ask: 1) Where are sirens distributed in the lower peninsula of Michigan? Did sirens arrive in Michigan naturally or were they anthropogenically introduced? And 3) Should Michigan's sirens be considered a distinct subspecies? From March to May, 2023, we will sample aquatic environmental DNA (eDNA) along five watersheds in Southwest Michigan where sirens are suspected to reside. Positive eDNA detections will indicate sirens occupy habitats immediately upstream of the sample site, illuminating their distribution, habitat associations, and dispersal routes. If eDNA is detected along aquatic corridors from Indiana, it is likely they arrived in Michigan naturally. To determine whether Michigan's population may be considered a distinct subspecies, we will compare mitochondrial DNA and morphometric data from the Michigan and Indiana populations to those of the Gulf Coastal Plain. This research will fill longstanding knowledge gaps in siren phylogeography and provide decision makers information critical to the conservation of lesser sirens in the Great Lakes region.

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## Sublethal effects of methylmercury on the physiology of small, coastal elasmobranch, *Mustelus canis*

Maria Auxiliadora Sabando Plaza, Mi-Ling Li, Jonathan Cohen, Aaron Carlisle

As mid to upper trophic level predators, elasmobranchs are well known to have elevated levels of mercury and its more toxic, organic form methylmercury (MeHg). The lethality of MeHg across different taxa has been well established, but its sub lethal effects have only been investigated in depth at the scope of human health. The sub lethal effects of exposure to MeHg, which are known to impact animal physiology and behavior, remain largely undescribed in elasmobranchs. In this study, we establish baseline information on how the physiology of a small, coastal elasmobranch (*Mustelus canis*, Smooth Dogfish) is impacted by chronic, sub lethal exposure of this widespread and potent contaminant. We assess sublethal effects of different levels of exposure using different physiological parameters including aerobic scope estimates measured via oxygen consumption, growth rate, body condition, and food consumption. Behaviors, such as foraging, prey capture, swimming, and behavior are critical for many species of elasmobranchs yet are likely impacted by exposure to MeHg. We also used findings from the present study to build a modified bioenergetics model to account for how energy allocation may be affected by long-term sub-lethal exposure on a young of the year elasmobranch species. Hence, assessing effects on physiology and behavior after sub-lethal exposure can give us an idea of how important physiological processes may respond to exposure to this widespread and potent contaminant.

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## Assessing fish biodiversity in living shoreline reefs with environmental DNA in Lake Calcasieu, Louisiana

Tanner Bahm, Kyle Piller

Living shorelines are implemented to reduce erosion and risks of flooding in low-lying areas along the northern Gulf of Mexico. In addition to stabilizing substrates, these constructed shorelines also can provide habitat for fish and invertebrates as well. A challenge presented by living shoreline reefs is biodiversity monitoring, as traditional surveys of fish populations in these areas including seining, traps, and visual SCUBA counts are generally ineffective in summarizing fish assemblages due to the habitat heterogeneity and high turbidity. An alternative and non-invasive approach that has been used to monitor otherwise difficult to sampling habitats is environmental DNA (eDNA).

In this study, we focused on the living shoreline reefs in Lake Calcasieu, an estuary in Cameron Parish, Louisiana. Water samples were collected from two reefs, constructed in 2017 and 2022 as well as adjacent non-reef habitat that served as the control. Following the collection of three 500ml water samples per reef and control, water samples were filtered (glass microfiber filters), extracted (Powerwater kit), amplified (12S mtDNA), sequenced, and analyzed using a predefined protocol and analytical pipeline. These methods allowed us to evaluate the biodiversity of fishes in the living shoreline reefs installed in Lake Calcasieu.

## Degree Day Accumulation and the Onset of Spring Activity by Amphibians in Ithaca, NY

*Patrick Baker, Rachel Hughes, James Hughes*

Onset of spring activity is a key life history transition for terrestrially-hibernating amphibians that breed in vernal ponds. The timing of migration from the upland habitat is stimulated by environmental cues that ostensibly forecast favorable conditions in their wetland destination. The Spotted Salamander (*Ambystoma maculatum*), Spring Peeper (*Pseudacris crucifer*), and Wood Frog (*Lithobates sylvaticus*) are widely distributed and well-studied amphibian species that frequently co-occur in woodland pond communities in the eastern United States and Canada. All three species initiate migration to breeding ponds in late winter/early spring, often within days of one another. Onset of activity in this group has inherent year-to-year variation that may also be influenced by climate change; however, recent studies have had contradictory outcomes when examining temporal changes in the phenology of their early spring activities. We compared dates of first record (DOFR) for early breeding amphibians in the vicinity of Ithaca, NY from the early 20th century (1900-1911) to recent observations (2016-2022). Spotted Salamanders, Spring Peepers, and Wood Frogs were observed to advance activity by 10, 12, and 13 d respectively compared to the early 1900s. We found that DOFR was associated with a rise in temperature that we characterize with a degree day model using a base temperature of 3°C (T3DD). First observations were recorded at an average accumulation of 36 T3DD (Spotted Salamanders and Spring Peepers) or 44 T3DD (Wood Frogs). Species-specific averages for T3DD did not differ significantly among species or between periods of observation.

## Eleutherodactylus rogersi (Bahamian Flat-headed Frog): Body size, dorsal patterns, and habitat on San Salvador, The Bahamas

*Kristin A. Bakkegard*

*Eleutherodactylus rogersi* (Bahamian Flat-headed Frog) is The Bahamas only endemic frog and one of three native species. Described in 1955 as a subspecies of *E. ricordi* before reclassification in 1965 as a subspecies of *E. planirostris*, it was finally recognized as a species in 2007. Data on *E. rogersi* are limited to the 1955 description by Goin, thus little is known about this frog. I measured the snout-vent length (SVL) and weight of 108 live *E. rogersi* captured on San Salvador, The Bahamas during October and November 2022 (winter) and 107 live *E. rogersi* during March 2023 (early spring). I also noted dorsal patterns and habitat. The mean SVL of winter frogs was 18.5mm (range 7.3 – 32.6mm, SD = ± 7.2) and mean weight was 0.61g (range 0.02 – 2.52g, SD = ± 0.58). The mean SVL of early spring frogs was 21.7mm (range 12.7 – 32.8mm, SD = ± 4.4) and mean weight was 0.74g (range 0.16 – 2.26g, SD = ± 0.44). In early spring, no frogs were found in the smallest size classes, suggesting that reproduction is seasonal, most likely late spring to early winter. While the dorsal patterns of many frogs matched Goin's description to include striped and unstriped morphs, some had more dorsal pigmentation than expected. This frog occupies a variety of habitats to include black coppice, white coppice, and around human-built structures and landscaping. They are found under cover objects during the day (rocks, boards, downed banana leaves, discarded building materials), and are terrestrially active at night.

## Investigating centennial changes in genetic diversity and demography for *Sphaeramia nematoptera* using low coverage whole genome sequencing

*Jemely Grace P. Baldisimo, Christopher E. Bird, Kent E. Carpenter*

The Philippines has the most marine species per unit area than anywhere else in the world, making it a center of marine biodiversity. However, local marine resources and reef-associated livelihoods are now threatened by climate change, habitat degradation, and overfishing. Genetic approaches are useful for biodiversity monitoring but are often excluded because it is costly and not widely understood or practiced. As a result, biodiversity changes are only discovered after major loss of biodiversity has already occurred. Specimens collected in the Philippines from 1907 to 1909 by the U.S.S. Albatross Expedition provide an opportunity to investigate changes in genetic diversity and demography for coral reef fishes. This study focused on a targeted Indo-Pacific marine ornamental fish species, *Sphaeramia nematoptera*. Fish tissue samples from Albatross and contemporary collections were processed using high throughput sequencing, genotyped, and analyzed using a bioinformatics pipeline developed for low coverage whole genome sequences. Our results provide information on genetic diversity and demographic inference that can provide insight on the extinction risk of *Sphaeramia nematoptera*. These results may also be useful for conservation planning and resource management, especially for exploited marine species.

## Acoustic Telemetry Reveals Movement Behavior and Distribution for Two Shark Species in the World's Most Extreme Tides

*Charles Bangle, Javon Lo, Daniel Hasselman, Frederick Whoriskey, Joanna Mills Flemming, Darren Porter, Gregory Skomal, Megan Winton, Bryan Franks, Chris Fischer*

Minas Passage, in the upper Bay of Fundy, Nova Scotia, hosts the world's largest tides, with a tidal range up to 16 m and peak current speeds exceeding 5 m/s. This area is also the only access for marine and diadromous fishes to foraging and nursery habitats in Minas Basin and is a testing and hosts a demonstration site for renewable tidal power generation technologies. To better understand the use of this unique habitat by upper trophic level species, we used acoustic telemetry detections provided by local collaborators to develop predictive species distribution models for two migratory sharks, the spiny dogfish (*Squalus acanthias*) and white shark (*Carcharodon carcharias*). Individuals of both species made repeated visits to Minas Passage during their seasonal occurrences in the upper Bay of Fundy. Detected spiny dogfish were exclusively mature females and most white sharks fell within a relatively narrow subadult size range. Extensive range testing using static arrays and drifters allowed us to develop methods to account for environmental effects on acoustic tag detection range. Boosted regression tree modeling demonstrated the influence of hydrodynamic and environmental conditions on the spatial distribution of both species over the course of the full tidal cycle. Seasonal presence was strongly associated with preferred temperature ranges for both species, while local distribution within Minas Passage was associated with physical oceanographic conditions. This information will be critical in assessing the probability of encounter between migratory sharks and tidal power development, fisheries, and other anthropogenic activities in Minas Basin.

## Local Adaptation and Climate Impacts on Habitat Use of the Bull Shark (*Carcharhinus leucas*)

*Shannon Barry, Matthew Ajemian, Charles Bangle, Laura Garcia Barcia, Jayne Gardiner, Marcus Drymon, Bryan Frazier, James Gelsleichter, Dean Grubbs, Neil Hammerschlag, Michael Heithaus, Matthew Smukall, Bradley Strickland, Tonya Wiley, Toby Daly-Engel*

The distribution of marine species is likely to be heavily altered by climate change, and some marine species have already been observed migrating poleward to novel habitats that were previously outside of their geographic range. Recently, bull sharks (*Carcharhinus leucas*) were found to have expanded their habitat in the northwestern Atlantic Ocean by establishing a novel nursery >800 km north of their historical range, in correlation with rising



sea surface temperature (SST). This raises the question: If shark populations are undergoing poleward expansion in response to climate change, then what will be the resulting impact to the genetic diversity and future viability of these species? This research seeks to investigate this question by identifying signatures of local adaptation of bull sharks within nursery habitats using Single Nucleotide Polymorphisms (SNPs) to delineate genetic structure and the resulting diversity metrics. These findings, plus telemetry data (acoustic tag detections) provided by collaborators in the Florida Atlantic Coastal Telemetry (FACT) network, will be mapped to a seascape of oceanographic factors such as salinity, depth, and SST, and scanned for gene-environmental correlations. These findings will then be incorporated into Genetic Species Distribution Models (GSDMs) for predicting how climate change will impact future habitat use by bull sharks, and made freely available to resource managers for adaptation to other species. These models will provide critical insight into how climate change may impact habitat use, and provide managers with a powerful new tool to understand and anticipate the effects of warming water on species' health and viability.

### Lineage-Specific Effects of Incubation Temperature on Phenotypic Disparity

*Jacob Barthelemy, Christopher Murray, Dr. Clifford Fontenot*

The effects of environment on phenotype in amniotes are not limited to the canalized effects of selection and drift of the past. Phenotypic plasticity may be favored in environmentally variable conditions. Previous research on turtles have elucidated effects of incubation temperature on phenotypic characteristics such as hatchling size, gonad size, and head width. It is hypothesized that lineage-specific phenotypic disparity within clutch can be maximized via variation in incubation temperature. *Glyptemys insculpta* and *Terrapene ornata* are closely related and provide close approximations of developmental pathways. In order to better understand thermal effects, I plan to quantify allelic diversity with ddRADseq data, as well as phenotypic disparity using geometric morphometrics of cranial and carapace morphology. It is predicted that clutches exposed to a wide breadth of incubation temperatures will exhibit more variation than clutches exposed to uniform temperature. Clutches will be incubated in the lab for maximum control of thermal variation and maximum intra-clutch survivorship.

### First Insights into the Movement Ecology of the Western Atlantic Pygmy Devil Ray Using Passive Acoustic Telemetry

*Kim Bassos-Hull, Atlantine Boggio-Pasqua, Krystan Wilkinson, Breanna DeGroot, Dean Dougherty, Peter Hull, Greg Byrd, Matthew Ajemian, Jessica Valek, Alexander Fogg, Michael Norberg, Tabitha Siegfried, Kirsty Ballard, Jeffery Guertin, Cody Mott, Frédéric Ménard*

The Western Atlantic pygmy devil ray, *Mobula hypostoma*, was recently listed as "Endangered" on the IUCN Red List based on reduced observations over time throughout its range. Little is known about *M. hypostoma* biology and ecology, including the current distribution and seasonality. Since 2013, Mote Marine Laboratory's Sharks and Rays Conservation Research Program has collected data on *M. hypostoma* in the eastern and northern Gulf of Mexico (GOM). Between 2019 and 2023, 18 devil rays were internally tagged with Innovasea V13 acoustic transmitters off the central west coast of Florida (N=7), off the Florida Panhandle (N=7) and off the central east coast of Florida (N=4). Preliminary acoustic detection data provided through iTAG and FACT collaborative networks revealed seasonal migrations between the central west coast of Florida (summer and fall) and the Florida Panhandle (fall and winter). The longest documented return migration was of a juvenile male tagged off Sarasota in August 2020, detected by the Mississippi River mouth (Louisiana, 691 km from tagging location) in October 2021, and detected back in Sarasota in July 2022. Future research efforts include additional tagging of *M. hypostoma* in the GOM and east coast of USA to examine movement patterns. Passive acoustic telemetry data together with citizen observations and fisheries-dependent data will be used to build an ecological niche model for this species in the Western Central Atlantic region. This study aims to fill critical knowledge gaps in *Mobula hypostoma*'s biology and ecology to inform management and conservation strategies of this endangered species.

### Skinks of a Different Stripe: Order out of Chaos in the *Trachylepis striata* Complex of African Mabuyine Skinks

*Aaron Bauer, Philip Skipwith, Eli Greenbaum, Daniel Portik*

*Trachylepis* constitute a diverse group of mabuyine skinks that are especially speciose in sub-Saharan Africa. Although species level relationships have been established for most species, those among *T. striata* and its relatives have remained elusive. We used a multilocus dataset (5 nuclear markers, 1 mitochondrial marker) to evaluate relationships among ~215 individuals of the *Trachylepis striata* complex and its sister taxon, *T. spilogaster*. Species assignment within this group is typically based on color pattern and misidentifications are common. Nuclear data analyzed by both tree-building and clustering methods (Admixture, PCA) strongly supported four monophyletic groups within the *T. striata* complex. 16S data yielded a broadly similar pattern, but assignment to species was in conflict for some samples. *Trachylepis striata sensu stricto* is restricted to southeastern Africa whereas *T. wahlbergii* and *T. punctatissima* are chiefly western and southern taxa, respectively, although all three occur in sympatry or near-sympatry in some areas. The fourth species in the complex is *T. loluensis*, which occurs in the Albertine Rift and the Eastern Rift systems. Two previously recognized taxa, *T. sparsa* and *T. mlanjensis*, are not supported by our data, but rather fall within *T. punctatissima* and *T. striata*, respectively. The complex diverged from *T. spilogaster* approximately 5 MY ago and divergence within the complex began approximately 2–2.5 MY ago. Color pattern appears labile within members of the group and is not necessarily reflective of relationship. Our results support substantial changes to previous conceptions of species assignment and biogeography of all constituent taxa.

### Influence of Temporary Emigration on Wood Turtle (*Glyptemys insculpta*) Detection and Abundance Estimates

*Allyson Beard, Donald Brown, Eric Hileman, Christopher Lituma, Michael Jones*

Reliable population estimates are critical to making informed management decisions for wildlife species. Standardized survey protocols have been developed for Wood Turtle (*Glyptemys insculpta*) population monitoring, a species of conservation concern. Protocols employ repeated surveys of defined areas, allowing for estimation of survey-specific detection probability (*p*) and site-specific abundance. Wood Turtles are highly mobile and the assumption of population closure during surveys is unlikely to be satisfied, but current protocol designs do not allow for separation of availability (*pa*) and detectability (*pd*). Our objectives were to determine if *pa* is random and whether *pa* and *pd* are influenced by demographic characteristics. We modified the Midwest version of the wood turtle survey protocol to include a double-pass design, allowing us to estimate *pa* and *pd* using a robust design capture-recapture model. The modified protocol was implemented in Minnesota and Wisconsin between 2017 and 2022. Preliminary results indicate that *pa* was non-random but not influenced by demographic characteristics, and that capture and recapture probability were influenced by turtle carapace length. The probability of emigrating from the survey area between surveys was 0.63 (CI = 0.52 – 0.73), and probability of emigrants not returning to the survey area during remaining surveys was 0.80 (CI = 0.69 – 0.88). Given availability, the probability of capture was 0.48 (CI = 0.36 – 0.61), and the probability of recapture during the second pass was 0.25 (CI = 0.19 – 0.32). We will discuss the implications of our study results for current abundance estimates and provide modeling recommendations to reduce bias.

### Leaky Moms, Tight Babies: Cutaneous Evaporative Water Loss in Response to Supplemental Hydration in Pregnant Rattlesnakes and Neonates After Birth

*Robin Bedard, Savannah Weaver, Emily Taylor*

Viperid snake populations may be at heightened risk from the predicted increase in drought due to the extreme hydration costs associated with female reproduction and their reliance on free-standing bodies of water or rain for hydration. Drought may negatively impact viper populations if females become increasingly water-stressed and resorb developing embryos to conserve water content because of mother-offspring conflict. Cutaneous evaporative

water loss (CEWL) is important to study because the skin is the primary site of osmoregulation in snakes as they experience extremely low amounts of water loss elsewhere. We conducted a study to investigate the plasticity of CEWL in response to reproduction and hydration in arid-adapted prairie rattlesnakes (*Crotalus viridis*). Our goal was to assess how supplemental hydration impacts mother CEWL during and after pregnancy and neonate CEWL after birth. We also assessed how neonate first ecdysis impacts rates of CEWL. We found that supplementally hydrated mothers experienced higher rates of CEWL compared to dehydrated individuals, but CEWL was primarily driven by ambient humidity and temperature. The day after birth, hydrated and dehydrated neonates experienced similar rates of CEWL, but neonate CEWL was associated with the mother's rate of CEWL. Additionally, we found that neonate CEWL increased after first ecdysis suggesting that neonates are born with "water-tight" skin. Investigating and quantifying the physiological implications of water shortage and dehydration can help us better understand the effects of drought and predict how wild populations of arid-adapted reptiles may respond to the predicted increase in megadroughts.

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### **Salamander eye size and investment correlate with ecology and life history**

*Nicole Bednarik, Rayna C Bell, Matthew K Fujita, David J Gower, Ryan K Schott, Jeffrey W Streicher, Kate N Thomas*

Eye size varies remarkably across animals and is tightly linked to visual performance. Consequently, differences in eye size are associated with shifts in environmental conditions and behaviors in many vertebrate species. Salamanders are an understudied group that represent an opportunity to investigate these potential ecological selective pressures within amphibians. Here, we measured the eye size and investment for 154 salamander species from all 10 currently recognized families, including species that differ in natural history traits hypothesized to be linked to variation in eye size. Salamander eye size and investment were significantly correlated with habitat type, with positive investment seen in terrestrial, semiaquatic, and scansorial species. The greatest variation in eye size was observed in aquatic species. Relative eye size was also associated with developmental mode (metamorphosing, direct developing, and paedomorphic life histories). These results mirror previous findings in frogs, indicating that ecology may be a driving factor behind eye size variation within amphibians.

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### **Phylogeography of Deep-Water Snappers in Honduras and Belize**

*Makenna Beehler, Ivy Baremore, Rachel Graham, Toby Daly-Engel*

Silk Snapper (*Lutjanus vivanus*) is a deep-water fish species with a range throughout the Western Atlantic Ocean and is heavily exploited in fisheries in Mesoamerica. While they are targeted in both artisanal and commercial fisheries, there has been a significant increase in artisanal fishing in these regions, surpassing commercial fisheries. Deep-water fisheries in Honduras and Belize are geographically connected to stocks in the wider Caribbean Sea, but largely unmanaged. Silk snappers have conservative life history traits like slow growth and late maturity compared with many shallow-water teleosts, making it more difficult for them to rebound from overexploitation. If the populations of snapper off these coasts are mixing to create one genetic population, regulating them as separate stocks in each country may cause overfishing past sustainability. The goal of this research is to use DNA samples taken from snappers in both coastal and island locations throughout Honduras and Belize to determine the number of evolutionary distinct groups and possible management units. We examined genetic structure using mitochondrial and nuclear DNA, and performed phylogeographic analysis using Bayesian tree-building and assignment-based approaches. These results shed new light on stock structure in deep-water snappers in the southern Caribbean, providing a context for management that spans international borders.

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### **Habitat Use and Reproductive Ecology of Sand Tiger Sharks, *Carcharias taurus*, in the Northwest Atlantic**

*Mikayla Beeson, Ara McClanahan, Shawn Harper, Carol Price*

Sand tiger sharks, *Carcharias taurus*, are large, coastal predators whose populations are globally distributed and aggregate at Northwest Atlantic coast shipwrecks, which act as artificial reefs supporting courtship, mating, and feeding along migratory routes. *C. taurus* have a spot pattern that is as unique as the human fingerprint, allowing for easy identification of individuals. Globally, sand tiger sharks have experienced significant declines, resulting in their listing as a vulnerable species by the IUCN Red List of Threatened Species. The Northwest Atlantic population has experienced more than 75% decline and is also listed as a species of concern by the National Marine Fisheries Service (Pollard and Smith 2009). We hypothesize that shark utilization of shipwreck reefs varies by season, age, sex, and reproductive status. We use non-invasive diver-collected photography to assess the migratory ecology and life history of *C. taurus* populations in collaboration with the NC Aquariums' Spot-A-Shark USA citizen science project. To do so, we analyze photographs of individuals using Wildbook machine learning algorithms to differentiate between individuals based on unique skin spot constellation patterns, forming a spatial and temporal record of observations. Repeat records may suggest site fidelity (Paxton et al. 2019). ROV video analysis provides new insights into *C. taurus* spatial distribution, proximity to wrecks, and reproductive status, emphasizing the need for effective population monitoring. Our results suggest that the Northwest Atlantic subpopulations are diversely spatially distributed between shipwrecks, and mixed-sex, with a higher than normal male-to-female sex ratio, providing vital information on habitat usage and social ecology.

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### **Going in Blind: A Look into the Molecular Evolution of Caecilian Vision**

*Ashmika Behere, Rayna Bell, Matthew Fujita, David Gower, Michael McGowen, Jeffrey Streicher, Kate Thomas, Ryan Schott*

Although vision is one of the predominant ways in which organisms interact with their environment, many animals inhabiting dark environments have reduced or even lost their visual systems. Caecilians are fossorial (burrowing) animals that may be on the precipice of vision loss, providing an excellent comparative system to investigate the selective pressures leading to the loss, or maintenance, of vision. It was initially believed that caecilians were blind because their eyes are small and often covered in layers of skin or even bone! However, recent studies of eye morphology and behavior support the hypothesis of a functional scotopic (dim light) visual system in caecilians. By contrast, these approaches did not find any evidence of a photopic (bright light) visual system supporting the hypothesis that caecilians have lost the bright light (cone) photoreceptors and the associated cone visual genes that would be required for photopic, and color, vision. To test this hypothesis, we searched for genes involved in the initial stages of dim and photopic vision (phototransduction genes) in three caecilian genomes. Unexpectedly, we found multiple complete and functionally conserved cone-specific phototransduction genes. We analyzed selection pressures acting on these genes and found them to have similar selective constraints as rod genes suggesting a conserved function. Further, we found evidence for positive selection of a subset of the phototransduction genes indicative of potential functional adaptation. Our results contribute toward a better understanding of why organisms living in dark environments may maintain an energetically expensive sensory system like vision.

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### **Vision in dim light and the evolution of colorful, nocturnal frogs**

*Rayna Bell, Jeanne Robertson, Ellis Loew, Matthew Fujita, David Gower, Ryan Schott, Jeffrey Streicher, Kate Thomas*

Some crepuscular and nocturnal animals are brightly colored and yet the adaptive significance of their colorful patterns in low light is poorly understood. In particular, nearly 80% of frogs are nocturnal and many of these species exhibit color patterns with bright hues and/or contrasting spots and stripes. Despite the prevalence of these conspicuous patterns, studies of their

potential function as conspecific visual signals have been limited by our understanding of anuran vision, particularly in dim light. Integrative studies of anuran visual systems indicate that frogs have relatively large eyes for their body size and several adaptations to enhance vision in dim light, indicating that vision is likely of high importance. Here we use visual modeling approaches to assess the extent to which distinct color pattern phenotypes in red-eyed tree frogs (*Agalychnis callidryas*) and forest reed frogs (*Hyperolius tuberculatus*) are perceptible by conspecifics as differences in brightness (luminosity) or color (chromaticity). Our results suggest that visual displays among anurans at twilight and after dark might be severely underappreciated, and more generally, that a better understanding of anuran visual systems provides a framework for testing hypotheses about the evolution and adaptive function of color pattern across the frog tree of life.

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## **Biodiversity of Philippine Fishes: A DNA barcode reference library based on voucher specimens highlights remaining taxonomic questions in the region**

*Katherine E. Bemis, Matthew G. Girard, Mudjekeewis D. Santos, Kent E. Carpenter, Jonathan R. Deeds, Diane E. Pitassy, Nicko Amor L. Flores, Elizabeth S. Hunter, Amy C. Driskell, Kenneth S. Macdonald III, Lee A. Weigt, Ned Rose, Jeffrey T. Williams*

Accurate identification of fishes is essential for understanding their biology and to ensure food safety for consumers. DNA barcoding is an important tool because it can verify identifications of both whole and processed fishes that have had key morphological characters removed (e.g., filets, fish meal); however, DNA reference libraries are incomplete, and public repositories for sequence data contain incorrectly identified sequences. During a nine-year sampling program in the Philippines, a global biodiversity hotspot for marine fishes, we developed a verified reference library of cytochrome c oxidase I (COI) sequences for 2,525 specimens representing 984 species. Specimens were primarily purchased from markets, with additional diversity collected using rotenone, SCUBA, or fishing gear. Species identifications were verified based on taxonomic, phenotypic, and genotypic data, and sequences are associated with voucher specimens, live-color photographs, and genetic samples catalogued at Smithsonian Institution, National Museum of Natural History. Herein, we provide an overview of The Biodiversity of Philippine Fishes dataset and highlight remaining taxonomic problems identified through the project.

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## **ROV observations of the rare spikefish, *Hollardia cf goslinei* (Triacanthodidae)**

*Katherine E. Bemis, James C. Tyler*

*Hollardia goslinei* Tyler 1968 was described from three specimens collected off Hawaii. It is the only representative of its genus in the Pacific and has remained poorly known: there are only 8 specimens in museum collections worldwide, and no new specimens have been collected since 1973. Curiously, however, it is commonly seen by remotely operated vehicles (ROVs) including the ROV Deep Discoverer deployed by NOAA Ship Okeanos Explorer. Herein, we report and describe five ROV observations of *H. cf goslinei*. The sightings occurred from 333 to 493 m, and from off Hawaii as originally described, but also further west and south, including off Jarvis Island, Palmyra Atoll, and Johnson Atoll. We describe the habitat that *H. cf goslinei* was observed in, noting that it occurred in relatively low oxygen waters. From the high definition videos, we observed behavior, including *H. cf goslinei* rapidly changing color from pink to white and *H. cf goslinei* flaring its small pelvic fan. These new sightings provide information on the biology of this poorly known species.

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## **Trunk Morphology and Swimming Modes of the Giant Oarfish, *Regalecus glesne* (Lampriformes: Regalecidae)**

*William E. Bemis, Gabriel Vinicius Felix Afonso, Hilton Eric, Robert Shepherd, Katherine E. Bemis*

Vertebrates evolved elongate bodies in two ways: 1) addition of vertebrae; or 2) longer vertebrae. The Giant Oarfish, *Regalecus glesne*, is the longest bony fish, reaching lengths > 8 m. Oarfishes are unusual because their vertebrae

vary in aspect ratio along the body. The laterally compressed body has many vertebrae (55 precaudal + 90 caudal = 145 vertebrae in a 3 m specimen studied) and even more dorsal fin rays (401 in one specimen or ~2.8 fin rays/vertebra). Anterior precaudal vertebrae are short and deep (aspect ratio of ~1.0) but they become progressively longer and shallower posteriorly (aspect ratio of ~0.2). Oarfishes swim by lateral undulation and by unidirectional or counter-propagating waves along the dorsal fin. Lateral undulation of the trunk relates to the number and length of individual vertebrae and trunk muscle anatomy whereas waves along the dorsal fin relate to the number of fin rays and the sizes of their inclinator muscles. Trunk muscles, consisting of folded myomeres with thick myosepta, power lateral undulation. Dorsal pterygiophores support one fin ray each; these rays have long processes at their bases to which large inclinator muscles attach. The number of pterygiophores per vertebra increases posteriorly, e.g., each of the most posterior vertebrae support 4-5 pterygiophores and fin rays. Similar combinations of elongate bodies and locomotion using both lateral undulation and counter-propagating waves evolved many times in different groups of midwater fishes but oarfishes present the most dramatic increases in vertebral aspect ratio and variation in the pterygiophore:vertebra ratio yet known.

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## **The Biology of *Latimeria chalumnae* and Evolution of Coelacanth**

*William E. Bemis, Ethan France*

In addition to his research on sharks, sea turtles, and many other marine species of the Mid-Atlantic Bight, Jack Musick was fascinated with coelacanth. As usual, Jack was ahead of his time. For example, together with others, Jack worked more than 30 years ago to make the first CT-scans of *Latimeria chalumnae* using a specimen preserved at the Virginia Institute of Marine Science (VIMS 8118). Results of that CT study were included in the 1991 volume *Developments in Environmental Biology of Fishes 12*, for which Jack served as lead editor. To honor Jack's work, friendship, and lifetime of contributions to our field, we will review what we have learned about living and fossil coelacanth since his 1991 book, including how molecular phylogenetic tools, histology, and micro-CT furthered contemporary study of this ever-fascinating group of fishes.

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## **A phylogenomic investigation of the evolution and biogeography of a widespread Southeast Asian snake (Homalopsidae: *Hypsiscopus*)**

*Justin M. Bernstein, Harold K. Voris, Bryan L. Stuart, Daryl R. Kams, Jimmy A. McGuire, Rafe M. Brown, Marcelo Gehara, Camilo A. Calderon-Acevedo, Angelo Soto-Centeno, Sara Ruane*

Phylogenomic data provide broad opportunities to examine species and populations at a fine scale. However, tree-based methods for acquiring divergence dates from genomic data are presumed to be more accurate than multilocus data, the latter of which can easily be incorporated into coalescent demographic models. These models may be a better alternative due to relying on priors that are customized specifically to the study group. In this study, we investigate the diversification of rice paddy snakes from Southeast Asia (Homalopsidae: *Hypsiscopus*). We perform coalescent demographic modeling to determine if divergence dates from models are closer to dates obtained using penalized likelihood methods from a genomic phylogeny or to dates from a multispecies coalescent tree using a mtDNA-driven tree. Additionally, we use ecological niche models to determine if environmental factors helped maintain the current diversity. We find that coalescent models yield dates congruent with the multilocus trees using two genes, inferring an origin date of 2.4 mya (phylogenomic data=3.6 mya). Our results support previous hypotheses that the tectonic uplift of Thailand's Khorat Plateau may have initiated *Hypsiscopus* diversification. Additionally, ecological niche models and niche overlap analyses suggest that environmental differences may also have influenced present day distributions. Our study expands on the diversification history of Southeast Asian snakes, and also suggests that datasets with few genes may be useful for preliminary estimates and biogeographic hypotheses.

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## Utility of Ultraconserved Elements from Formalin-fixed Fish Specimens: A Case Study of Enigmatic African Catfishes

Maxwell Bernt, Melanie Stiasny

Despite advancing methods in molecular phylogenetics, the majority of fish specimens in natural history collections are fixed in formalin and have long been considered unsuitable for genetic analysis. Recently, however, high-throughput sequencing approaches have shown promise in recovering genomic data from DNA-degraded museum specimens, including samples fixed in formalin. Here, we explore the efficacy of target enrichment of ultraconserved elements (UCEs) for formalin-fixed fish specimens and assess the utility of these sequence data in phylogenomic analyses. We use seven museum specimens of rare clariid catfishes and incorporate these samples into a UCE-based phylogeny of the family. These samples range in age from 20 to 98 years and include the potentially extinct *Xenocliarias eupogon*, and the troglitic *Uegitglanis zammaraoui*. We discuss methods of quality control and locus filtration that optimize phylogenetic inference for degraded sequence data. Our results show consistent and well-supported placement of these seven samples and highlight the largely untapped potential of museum specimens in fish systematics.

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## Monitoring Sublethal Effects of Chytrid and Ranavirus Infection in Green Frogs, *Rana clamitans*

Catherine Bevier, Matthew Chatfield, Cheryl Frederick, Tristan Burgess

Emerging infectious diseases caused by pathogens such as chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) and ranavirus are one source of amphibian population decline and extinction. Some species, such as the Green Frog, *Rana clamitans*, tolerate infection and serve as models to better understand disease vulnerability, tolerance, and resistance. Such species, however, may be susceptible to sublethal effects of pathogen infection that may be exacerbated by environmental stressors. We have monitored populations of male *R. clamitans* over five years in three locations in Maine that vary in habitat quality and land use. Isolated island populations in relatively undisturbed habitats were not consistently infected with either pathogen, but individual frogs from inland populations in areas with variable degrees of residential development and agricultural activity tested positive for both pathogens. We are evaluating morphological, immunological, and behavioral characters, including calling activity, to determine if males in these populations exhibit traits that could compromise immune defense or reproductive fitness. Calling activity, in particular, is simple to monitor using passive sound recorders, and we are using these data to explore the relationship between general infection status and chorus activity for each population. We also compared breeding coloration, body condition, and morphological symmetry among populations and between infected and uninfected males. Preliminary results suggest that populations differ in these characters among the three locations, but there is little evidence of significant differences in these features between infected and uninfected males.

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## Beyond Color and Pattern: Elucidating the Factors Associated with Intraspecific Aggression in the Mimic Poison Frog (*Ranitomeya imitator*)

Eli Bieri, Andrew Rubio, Kyle Summers

Assortative or disassortative aggression – when individuals display more aggression towards conspecifics with similar or different phenotypic characteristics – can either maintain polymorphisms or facilitate gene flow between populations depending on which direction the aggression is aimed. Deciphering which factors elicit or prevent aggression is crucial to improving our knowledge of the origin and maintenance of reproductive barriers and subsequent speciation. The Peruvian mimic poison frog, *Ranitomeya imitator*, is a monogamous and territorial species that has evolved into four distinct color-pattern morphs in a mimetic radiation. Here we use historical landscape genetic data and competition trials between male individuals sourced from different populations and color-pattern morphs to show that the level of aggression between individuals is not associated with color morph or body size but rather with source population. Individuals spent more time in combat with individuals from their own deme (genetically homogeneous population),

irrespective of color morph or size. These findings indicate that genotypic similarity is correlated with increased aggression in *R. imitator*, though the mechanism by which *R. imitator* males identify conspecifics as territorial threats remains unclear. As body size and color morph were not significantly associated with aggression levels, this study emphasizes the necessity of further research to identify whether other phenotypic traits are influencing territorial behavior between male frogs, and if these factors play a role in increasing gene flow, or conversely, the formation of reproductive barriers between populations.

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## Genetic Population Structure of bullnose ray *Myliobatis freminvillii* Lesueur, 1824 in the Western North Atlantic

Ellen Biesack, Christian Jones, Jan McDowell

The bullnose eagle ray *Myliobatis freminvillii* Lesueur, 1824 occurs in coastal waters of the western Atlantic from Cape Cod, Massachusetts to Argentina and on the US Gulf Coast. *M. freminvillii* is exploited in small-scale fisheries and occurs as bycatch in commercial fisheries. The species is listed as Vulnerable by the IUCN Red List of Endangered Species due to declining populations, however, to date no studies have examined its population structure to inform species-specific conservation or management plans. The preliminary dataset presented here is comprised of mitochondrial ND2 sequences for *M. freminvillii* collected from the east coast of the United States as well as corresponding morphology data for a subset of individuals. This study will provide a first look at the genetic structure of this vulnerable species in the western north Atlantic and inform upcoming sampling efforts focusing on any potential genetic breaks and/or panmictic populations. An understanding of *M. freminvillii* population structure will provide important context for bycatch reduction plans, multi-species fishery management plans, and other ongoing conservation efforts for rays in the coastal Atlantic.

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## Phylogeny and Patterns of Diversification in Angolan Geckos

Lacey Bishop, Jordan Valentine, Matthew Heinicke

Phylogenetics provides information pertaining to species relationships that can be used to assess regional historical biogeography. Our study evaluated whether the gekkonid lizard fauna of Angola has similarities in geographic patterns and times of divergence across genera, influenced by common biogeographic processes relevant to the region. We hypothesized that lizards with more similar habitat requirements would show the most similar historical biogeographic patterns. The project uses both newly synthesized sequence data and preexisting sequence data from Genbank that covers all genera of Gekkonidae known to occur in Angola. Sequence alignments were generated for each group and used to infer evolutionary relationships using Maximum Likelihood. These were used in combination with fossil calibrations to estimate times of divergence within each group. Results from the timing analysis were compared to see if Angolan geckos had common patterns of diversification, with the data suggesting that diversification patterns are lineage-specific and not generalizable across gekkonids. The role of the Great Escarpment in separating lowland from highland lineages is discussed.

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## Gone with the frogs - the hidden diversity of amphibian parasites

Gabriela B. Bittencourt, Jeff W. Streicher, Rebecca Higham, Simon P. Loader, John Lyakurwa, Santiago Ron, Maria Kamouyiros, Beth Okamura

Challenges to life on earth resulting from habitat loss, changing climate, pollution, invasive taxa and emerging disease are widely acknowledged to be causing a sixth mass extinction. No group exemplifies accelerating extinction risks better than amphibians whose alarming rates of species extinction and population declines pose severe consequences for global biodiversity. However, an underappreciated correlate of extinction is the linked extinction of their uncharismatic affiliate taxa. Like all animals, amphibians host an array of parasites, the 'pathobiome', whose diversities and abundances may change according to host and environmental conditions which, in turn, may impact amphibian health. Indeed, some amphibian declines are linked with chytrid fungus, ranavirus, and perkinsid infections. We report on the use of next generation sequencing to determine how amphibian pathobiomes



have changed over time and across space in biodiversity hotspots (Australia, Ecuador and Tanzania) using a combination of present-day and historical (museum) material, and environmental DNA. We will highlight insights on potential disease emergence, the diversity and potential co-extinction of affiliated pathobiome constituents, and amphibian population declines.

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### **Not Too Sharp: Evolutionary Consequences of Venom in Catfish Fin Spines.**

*Corinthia Black, Milton Tan*

Biomechanical constraints influence the evolution of phenotypes; however, what happens when these constraints are lifted is inadequately understood. In many fishes, venom is used as a defensive mechanism, with the most common delivery system being venom glands associated with fin spines. The functional demands of venom delivery suggest that fin spine shape may be morphologically constrained. We use catfishes as a model system to test if venom acts as a constraint on spine morphology. Venom has several origins and losses in catfishes, providing opportunities to study how phenotype changes when functional constraints are removed. Using automated landmarking techniques and phylogenetic comparative methods, we test if relaxed constraints on fin spine morphology result in more disparate phenotypes and determine if these limitations influence evolutionary rates of morphological change in catfishes.

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### **Creating a Reintroduced Population Using Captive-Bred Frogs: Wild Establishment by Zoo-Bred *L. sevosus* and its Research Opportunities**

*Allison Bogisich, Karen Candia, Steven B. Reichling, Jessica Cantrell, Cassandra Collins, James R. Lee, Sinlan Poo*

For almost 20 years, zoological institutions have collaborated with other environmental non-profits, academic institutions, and government agencies to prevent extinction of the critically endangered Dusky Gopher Frog (*Lithobates sevosus*). These partnerships have made strides toward the conservation of *L. sevosus* both ex situ and in situ. Novel methods of ex situ breeding developed at partnering zoos using captive colonies—both assisted and natural—have resulted in 8,175 tadpoles ( $n = 3,830$ ) and juveniles ( $n = 4,345$ ) being reintroduced at the Ward Bayou Wildlife Management Area in Mississippi since 2017. Information from captive populations has provided unique opportunities to document and study amphibian reproduction in ways that would be nearly impossible to replicate in the wild. Through monthly monitoring at the reintroduction site from Feb-Jul 2022, we documented *L. sevosus* occupying their fossorial niche in the longleaf pine habitat. We recorded successful wild reproduction of *L. sevosus* at this reintroduction site for three consecutive years, from 2021-2023. This marks the first successfully reproducing population of *L. sevosus* founded solely from captive, zoo-bred offspring. These results are crucial not only for their implications for the continued recovery of *L. sevosus*, but also because they provide an opportunity for further development and refinement of translocation and reintroduction plans for other amphibian species. Finally, these findings contribute to the broader evaluation of whether using animals from captive “source populations” to establish new wild populations can effectively produce long-term, self-sustaining populations of other endangered species.

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### **DEI: The Global Women in Herpetology Project: Putting a Spotlight on the Diversity of Women Working with Amphibians and Reptiles**

*Allison Bogisich, Umilaela Arifin, Itzue W Caviedes-Solis, Sinlan Poo*

Women in many professions, including herpetology, are generally still under-represented. The number of women is lower than men at all career stages, and data shows that representation decreases as the career stage increases. Societal structures, cultures, norms, and traditions have been identified as factors contributing to gender inequality. However, the exposure to diverse role models and broader representation within STEM has a positive effect in the recruitment and retention of students and early career biologists, especially from underrepresented groups in STEM. To increase the visibility of women in herpetology across disciplines, regions, and cultural backgrounds,

we started the Global Women in Herpetology Project. This project aims to place a spotlight on the diversity of women in our profession around the world and encourage younger generations to enter the field of herpetology. As part of this effort, we are putting together a book that is a compilation of short stories from 50 herpetologists from 50 countries across Africa, Asia, Europe, North and South America, and Oceania. In these stories, each author shares a personal narrative that intersects their gender, cultural background, and professional journey. To highlight these authors, we established a website and social media accounts, so that the project is able to reach a wider audience. The profits of this book will be used to establish a conference scholarship for students in underrepresented regions around the world.

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### **Trigeminal Innervation and Blood Supply to Boid and Pythonid Pit Organs**

*Helen Bond Plylar, Alan H. Savitzky*

Boas and pythons possess arrays of pit organ located either between or within the rostral and/or caudal supra/infralabial scales. These pit organs are sensitive to radiant heat and receive afferent innervation from the ophthalmic, maxillary, and mandibular branches of the trigeminal nerve. There is obvious wide variation in external pit morphology, which presumably constrains thermally mediated behaviors and may influence thermoreceptive efficiency in certain environments. While broad patterns of innervation have been described for a few pythonids, no studies describe such patterns in boids, and none examine inter/intrafamilial variation in innervation. Blood supply to the pit organs has been even less well studied and also likely impacts thermoreception. Because pit organs are used to detect radiant heat and to distinguish objects from background, a temperature decrease at the pit membrane may improve object detection and prevent thermal “after-images”. It is hypothesized that increased blood supply to the pit membrane maintains an optimal thermal state. Several histological studies have examined vessels associated with crotaline pit organs, describing extensive capillary networks within the pit membrane. Accompanying physiological studies suggested that blood flow through those vessels does indeed cool the pits, increasing sensitivity to radiant heat. We used vascular casting and diceCT to examine the cephalic blood supply and innervation of pit organs in three pythonids (*Antaresia childreni*, *Morelia spilota*, *Python regius*), and two boids (*Corallus hortulanus*, *Boa constrictor*). Our findings reveal both interspecific differences in blood supply to the pits, and differential innervation of the anterior supralabial pits.

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### **Histotroph Content and Physiological Effects of Embryonic Maternal Investment in Two Matrotrophic Elasmobranch Species**

*Annais Bonilla-Johnson*

Elasmobranchii's diverse reproductive strategies pose an unknown amount of risk to pregnant females from the level of embryonic maternal investment. This study aims to investigate the frequency and nutritional content of intrauterine histotroph secretions of Matrotrophic stingrays in Eastern Gulf of Mexico and assess the effects of embryonic maternal investment on the physical performance of gravid females throughout gestation.

Mature female *Hypanus sabinus* and *Rhinoptera bonasus* will be collected throughout all stages of gestation with seines, gillnets, and otter-trawls independently and in collaboration with Fish and Wildlife Research Institute's Fishery-Independent Monitoring Program. These specimens are euthanized and dissected to remove developing embryos and ova for organic dry weight procedures and to quantify embryonic maternal investment at all stages of gestation. Histotroph will be assayed for total energetic content, carbohydrates, and fatty acids, proteins, and lipids.

Lastly, 10 *H. sabinus* will be collected at early gestation and transported to Florida State University Coastal and Marine Laboratory facilities for respirometry trials. The individual's average oxygen consumption rate will be measured over a six-hour period and used to assess physical performance. Trials will be repeated every three weeks for six months to identify changes in physical performance over gestation.

Identifying intraspecific and interspecific variation in fluids that provide embryonic nourishment is integral to understanding how these reproductive

strategies contribute to elasmobranch fitness. Negative physiological effects during gestation would directly impact a female and her litters' ability to survive in the wild during their most vulnerable life stage.

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### **Walk this way: sexual segregation in the Blacktip Shark, *Carcharhinus limbatus*, off the United States East Coast**

*Beth Bowers, Bryan Frazier, Stephen Kajiura*

Sexual segregation is common in elasmobranchs (sharks, skates, and rays) and is hypothesized to result from sex-specific differences in body size and/or reproductive strategies. These sex-specific differences can lead to differences in habitat, diet, activity budgets, temperature preferences, and predation risk. Although information is lacking on sexual segregation in the United States East Coast (USEC) blacktip shark population, general biology and life history characteristics suggest that sexual segregation likely occurs. Sexual segregation can lead to biased commercial and recreational landings of a particular sex and, if landings are large, may lead to an unbalanced population, hindering management. Thus, it may be necessary to consider sex-specific differences in space use and environmental drivers of movements to understand how landings may be affected by potential sexual segregation. Here, we examine seasonal distribution, abundance, and space use as well as sex-specific and sex-combined environmental drivers of blacktip shark movements. We show that the USEC blacktip shark population exhibits sexual segregation, where females exhibit greater fidelity to the mating and nursery grounds in Georgia and South Carolina (spring, summer, and autumn) and males dominate more extreme latitudes along the migratory route, off southeastern Florida (spring) and from North Carolina to New Jersey (summer and autumn). Photoperiod reliably predicts the latitudinal movement of sexes overall as well as seasonally (summer, autumn, and winter). However, during spring chlorophyll-*a* is the most reliable predictor of movement for sexes combined. Management should consider seasonal sex-specific differences in distribution and abundance when assessing this stock in the future.

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### **Sneaky Male Strategies (Kleptogamy) Recorded in Cottonmouths, *Agkistrodon piscivorus***

*Frederick Boyce, Terence Farrell*

Kleptogamy, or "sneaky male syndrome," has been documented in many animals, notably fish. Smaller males of some species employ transient sexual mimicry to deceive guarding males, while in species where males compete for females via agonistic contests, smaller males can sneak past competing males while they are distracted. In 1989 Schuett and Gillingham posited that "sneaky strategies might evolve in species of snakes where male-male agonistic behavior occurs in response to intense competition for mates." We present video and photographic documentation of two separate instances of such behavior in *Agkistrodon piscivorus* that occurred at the same location in coastal North Carolina in 2020 and 2022. Both occurred in late summer at a birthing/attendance site, a small cavity in a wooded ditch habitat filled with many complex and varied structures. In addition to the "sneaky" male, each instance involved two larger suitor males and a single female. Birthing had recently occurred and neonates were present. The sneaky male repeatedly and implausibly challenged both the larger males to wrestle but avoided becoming engaged. The larger males eventually wrestled each other to apparent exhaustion, allowing the persistent sneaky male to court the female without interference. Further investigation is warranted, but these observations suggest that smaller males may intentionally goad larger agonistic males into expending even greater amounts of energy, enabling smaller males to access females once the brawnier ectotherms are completely exhausted. Microhabitats with an abundance of complex structures may also play a role in creating favorable outcomes for these smaller male suitors.

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### **Louisiana State University Museum of Natural Science Ichthyology and Herpetology Collections**

*David Boyd, Prosanta Chakrabarty, Christopher Austin*

The LSU Museum of Natural Science, located in Baton Rouge, Louisiana, houses more than 128,000 fluid-preserved reptile and amphibian specimens

and more than 21,000 lots of fishes comprising some 388,000 individual specimens, with tens of thousands of associated tissue samples. The herpetology collection represents approximately 2920 species of amphibians and reptiles while the fish collection contains 2518 species. The herpetology collection has strong Louisiana holdings, owing in large part to the recent accession of the Tulane University collection, as well as especially high numbers of specimens from Mexico and Papua New Guinea. The accompanying tissue collection dates to the 1960s and includes tissues from 70 countries. The ichthyology collection is likewise largely made up of local fauna with a little more than half of its specimens from Louisiana's waters and the rest from primarily outside the United States. The fish tissue collection has grown from 27 samples in 2008 to more than 10,000 today. The LSU "wet" collections are an accessible and important resource for collections-based research on fishes, amphibians, and reptiles of the Southeastern United States and Central America as well as for molecular systematics of neotropical fishes and the herpetofauna of Papua New Guinea.

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### **Molecular evolution of non-visual opsin genes across environmental, developmental, and morphological adaptations in frogs**

*John L. Boyette, Rayna C. Bell, Matthew K. Fujita, Kate N. Thomas, Jeffrey W. Streicher, David J. Gower, Ryan K. Schott*

Non-visual opsins are transmembrane proteins expressed in the eyes, skin, and brain of many animals. When paired with a light-sensitive chromophore, non-visual opsins form photopigment systems involved in various non-visual, light-detection functions, including circadian rhythm regulation, light-seeking behavior, and detection of seasonality. Previous research has primarily explored the diversity and function of non-visual opsins in model organisms, with few studies investigating their molecular evolution in non-model species. Here we explored molecular evolution of non-visual opsin genes in anuran amphibians. With lifestyles ranging from fossorial to aquatic, anurans inhabit a diverse array of light environments, which makes them a compelling system for studying the evolution of light detection mechanisms. Using whole-eye transcriptomes from 79 anuran species, as well as genomes and multi-tissue transcriptomes from an additional 15 species, we 1) identify which non-visual opsin genes are expressed in the eyes of anurans; 2) compare selective constraint among non-visual opsin genes; and 3) test for potential adaptive evolution by comparing selection between discrete ecological classes in anurans. We consistently recovered 14 non-visual opsin genes from anuran eye transcriptomes, compared to 18 genes that we recovered genome wide, and detected positive selection in a subset of these genes. We found variation in selective constraint between discrete ecological and life-history classes, which may reflect functional adaptation in non-visual opsin genes. Although non-visual opsins remain poorly understood, these findings provide insight into their molecular evolution and set the stage for future research on their potential function across taxa with diverse environmental, developmental, and morphological adaptations.

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### **Developing Large-Scale Monitoring of Diamondback Terrapin Populations**

*Danielle Bradke, Brian Crawford, John Maerz*

Large-scale monitoring is typically costly, time consuming, and logistically challenging – especially for cryptic species in relatively inaccessible habitats. However, developing feasible and effective landscape-scale monitoring is important to evaluate broad-scale management and track population changes over time. The diamondback terrapin (*Malaclemys terrapin*) is considered a high priority species under the State Wildlife Action Plan in Georgia, and the Department of Natural Resources is interested in developing a long-term monitoring program for this species. The primary current monitoring method relies on annual capture-recapture surveys, but this is too labor intensive to apply at a large spatial and temporal scale and is therefore limited to a few sites. There is a need for additional, less labor-intensive, methods that can be applied at many sites and integrated with capture-recapture surveys to monitor terrapin population responses. We investigated the efficacy of unmarked count surveys for use in tidal creek habitat. To do this, we conducted repeated counts of terrapins between April – July 2021 and 2022 at 120 survey points

within tidal creeks spanning the entire Georgia coast. We detected terrapins at 246 out of 636 (39%) surveys representing 38 out of 58 (66%) study sites. In total, we counted 2,518 terrapins (Mean=0.34 terrapins/scan; range=0–17). We used N-mixture models implemented in a Bayesian framework to model relative abundance within study sites and to assess precision and temporal variability among estimates. We discuss the potential of an integrated model using count data and capture-recapture data to improve precision and detect broad-scale population changes.

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## Updated Diamondback Terrapin Nesting Frequency Estimates and Population Projections

*Danielle Bradke, Dare Scott, John Maerz*

Life-history traits are directly linked to the vital rates that drive population growth and persistence. Reproductive characteristics, such as clutch size and reproductive frequency, drive recruitment rates of new individuals into a population. Therefore, informative projections of population persistence depend on having accurate estimates of these traits. While reproduction is one of the most well-studied aspects of many aquatic turtles, nesting frequency is one aspect that lacks data because, although it is relatively easy to observe nesting females, individual detection is very low. A recently published population viability analysis for diamondback terrapins (*Malaclemys terrapin*) in Georgia assumed an average annual nesting frequency of two clutches, based on existing literature. However, reports of higher captive reproductive frequency coupled with low individual detection rates among wild nesting females indicate nesting frequency in the wild is likely underreported. We used 5 years of individual nesting history data from the Skidaway Audubon Diamondback Terrapin Project, a citizen science project, to describe the naïve frequency of nesting events of diamond-backed terrapins on Skidaway Island, Georgia. While mean annual nesting frequency was 2.1 clutches, 34.5% of females were observed nesting 3 or more times in a single year with a maximum of 6 nesting events in a single season. We then developed a model to account for detection and estimate true nesting frequency. We report updated population projections, based on our estimates, and compare projected population growth and persistence probabilities to those previously published. These results will inform resource allocation for diamondback terrapin population management.

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## Deep Dwellers: Understanding the Residency Patterns and Space Use of Arctic Skates (*Amblyraja hyperborea*) in Scott Inlet Canada

*Jarius Bradley, Nigel Hussey, Aaron Carlisle*

Understanding residency patterns and space use of a species is key to implementation of sustainable and productive conservation management practices. The characterization of movement patterns of species within an ecosystem can provide insight into why animals use particular habitats such as for feeding or reproduction, or how movements are regulated by various biotic or abiotic factors. Furthermore, by describing the fine scale movements of a species within an ecosystem, patterns of habitat use, movement corridors, and areas of core activity can be identified, at both the population and individual levels. Due to the logistical challenges of working in the Arctic, these types of studies have historically been limited compared to temperate or tropical climates. The Arctic Skate (*Amblyraja hyperborea*) is a high latitude, deep water species that is commonly caught as bycatch in both Canadian commercial and indigenous community fisheries. The life history characteristics of skates makes them vulnerable to overexploitation, and careful management is required. In this study, we used acoustic telemetry consisting of 83 receivers to study patterns of movement and residency of Arctic skates in Scott Inlet, Canada. Our goal is to characterize residency patterns and space utilization of 52 skates across 6 years throughout the community fishing ground. The intent is to elucidate both how the skates distribute themselves within the region as well as identify how sex, size, and reproduction may impact these dynamics. Through understanding these movement metrics, bycatch of Arctic skate can be reduced, and appropriate management formulated.

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## Perceptions Regarding the Recreational Cobia Fishery in Relation to Manta Ray Conservation

*Raquel Braun, Julia Wester, Catherine Macdonald, Jessica Pate*

Along the East coast of Florida there is a popular recreational cobia (*Rachycentron canadum*) fishery. Cobia in the region often associate with large chondrichthyan species like giant manta rays (*Mobula birostris*) during their migration periods. Among local recreational anglers, a well-known method of finding cobia involves tracking ray migrations and fishing near them. Mantas are listed as Endangered on the IUCN Red List due to their slow maturation, low fecundity, and vulnerability to fishing-related mortality. While it is prohibited to target and harvest mantas in Florida waters, due to the related cobia fishery and their reliance on nearshore habitats, they are often sighted trailing fishing gear reasonably attributable to recreational anglers. This study provides insight into angler perceptions, knowledge, and behavior regarding the recreational cobia fishery and the effect it has on mantas through semi-structured interviews with expert anglers. Collected data were analyzed to establish a best practices guide for fishing for cobia to reduce harm to mantas. Major findings of the study include positive attitudes from anglers toward cobia and manta conservation, a reportedly drastic change in cobia average size and distribution, and a high occurrence of accidental hooking of mantas while fishing for cobia. Anglers also report that the cobia fishery, which was once dominated by a small group of experienced anglers, is now more popular and accessible due in part to social media. This uptake in accessibility and increase in fishing pressure is negatively impacting both the cobia fishery and the manta population in South Florida.

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## It's frustrating being a user: High investment and low yield

*Patricia Brennan*

As a reproductive evolutionary morphologist, I have worked with almost all major vertebrate groups, more recently snakes. My research requires access to fresh/frozen specimens. As part of my work, I routinely contact many zoos and museums to acquire samples for my research, with varying success. In many cases, the research mission in many zoos is not spelled out, and there are no updated contacts to reach out to inquire about research collaboration, each zoo may require a different permit, necropsies are often destructive, there are no personnel available or money to process special requests, data on necropsies can be hard to access, and success is often dependent on personal connections. While my research cannot use already preserved museum specimens, collaborations with museums can be fruitful if specimen sharing is possible, for example, coordinating specimen acquisition and collection, and allowing dissection and removal of genitalia prior to preservation. Some possible solutions to improve sharing and access to specimens for research from both zoos and museums include the widespread use of the common research form from AZA to request samples, implement a pay scale for researchers to budget for in grant applications, make research information easy to find on websites, and clarify the research mission. Developing a specimen bank across organizations would also be an excellent step forward to increase access to samples that may otherwise go to waste.

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## Larval Growth Rate and Amphibian Metamorphosis: how Plethodontid Salamanders are Exceptional

*Maldonado Brittany, Claire Crookston, Tyler Brock, Corey Samples, Christopher Beachy*

Metamorphosis of larval plethodontids is unique among all animals in that variation in growth rate fails to affect metamorphic timing. However, there are only a few studies (four) and only one of them for a species in the Spelerpini (i.e., *Eurycea wilderae*). Coincident with a sampling effort for a different project, we captured 20 hatchling larval *Eurycea guttolineata*. This is a rare event. We took this opportunity to conduct a larval growth experiment in the lab. We fed larvae either hatchling brine shrimp or California blackworms. One group of larvae was fed these items ad libitum and another group had a restricted access to food. The intention was to have a rapidly-growing group of larvae and a slow-growing group of larvae. Food treatments had their intended effects and we recorded duration of larval period and size at metamorphosis. Individuals were killed and preserved at the end of the experiment for sex and allocation

(fat body size) determination. Growth variation caused variation in metamorphic size, how there was not an effect on metamorphic timing. This remarkable result remains restricted to the Plethodontidae and we hypothesize that this is related to diversity in life cycle expression in this diverse family.

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### Patterns and Consequences of Hybridization in an Imperiled Headwater Fish, the Leon springs pupfish, *Cyprinodon bovinus*

*Robert Bretzing, Andrew Fields, Elizabeth Hunt, Megan Bean, Ryan Smith, Kevin Conway, Christopher Hollenbeck, David Portnoy*

The Leon Springs pupfish (*Cyprinodon bovinus*) is a federally endangered freshwater fish that inhabits small spring-fed systems in West Texas. Currently, *C. bovinus* is constrained to a series of pools located in the Diamond Y Preserve in Pecos County, Texas. Furthermore, an invasive congener, the sheepshead minnow (*C. variegatus*) currently threatens the persistence of this species. Therefore, an assessment of standing genetic diversity and levels of hybridization was conducted to help guide conservation efforts. Reduced representation genomic sequencing was used to characterize the genetic diversity of *C. bovinus* and *C. variegatus* sampled throughout its range in Texas. Individuals were characterized as pure, hybrid, or backcrossed using composite genotypes, and levels of standing genetic variation were assessed within and between wild *C. bovinus* samples and a reserve population. No contemporary hybrids were found in the wild populations and there was evidence of heterogeneity between wild samples. Estimates of effective population size were lower for wild samples of *C. bovinus* than the refuge population.

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### The Past, Present, and Future of the U.S. Atlantic Shark Fishery: Has Management Been Successful?

*Karyl Brewster-Geisz*

The Highly Migratory Species Management Division of the National Marine Fisheries Service (NOAA Fisheries) has managed the U.S. federal shark fisheries in the Atlantic Ocean, including the Gulf of Mexico and Caribbean Sea, for the past 30 years. While I was not part of the process in 1993, I have been deeply involved in the management of the shark fishery since the mid-1990s. In those years, I have seen shark science progress and broaden our understanding of various shark species through the use of various tools and technologies. I have witnessed the change in the public perception of sharks in general. Whereas before the shark fishery was fairly unknown outside of a few enthusiasts, now, partly because of new technologies and social media, the public is much more engaged - whether that means protesting recreational shark tournaments or asking Congress to intervene directly with the management of the species. I have participated in pushing regional fishery management organizations to care more about sharks. Now, because of science, there are multiple international agreements that have taken action to conserve and manage sharks and their fisheries. One thing that has not changed is the overarching goal of U.S. management to prevent overfishing and rebuild overfished stocks while achieving optimum yield. Overall, has management been successful? I will provide a 30-year retrospective of U.S. Atlantic shark fisheries management, focusing on how management has adapted to the changing science, and provide a vision of the future.

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### Characterization of Elasmobranch At-Vessel and Post-Release Mortality Across Gear Types and Taxonomy

*Bethany Brodbeck, Edward Hale, Aaron Carlisle*

Elasmobranchs are widely recognized as susceptible to overexploitation due to their k-selected life history characteristics, making accurate stock assessments even more imperative to maintaining healthy populations. Unfortunately, at-vessel mortality (AVM) and post-release mortality (PRM) rates are available for very few elasmobranch species within various fisheries, hindering management efforts. Marine fisheries management must often use conservative PRM estimates when data are unavailable or substitute rates from different species and/or gear types. However, AVM and PRM rates can be highly variable among species, within species, and across fisheries, and using proxy estimates will

necessarily bias stock assessments. To assist future management efforts, we performed a meta-data analysis on all available literature regarding AVM and PRM for elasmobranchs ( $n = 77$ ). We used these data to characterize mortality rates for various gear types (e.g., trawl, longline, gillnet, purse seine, and recreational rod-and-reel) by taxonomy, looking at both orders and families. We also characterized variation in methodology within each gear type, which demonstrates an important need to standardize efforts and reporting. Lastly, we created a vulnerability index that considers our state of knowledge, to better inform future studies on where they should potentially focus their efforts. Our analysis demonstrates our overall lack of knowledge and a need for more, high-quality data on elasmobranch AVM and PRM.

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### Does exposure to roundup affect regeneration in salamanders?

*Robert Brodman, Brittany Tillman*

Herbicides change have been implicated as causes of amphibian declines. Salamanders are well known for their ability to regenerate tails and limbs. However, little is known about how exposure to herbicides may influence regeneration in salamanders. We studied the effects of tail clipping and herbicide application on Spotted Salamander (*Ambystoma maculatum*) larvae with a  $2 \times 2$  factorial experimental design with tail clipping and exposure to herbicide as factors. We collected salamander eggs and exposed half to Roundup at 3ppm. Larvae were reared in the lab at 16C until all the eggs hatched. Then half of the control and herbicide treated larvae had 2-3mm of their tails clipped. Survival, SVL, limb development, and re-growth of tail were monitored over the next six weeks and SVL and tail length were assessed in juveniles after 6 months. The larvae in Roundup treated groups had reduced survival, slower tail regeneration, and increased SVL compared to no herbicide controls. There was no difference in survival, SVL, or limb development of tail clipped larvae compared to controls. All clipped larvae fully regenerated tails within 3 weeks. This suggests that while herbicides affect mortality, it does not slow regenerative abilities of salamander larvae. After 6 months there was no difference in SVL or tail length of juvenile salamanders in Roundup treated groups compared to control and no difference in SVL of juveniles whose tails were cut compared to control. However, the tail of juveniles whose tails were cut were shorter than control juveniles.

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### Resolving Phylogenetic Conflicts Among Paleozoic, Mesozoic, and Living Chondrichthyans

*Allison Bronson, John Denton, Alan Pradel, John Maisey*

The past several decades of research in chondrichthyan evolutionary morphology have lent increasing support to the longstanding idea that acanthodians are stem chondrichthyans, and that sharks and their allies represent a highly specialized crown group of gnathostomes. However, despite technological improvements in both analytical techniques and in description of specimens, analyses continue to disagree about the evolutionary position of major chondrichthyan lineages (for example, symmoriforms and holocephalans). We generated a morphological matrix including 48 genera of fishes and explored this dataset using parsimony and maximum likelihood phylogenetic analyses. At first glance, our results add to the cacophony of disagreement surrounding the symmoriform group, but we explore the root causes of these differences and emphasize why highly detailed morphological descriptions are critical for resolving the chondrichthyan phylogeny.

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### Concurrent Threats Jeopardize the Viability of Virginia's Hellbender Populations

*George Brooks, William Hopkins, Holly Kindsvater*

Detecting declines and quantifying extinction risk of long-lived, highly fecund vertebrates can be challenging. In addition to the false sense of security introduced by large clutches, the imperiled status of long-lived species can often be masked by extinction debt. Here we develop a demographic model for the Eastern Hellbender (*Cryptobranchus alleganiensis*). We examined the individual and interactive effects of three of the leading threats hypothesized to contribute to the species' demise: habitat loss due to siltation, high rates



of nest failure, and excess adult mortality caused by fishing and harvest. We parameterized the model using data on their life history and reproductive ecology to model the fates of individual nests under both deterministic and stochastic environmental conditions. Our model suggests that high rates of nest failure observed in the field are sufficient to drive hellbender populations towards a geriatric age distribution and eventually to localized extinction, but that this process takes decades due to the species' longevity. The combination of limited nest site availability, nest failure, and stochastic adult mortality can interact to increase the likelihood and pace of extinction. Our model helps to identify tipping points beyond which extinction becomes certain and management interventions become necessary. Our approach can be generalized to understand the interactive effects of various threats to the extinction risk of other long-lived, highly fecund vertebrates. As we face unprecedented rates of environmental change, holistic approaches incorporating multiple concurrent threats and their impacts on key aspects of life history will be necessary to proactively conserve species.

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## **Fisheries for Teleosts Associated with Deep-Sea Sponge-Coral Ecosystems on the Blake Plateau off the Southeastern U.S.**

*Myra Brouwer, Walter Buble, Claudia Friess, Sarah Goldman, Kimberly Hendricks, Adam Lytton, Kelly Robinson, George Sedberry, Byron White, David Wyanski, Sean Yackley*

A deepwater (> 300 m) demersal fishery developed off the southeastern U.S. in the 1980s, targeting Wreckfish (*Polyprion americanus*) in sponge-coral and hard-bottom habitat on the Blake Plateau. Declining catch and increasing regulations in shallow-water fisheries motivated fishermen to develop alternatives in deep water > 150 km from port. Subsequent expansion in the Wreckfish fishery led to management including gear restrictions, spawning-season closure, annual catch limits and individual quotas for the commercial Wreckfish fishery and a vessel limit, seasonal opening and annual catch limits for the recreational sector. Management resulted in a small sustainable fishery for Wreckfish, in contrast to fisheries elsewhere in the wide range of this species, where the fishery collapsed. Several bony fishes exploited in other countries are also landed with Wreckfish in the U.S., but no management plans have been developed for them. Blackbelly Rosefish (*Helicolenus dactylopterus*), Red Bream (*Beryx decadactylus*) and Barrelfish (*Hyperoglyphe perciformis*) are, like Wreckfish, desirable fishery species that are long-lived (30, 69, 85 and 91 y, respectively). They are associated with hard bottom that supports a higher diversity of fishes than nearby habitats of unconsolidated sediments. Because of their life histories and limited habitat, they can be easily overfished. Genetic analysis of some of these species shows connectivity between the U.S. and other areas where they are fished, which could result in depletion without international management. Regional management for the southeastern U.S. that includes all deep-sea species landed, and consideration of fisheries in other parts of species' ranges, would minimize the threat of overfishing.

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## **Influence of Recent Climate Change on Morphology of a Widespread Lizard, *Sceloporus occidentalis***

*Dylan Bryant, Matthew Lattanzio*

Recent climate change has been implicated as a major driver of species' extinctions throughout the world, revealing a need to investigate the mechanisms by which taxa cope with shifts in temperature and precipitation regimes. Over the past few decades, several studies have suggested that changes in mean body size within a species are universal responses to ongoing climate change. However, the potential for variation in the direction of changes, particularly between sexes, has received scant attention. Moreover, consideration for ecologically-relevant morphological traits outside of body size is also lacking. Using historical (1960-1973) and modern (2021) samples of adult Western Fence Lizard (*Sceloporus occidentalis*) populations in California, we assess the extent that long-term changes in several morphological traits coincide with shifts in climate over time. Currently, we have completed some preliminary analyses and are finishing measurements on remaining museum specimens. Based on preliminary findings we expect 1) the overall magnitude of trait changes to vary among and within localities, 2) male traits sizes

to increase and female traits to decrease or remain unchanged over time, leading to an overall 3) increase in sexual-size dimorphism (SSD) within *S. occidentalis* linked to recent climate change. Ultimately our findings should reveal the multivariate nature of climate change's impacts on a species, as well as the extent that distinct populations diverge in any response patterns. We anticipate our findings will also provide novel insight into the extent that phenotypic patterns normally attributed to sexual selection (e.g., SSD) also coincide with environmental variability.

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## **Verification of Giant Sea Bass (*Stereolepis gigas*) Spawning: Through Auditory Observations**

*Elizabeth Burns, Larry Allen, Michael Franklin*

Giant Sea Bass (GSB), *Stereolepis gigas*, is the largest marine bony fish off the coast of California, an apex predator, and is currently classified as critically endangered by IUCN Red List. Despite recent studies on GSB, there is no documentation of their spawning and related reproductive behaviors in their natural environment due to their depressed population size. Previous studies have shown GSB capable of producing a variety of sounds (many sounding like a "boom"). Past studies have observed that the closer in proximity you are to a GSB spawning aggregation the louder and more numerous GSB sounds are heard. In these spawning aggregations, "booms" have been observed to be linked with antagonistic behaviors between GSB males, indicating that sound production is most likely part of spawning. In the summer of 2019 novel reproductive sounds labeled "snares" & "bursts" were recorded during successful spawning events of captive GSB. These novel sounds have only been observed and recorded during spawning. Using Raven Pro we examined audio samples gathered in the summers of 2014, 2015, and 2019 and identified "snare" and "burst" sounds recorded at GSB spawning aggregations. We then tested recorded when these sounds occurred, to see if they occurred during time of spawning. We hypothesize that GSB vocalization occurs frequently during spawning and is used in combination with courtship behaviors to signal reproduction readiness. I argue that by identifying these behaviors, we will be able to verify GSB spawning when these auditory behaviors are observed during their period of spawning.

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## **Age, Growth, and Origin of Kokanee Salmon in California Lakes and Reservoirs**

*Lee Burrows, Levi Lewis, Malte Willmes, Andrew Rypel, Gabe Singer, Kyle Murphy*

Kokanee Salmon are a landlocked, adfluvial variety of Sockeye Salmon (*Oncorhynchus nerka*) that has been introduced to California lakes since the 1940s. This popular non-native sportfish now functions as a key trophic link in the pelagic food web of many lacustrine ecosystems; however, little is known regarding variation in the demography of Kokanee across different lake ecosystems. Here, we used otolith-based analyses, including image analysis and Sr-isotope geochemistry, to quantify patterns in size-structure, age-structure, growth rates, and natal origins (i.e., wild versus hatchery) of Kokanee from four California lakes that span a broad range of elevations and environmental conditions: Lake Tahoe, Lake Berryessa, New Melones Lake, and Stampede Reservoir. Otolith macrostructures (annual bands) indicated most individuals were 2-3+ years old, with Stampede having proportionally more fish from younger age classes (e.g., 2+ yrs), Lake Tahoe having more fish from older age classes (e.g., 3+ yrs), and New Melones and Berryessa in between. Kokanee from Lake Berryessa exhibited the largest body sizes across age classes, indicating more rapid growth relative to other lakes. Kokanee from Lake Tahoe and New Melones exhibited intermediate size-at age, whereas those from Stampede Reservoir exhibited the smallest sizes-at age and respective growth rates. Results from geochemical analyses indicated that hatchery-origin fish predominate in Lake Berryessa and Lake Tahoe, whereas most individuals in New Melones Lake and Stampede Reservoir were wild-spawned. These results elucidate how growth and reproduction of Kokanee vary among major lake ecosystems in California, thus further informing the management of this valuable fishery.

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## Development of the Bonnethead shark (*Sphyrna tiburo*)

Steven Byrum, Gareth Fraser, Gavin Naylor

The hammerhead sharks (family Sphyrnidae) are a charismatic and immediately recognizable monophyletic group of sharks, easily identified by their flattened and laterally expanded heads. Though there has long been an interest in hammerhead development, there are currently no explicit staging tables published for any members of the group. The bonnethead shark *Sphyrna tiburo* is the smallest member of Sphyrnidae and is abundant in waters along the Gulf Coast and Southern Atlantic. Due to their relative abundance, close proximity to shore, and brief gestation period, it has been possible to collect multiple embryonic specimens at varying stages of development. Here, I compare the developmental trajectory of the bonnethead with those of other chondrichthyans to establish a staging series for this species. With an emphasis on the emergence of the unusual cartilaginous skull (chondrocranium) in the bonnethead, I have employed several morphological (CT, histology) and molecular (localized protein and gene expression) techniques to characterize the developmental deviation in head shape at precise embryonic stages. This work will serve as a platform for future comparative developmental research both with hammerhead sharks and across chondrichthyan fishes, underpinning the extremes of morphological diversity in this group of fishes.

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## Minorities In Shark Science (MISS) Impact Study and Evaluation

Camila Caceres, Traci English-Clarke, Triana Arguedas, Jasmin Graham

Minorities In Sharks Science (MISS) is an NGO that promotes diversity and inclusion in the field of shark science. Since 2020, MISS has gathered over 500 members across 30 countries, provided \$95,000 in scholarships and 19 free workshops and trainings. In 2022, in order to determine MISS's impact in the field of shark science, an online survey was completed by over 150 members to measure young BIPOC scientists' perceptions of whether and how MISS has changed the field of shark science, as well as how MISS has helped them to effect change themselves. This study included surveys of participants from a selection of MISS programs (summer camp, the MISS workshop, and MISS Fellows), as well as members of MISS/Friends of MISS (FOM) and members of the American Elasmobranch Society (AES), along with interviews with staff at organizations that have partnered with MISS to provide fellowships. Preliminary results suggest that MISS members overwhelmingly perceive that MISS has increased the number of both racial and gender minorities in shark science, helped to reduce barriers for racial and gender minorities in the field, and increased scientists' awareness of racial and gender issues in shark science.

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## Do Florida cottonmouth snakes need freshwater?

Karen Caceres, Mark Sandfoss, Natalie Claunch, Zachary Steele, John Whiteman

Water availability in the environment varies widely across space and time, influencing water budgets of animals and driving functional adaptations. Obtaining adequate amounts of freshwater for water balance is particularly challenging in desert, polar, and marine environments. Ectotherms change their daily and annual activity patterns, habitat selection, and behavior to maintain water balance. The ability to obtain water exerts selective pressure on all aspects of ectotherms' ecology and behavior. Therefore, when organisms expand into habitats that have limited water availability compared to their typical habitat, physiological, morphological, and/or behavioral adaptations are required to maintain water balance. Florida cottonmouth snakes (*Agkistrodon conanti*) populations are usually associated with mesic freshwater habits; however, there are atypical populations inhabiting coastal islands in Florida that lack a permanent source of freshwater. A. conanti has rarely been spotted in the ocean, and cannot rely on saltwater for its water balance as they do not possess the necessary physiological adaptations to extract salt obtained from prey or from drinking seawater directly. Here, we predict that these atypical populations have a high reliance on metabolically produced water that can satisfy their required water balance compared to mainland populations. We

test this prediction by measuring a tracer of metabolic water in blood samples, then estimating the fractional inputs from drinking/food water versus metabolic water in the body water pool.

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## Diet and Feeding Ecology of the Whitespotted Eagle Ray (*Aetobatus narinari*) from Florida Coastal Waters Revealed via DNA Barcoding

Brianna Cahill, Ryan Eckert, Breanna DeGroot, Kim Bassos-Hull, Joshua Voss, Matthew Ajemian

The whitespotted eagle ray (*Aetobatus narinari*) is a highly mobile predatory batoid distributed throughout shallow, warm-temperate to tropical Atlantic waters from North Carolina to Brazil. The species' strong, plate-like dentition facilitates consumption of hard-shelled prey. Due to winnowing behavior, shell fragments are rarely retained in the stomach contents, creating a significant challenge to prey identification based on soft tissues alone. Here, we report on the first analysis of whitespotted eagle ray diet in Florida waters using visual-based gut content analysis complemented with DNA barcoding. Gut contents were obtained via gastric lavage from 60 individuals collected in the Indian River Lagoon and off Sarasota, Florida. Of the 221 unique prey samples collected, 185 were deemed suitable (following DNA extractions, PCR and confirmation using gel electrophoresis) for sequencing. Approximately 88.1% of samples yielded positive species matches in either the Barcode of Life database or Genbank. Results from the sequenced data indicate that whitespotted eagle ray diet in Florida is mainly comprised of bivalves and gastropods, with relatively higher occurrences of giant false donax (*Iphigenia brasiliana*), predatory conchs (i.e., *Buccinoidea*), and cockles (i.e., *Cardiidae*) from both databases. Although in lower occurrences, there was evidence for consumption of hard clam (*Mercenaria mercenaria*), a major shellfish aquaculture and restoration species in Florida, and hermit crabs (*Diogenidae* and *Paguridae*). Such wide-ranging prey species from various trophic guilds (i.e., benthic scavengers, filter-feeders, and carnivorous gastropods) and locations (i.e., deep-burrowing infauna to epifauna) highlight the whitespotted eagle ray's diverse role in top-down regulation of coastal benthic communities.

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## A Tail of Two Gobies: Comparative Differential Gene Expression between Two Euryhaline Co-Occurring Gobiiformes

D. Cooper Campbell, Henry Bart Jr.

The order Gobiiformes inhabits waters around the globe that vary widely in salinity. From climbing waterfalls to deep-water reefs, this unique group of benthic fishes has members that can tolerate every amount of salt in their habitat from a pinch to a cup or more. While a great deal of research has been done to understand the physiological mechanisms of salinity tolerance in fishes, less work has focused on understanding the genetic mechanisms underlying salinity tolerance and how they vary among non-model fishes. With this in mind, the goal of my research is to describe differential gene expression in two species of Gobiiformes – *Ctenogobius boleosoma* and *Evorthodus lyricus* – two species that co-occur in the Gulf of Mexico across a variety of salinities from river mouths to outer barrier islands. RNA-seq methods will be used to obtain and describe differentially expressed genes within these two species and identify novel genes associated with salinity tolerance as well. Our tolerance data will be obtained using the satellite animal facility at the Tulane University Biodiversity Research Institutes wet lab, where we will house and experimentally expose fish to differing salinities before collecting tissues for submission to RNA-sequencing services. We expect to see differences in expression in genes like *ATP1A1*, *KCNJ5*, and *CACNA1D*, which code for subunits of the Na,K-ATPase sodium-ion channel.

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## Black and Yellow Aposematic Fellow: Evidence for aposematism and unpalatability in the Nearctic Gray Tree Frog (*Hyla versicolor*).

Joseph Cannizzaro IV, Gerlinde Hobel

Animals that deter predatory attacks and reduce predation pressure are at a selective advantage. Aposematism or the advertisement of bright colors that

signal unpalatability or chemical defense is one of many methods animals employ to dispirit predation. Recognizing form and function of animal weaponry is paramount to understanding the ecological and evolutionary forces behind predator and prey dynamics. The Gray Tree Frog (*Hyla versicolor*) is an enigmatic Tree Frog native to the eastern half of North America. *H. versicolor* has a conspicuous black and yellow spotted pattern on its thighs and flanks. Furthermore, *H. versicolor* has been reported to harbor extremely noxious defensive skin secretions. We investigated aposematism in *H. versicolor* by conducting a palatability study to test whether *H. versicolor* skin secretion is unpalatable to a model predator. Furthermore, we conducted a clay model field study to test if the black and yellow thigh pattern is aposematic warning coloration and reduces predation. We found *H. versicolor* skin secretion to be significantly unpalatable, and that the black and yellow thigh pattern significantly reduced clay model predation.

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### Sex-Specific Patterns of Timing and Frequency of Ecdysis in Free-Ranging Timber Rattlesnakes (*Crotalus horridus*).

*Max Carnes-Mason, Steven Beaupre*

Shedding is an important component of the yearly energy budget of low-energy squamates. Reports of free-ranging patterns of ecdysis in snakes are sparse but serve as an important prerequisite to understanding the magnitude of energetic effort animals devote towards shedding. Adult Timber Rattlesnakes (*Crotalus horridus*) were tracked via radio telemetry over 25 active seasons. We recorded the timing of ecdysis within the year and the frequency of shed events within individuals at a population scale. Between 1995 and 2020, we collected 10,500 observations across 175 telemetered individuals in the field. From our long-term data, we identified 244 unique shed events from 94 individuals (207 individual-years). In our population all individuals shed once or twice per year regardless of sex (mean frequency of sheds per active season:  $1.18 \pm 0.075$  (N=100 males);  $1.13 \pm 0.064$  (N=107 females)). While there was no significant difference in shed frequency between sexes, we found evidence for differential drivers of frequency. Males in better body condition ( $\log(\text{Mass}) / \log(\text{SVL})$ ) were more likely to shed two times in a season. In females, reproductive condition but not body condition was a significant predictor of shed frequency with gravid animals shedding less frequently than non-gravid counterparts. Comparison of population-wide patterns in the timing of shedding to a histogram of observations of the timing of courtship behaviors within the year (91 observations of courtship and mating) suggests a reproductive function of ecdysis in *C. horridus*. Explanations include possible exuviation of chemosensory-signaling molecules at skin sloughing.

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### Genetic Time Travel in Philippine Fishes

*Kent Carpenter, Angel Alcala, Pinsky Malin, Christopher Bird, Abner Bucol, Eric Garcia, Brendan Reid, Sharon Magnuson, Mudjekeewis Santos, Rene Abesamis, Jeffrey Williams, Cleto Nanola Jr., Richard Muallil, Whalen John, Jemelyn Baldissimo, René Clark, Ivan Lopez, Martin French, Roy Roberts, Kyra Fitz, Jordan Rodriguez, Chandy Jablonski, Marial Malabag, John Schaefer*

Human impacts on marine fish populations in the Philippines have intensified over the past century, particularly from fishing pressure and nearshore habitat destruction. Fortunately, inadvertent historical consequences from early Philippine-American interactions allow comparisons of contemporary fish populations with those existing in the early part of the 20th century. The U.S. government sent a scientific research vessel, the U.S.S. Albatross to the Philippines from 1908 to 1909 to survey fisheries resources. Over 91,000 specimens of fishes were collected in over 28,000 lots curated at the Smithsonian U.S. National Museum of Natural History; each lot putatively containing a single species from a single location. Fortunately, formalin fixation that makes DNA sequencing extremely difficult was not common for fish preservation during the time of the U.S.S. Albatross collections. Instead, the expedition utilized readily available ethanol – essentially high-powered raw rum – from the sugarcane industry as the preservative. Ethanol is now commonly used to preserve tissues for DNA extraction and sequencing. DNA in these over century-old specimens has degraded but a combination of RAD, baited, and low coverage shotgun sequencing has resulted in viable population level DNA sequences that is compared with sequences from contemporary

species duplicates collected at the same general locality as those collections from the U.S.S. Albatross. Preliminary data indicates losses in genetic diversity in contemporary duplicate populations.

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### Biofluorescent diversity in marine Teleosts and Elasmobranchs

*Emily Carr, John Sparks*

Many fishes have been discovered to possess a novel feature known as biofluorescence, the passive process of light absorption and re-emission at longer wavelengths. Whereas these wavelengths are beyond human's visible spectrum, the bulk of the diversity of biofluorescence in bony and cartilaginous fishes has only been described in the past decade. While there are many studies that describe fluorescence in new species, there is a lack of complete analysis of the phylogenetic diversity of biofluorescence. This study creates a comprehensive account of all known biofluorescent Teleosts and Elasmobranchs, a total of over 450 species spanning over 90 families. We also investigate phylogenetic patterns in fluorescent color including only red, only green, or both red and green.

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### Jack of All Trades: AES President, Editor, Tarpon Whisperer

*Jeffrey Carrier*

While Jack Musick's scholarship was widely known and admired, and his mentorship of his many students provided us with numerous successful and highly recognized scholars, a fitting legacy in its own right, perhaps lesser known are some of the other, less visible areas where Jack achieved similar successes. His term as AES President, his co-editorship of numerous volumes devoted to sea turtles and the biology of sharks and their relatives, and his never-ending quest for game fish taken on fly were equally as important as his primary loves of basic research and time at sea. These lesser-known accomplishments to be briefly summarized will provide a pleasant diversion from the more customary research topics to be presented in this Symposium and may be somewhat more entertaining as Jack's love of bureaucracy is further revealed.

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### Fishes of the Brazilian Coast

*Alfredo Carvalho-Filho*

Have you ever wondered what is the world's largest poorly known marine ichthyofauna region? There are dozens of really cool papers on fish from the Caribbean, Gulf of Mexico, North Atlantic, East Atlantic, Northwest Atlantic, just to name a few in that part of the world, but none on the Southwest Atlantic! The Brazilian coast is one of the longest in the world, the third in the American Atlantic. Along the almost 9,000 kilometers of coastline, 60% of Brazilians live, around 120 million people, almost double the total population of the United Kingdom! Millions eat fish every week, hardly knowing what they are eating! "Fishes of the Brazilian Coast" will fill this knowledge lack. This work presents diagnoses, photos and details that will allow the identification of 1,008 species of about 1,160 total coastal marine taxa of the region, which also has about 1,000 additional species from deep waters. Habitat, behavior, natural history, distribution, fisheries and edibility are also presented. From the shallowest estuaries and reefs up to 200 meters deep, this unusual work is aimed at professional ichthyologists as well as sport fishermen and people attracted by a very rich fish fauna. Professionals will find numerous novelties sprinkled throughout the text; anglers will find it enjoyable, with its well readable sections supplemented with illustrations. The large literature section (over 1,000 entries) is divided into background information, taxonomy sources covering multiple families, and references for each family. [www.brazilianmarinesfishes.com](http://www.brazilianmarinesfishes.com)

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### Migratory variability and seasonal habitat use of great hammerhead sharks in the Florida Keys

*Grace Casselberry, Gregory Skomal, Lucas Griffin, Jacob Brownscombe, John Finn, Joseph Dello Russo, Steven Cooke, Andy Danylchuk*

Limited data are available regarding great hammerhead shark (*Sphyrna mokarran*) spatial ecology, especially in the Florida Keys and southwestern

Florida, despite these areas being designated essential fish habitat. Fourteen mature great hammerhead sharks were tagged with acoustic transmitters in the lower Florida Keys. Movement data were collected from April 2019 through July 2022 throughout coastal Florida via data sharing with acoustic telemetry networks. Movements outside of the Florida Keys had high individual variability, with some sharks moving north during the summer months ( $n=9$ ) while others remained in the Keys year-round ( $n=5$ ). Network analysis with community detection algorithms quantified regional connectivity between the Florida Keys, coastal Gulf of Mexico, and coastal Atlantic Ocean. The Fast-Greedy algorithm revealed individual hammerheads rarely traverse both the east and west coast of Florida, instead the lower and upper Keys serve as an area of mixing for individuals that then move northwest into the Gulf of Mexico or northeast along Florida's Atlantic Coast. Sharks that were detected in the Florida panhandle comprised a more isolated community from the rest of the network, with infrequent returns to the Keys. Individuals that remained within the Florida Keys year-round displayed significant seasonal shifts in habitat use. High residency in channels in the spring and on artificial reefs in the summer and winter may be driven by prey availability and water temperature. These data improve our understanding of regional connectivity for great hammerheads in Florida and long-term fine scale habitat use, which is largely lacking for the species.

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### **Chytrid in the Clouds: Two Years of Mark-Recapture of Direct-Developing Frogs in the Amazonian Andes**

*Alessandro Catenazzi, M. Isabel Diaz, Jonathan Adamski, Hamlet Maza Camacho, J. William Tito Nina*

Anuran assemblages in montane forests of the Amazonian Andes have declined dramatically following epizootics of chytridiomycosis over the last four decades. While the fungal disease has extirpated or caused the likely extinction of many aquatic-breeding frogs, direct-developing frogs associated with terrestrial lifestyles have persisted and cohabit with chytrid, possibly because they rarely encounter the aquatic chytrid zoospores. In order to understand chytridiomycosis transmission and dynamics in terrestrial frog assemblages, since May 2021 we are conducting a mark-recapture study of all anurans along twelve 100-m long transects in the montane forests of southeastern Peru. The susceptibility of several of the study species has previously been assessed in laboratory infection trials, but how infection affects hosts in the wild, and the community level responses to enzootic conditions are poorly understood. We have marked over 2800 frogs of 17 species, with most frogs captured once or twice (15%), but some frogs captured up to six times over a period of up to 607 days. Recapture rate averaged 6% across all transects, and ranged from 2–40%, while disease prevalence varied from 0–80%. Our data show that frogs are able to clear disease infection in the wild, because several frogs that were infected when first captured (or during a recapture) appeared uninfected during one or several successive recaptures. Data on movements, infection history, changes in spatial distribution and abundance will inform models on disease dynamics, and increase our understanding of how a water-dependent fungus can spread among terrestrial organisms.

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### **Measuring local adaptation in natural shark populations using immunogenetic diversity**

*Eloise Cave, Michael Criscitiello, Jeannine Ott, Toby Daly-Engel*

To better understand how populations adapt to changing environmental conditions, it is useful to study functional genes that vary in response to environmental pressure, to shed light on how elasmobranchs cope with climate change. One highly-adaptive gene region is the major histocompatibility complex (MHC), which has been shown to vary in response to pressure like sea surface temperature (SST). Our overall objective is to determine if MHC loci show evidence of genetic local adaptation in sharks, which can be used to pinpoint populations at risk. This requires comparing functional loci like the MHC to putatively neutral loci like single nucleotide polymorphisms (SNPs). Here, we aim to determine the genetic diversity and structure of SNP loci in tiger sharks (*Galeocerdo cuvier*) for comparison to MHC loci. SNPs were generated on 283 tiger sharks by Double Digest Restriction Site Associated DNA sequencing (ddRAD-seq) and sequenced on a Nextseq 550 and a Novaseq 6000. The MHC class II a and b regions will be sequenced using

targeted next-gen sequencing to identify SNPs that occur only in the MHC region. This study will allow us to examine the distribution and diversity of SNP and MHC loci across a seascape, where higher MHC genetic structure and lower genetic diversity is interpreted as evidence of genetic local adaptation that may lead to better population-specific management and conservation efforts.

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### **Skink's country: integrative taxonomy of Angola *Trachylepis* (Squamata: Scincidae)**

*Luis Ceriáco, Mariana Marques, Diogo Parrinha, Arthur Tiutenko, Jeffrey Weinell, Brett Butler, Aaron Bauer*

The genus *Trachylepis* (Reptilia: Scincidae) is one of the more diverse genus of reptiles in the African continent. In Angola, southwestern Africa, the genus is currently represented by 21 species, most of them which are part of challenging nomenclatural and taxonomical species complexes. This problematic taxonomy affects our comprehension of the diversity and evolution of the genus as a whole in the African continent. Here we present the main results of a detailed integrative taxonomic revision of the Angolan *Trachylepis*. For this we used molecular phylogenetic analysis with a combination of mitochondrial (16S, ND2) and nuclear (RAG1) markers, as well as morphological data and an extensive historical and nomenclatural revision. As result we increased the number of *Trachylepis* species occurring in Angola, found six new species to science and revalidated forgotten species. The description of the new species and the revision and revalidation of the Angolan species already described contributes to a better understanding of the taxonomy and biogeography of the genus, as well as to the general biogeographic patterns and evolution of the Angolan and southwestern African herpetofauna.

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### **Predicting the long-term viability of Reticulated Flatwoods Salamander (*Ambystoma bishopi*) populations under future climate changes**

*Houston Chandler, George Brooks, Yan Jiao, Carola Haas*

Amphibians that breed in ephemeral wetlands typically exhibit cyclical dynamics whereby relatively high adult survival allows populations to persist through episodes of boom-and-bust recruitment. However, global changes that alter the normal cycle of events (e.g., the frequency of reproduction) can threaten the viability of these populations. We developed a stochastic Integral Projection Model for the Reticulated Flatwoods Salamander (*Ambystoma bishopi*) using demographic functions derived from 10 years (2010–2020) of drift fence monitoring on Eglin Air Force Base, Florida. We used this model to examine the responses of two salamander populations to climate change by incorporating projections from three Global Circulation Models under two emission scenarios. Using just wetland hydroperiod as a metric of reproductive success in the two populations, 16.7% and 33.3% of simulations indicate a high probability ( $>0.5$ ) of extinction by the year 2100. However, when also accounting for potential mismatches between salamander phenology and wetland hydrology, the number of simulations predicting high extinction probability increased to 50.0% and 67.7% at the two sites. In all simulations, both complete and partial reproductive failure were common, and our results indicate that there is a reasonable chance that these small flatwoods salamander populations will go extinct by the end of the century. Conservation and management actions should focus on maintaining high adult survival, managing habitat to extend wetland hydroperiod, and promoting clusters of high-quality breeding wetlands that can undergo natural extinction and colonization dynamics.

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### **Diversity and conservation of large and rare Sciaenidae species (Sciaenoidei, Acanthuriformes).**

*Ning Labbish Chao, Ming Liu, Meng-Hsien Chen, Chi-Wei Chang, Ying Giat Seah*

Sciaenidae includes about 300 species in 70 genera that inhabit mostly warm coastal waters with river runoffs. The minimum matured size is less than 7 cm TL (*Stellifer magoi*) to a maximum length of over two meters (e.g., *Argyrosomus regius*, *Argyrosomus hololepidotus*, *Bahaba taipingensis*, *Pseudolithus*



senegallus and *Totoaba macdonaldi*). Most species are fished for food, which has caused the wild population collapse, e.g., the Chinese yellow croaker (*Larimichthys crocea*) in the 1970s. Large croakers are popular for sport fishing and aquarium exhibitions; some have been successful in aquaculture. Their large and thick gas bladders have become highly valued and demanded in the Asian market, thus suffering more fishery pressure. The taxonomy of some large and widely distributed croakers still needs clarification. Large *Atractoscion* species of the West Indian Ocean, Australia, and the East Pacific share a uniquely shaped gas bladder with American *Cynoscion*, but differ by lack of sharp canine-like teeth at the tip of the upper jaw. The vulnerable *C. acoupa* of the central West Atlantic coast, heavily explored for their maw, also lacks sharp teeth. Widely distributed Indo-West Pacific species *Argyrosomus japonicus* and *Megalonibeia* (or *Protonibeia*) *diacanthus* may contain additional cryptic species. Southeast Asia endemic “Bahaba” *polykladiskos* is a sister species of the Freshwater *Boesemania microlepis*; their gas bladders (maw) are highly valued and most likely overfished. Little information is available on the rare *Panna perarmatus* of Borneo. The Central West African coastal *Pseudotolithus senegallus* is vulnerable, and the conservation task can be complicated in multi-national waters. Many small to medium-sized croakers are found in fishery by-catches and are challenging to identify, e.g., *Johnius* and *Stellifer* species. Amid the taxonomic uncertainty of *Sciaenidae*, an innovative conservation strategy is in demand.

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### Effects of Northern Snakehead (*Channa argus*) mucus and scales on terrestrial friction during amphibious behaviors

Francis Chiles-Lopez, Noah Bressman

The mucus coat serves many important functions for aquatic fishes, but its functionality for terrestrial behaviors in fishes have not yet been investigated. The Northern Snakehead (*Channa argus*) is an invasive fish to the Chesapeake Bay and among the largest amphibious fishes, so this species is likely impacted more by terrestrial friction than most amphibious fishes. The goal of this project was to investigate how snakehead mucus affects friction during terrestrial locomotion, assess if their mucus evolved to facilitate terrestrial behaviors compared to that of a fully-aquatic fish (Common Carp, *Cyprinus carpio*), and determine how snakehead scales influence friction anisotropy compared to a scaleless fish (Blue Catfish, *Ictalurus furcatus*). Using freshly euthanized fish on two substrates, we pulled fish in forward and backward directions using a force meter until the fish began to move, to indicate the force needed to overcome static overcome. We tested each fish its mucus coat intact and then again with it wiped off, allowing for paired comparisons. Snakehead mucus significantly reduced friction and was significantly more slippery than carp mucus. Additionally, scales exhibited frictional anisotropy properties, with less friction in the forward direction and more in the backward direction, mirroring snake scale anisotropy. Amphibious fishes like snakeheads may have evolved particularly slippery mucus to aid in terrestrial locomotion by reducing friction and energy required to move overland, potentially between bodies of water. This study also determined an artificial lubricant with similar properties to snakehead slime, which can aid in amphibious fish-inspired robotics and bio-materials.

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### Developing New Systems for Riparian Ecosystem Monitoring

Christian Ching, Jeffery W. Streicher

Riparian ecosystems maintain and protect biodiversity within both terrestrial and freshwater habitats. These unique transitional areas support diverse assemblages of fauna and flora, provisioning ecosystem services, benefiting conservation and mitigating species decline. However, riparian wetlands are declining at an accelerating rate, becoming one of our planet's most threatened ecosystems facing threats from invasive species, land use change, over-extraction of water, eutrophication, contaminants, and climate change.

Globally, monitoring strategies of riparian areas have largely focused on hydrology, water quantity and quality, rather than biodiversity. Previous work has also been concentrated in temperate regions, leaving understandings of ideal conservation interventions, and monitoring protocols in tropical riparian systems lacking. Methods of assessing riparian system health have generally used vegetation surveys, hydrological or remote sensing data. While these have the potential to map riparian coverage and hydrological services, they

have limited capacities for tracking biodiversity composition and change through time. Given these factors, our project leverages new technologies to determine faunal assemblages (amphibians and fishes) within a tropical riparian system.

Using passive acoustic monitoring and environmental DNA analyses, our study aims to build a tropical riparian biodiversity monitoring framework. Based in the Pursat Basin, Cambodia, we have determined sites along a river connecting the Cardamom Mountains to the Tonle Sap Lake. We have compiled species lists and will be undertaking field surveys and sampling collection-based specimens, contributing towards genetic reference libraries.

Our biological data will be integrated into hydrologic models to improve upon existing riparian health metrics, aiming to inform riparian ecosystem management.

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### Rhodopsin Exon 1: Variation across the Anuran Tree of Life

Christian Ching, Kate N. Thomas, David J. Gower, Ryan K. Schott, Ryan K. Schott, Jeffery W. Streicher, Jeffery W. Streicher, Jeffery W. Streicher

Anurans (frogs and toads) exhibit a diverse range of visual ecologies. However, the molecular mechanisms underpinning visual-system evolution have been examined in only a small proportion of the over 7,500 living species. Rhodopsin (RH1) is a visual opsin gene typically expressed in dim-light sensitive, rod photoreceptors. Its first exon has been used extensively as a phylogenetic marker in anuran amphibians, with thousands of sequences publicly available. These data represent an important opportunity to examine variation across numerous species and ecologies. We carried out molecular evolutionary analyses on an anuran RH1ex1 dataset including 1,423 species. We found considerable sequence variation across species, including at spectral-tuning sites known from both RH1 and other visual opsins. Some previously unreported substitutions may be related to visual adaptations in species with distinct visual ecologies. Our results suggest that with increasing phylogenetic sampling, we will gain much needed insight into the spectral-tuning site diversity of anuran visual pigments and, more broadly, that studies of vertebrate opsins with limited or sparse sampling may miss important biological variation.

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### Taxonomic Overview of Species of *Cirrhinus* (Cyprinidae: Labeoninae)

Patrick Ciccotto

The cyprinid genus *Cirrhinus* Oken is composed of medium- to large-sized fishes distributed throughout South, Southeast, and East Asia, and introduced elsewhere. These fishes inhabit a variety of freshwater ecosystems and are of major importance in wild-capture fisheries and aquaculture. Despite its ecological, cultural, and economic importance, the genus *Cirrhinus* and its constituent species have long and problematic taxonomic histories owing to superficial morphological similarities among members of the genus and with other labeonin genera. Molecular phylogenetic analyses have provided valuable insight into the evolutionary relationships of these species within the subfamily Labeoninae and in conjunction with thorough examinations of oromandibular structures, have begun to resolve taxonomic issues within the group. This has included the revalidation of the genera *Henicorhynchus* Smith and *Gymnostomus* Heckel, previously considered synonyms of *Cirrhinus*, but remaining putative species of *Cirrhinus* still constitute a polyphyletic group. Examinations of museum specimens of these species reveal substantial variation in oromandibular structures and other morphological characters. This morphological variation and its taxonomic implications among putative members of *Cirrhinus* are discussed.

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### DEI: Teaching Phylogenetic Comparative Methods Using Experiential Learning Practices Through the Lens of Promoting Diversity, Equity, and Inclusion

Patrick Ciccotto

Phylogenetic comparative methods are crucial to addressing broad questions in organismal and evolutionary biology. However, the theory and application of these methods can prove challenging to teach at the undergraduate level, particularly through traditional passive learning approaches that can

discourage course participation in members of marginalized communities. Understanding how to generate appropriate hypotheses, how phylogenetic trees are constructed and how to interpret them, why comparisons across species necessitate the inclusion of phylogenetic relationships, and how to interpret p-values associated with phylogenetically corrected statistical tests are among several issues that lead to the misunderstanding and misinterpretation of these methods by students. I present a case study in which undergraduate students in an upper-level evolutionary biology course conducted a novel, semester-long collaborative project comparing eye diameters of darters, a group of North American freshwater stream fishes, between riffle and pool habitats, with emphasis on addressing these aforementioned issues. Learning outcomes and assessments are described in the context of the experiential pedagogy of undergraduates in phylogenetic comparative methods and other concepts covered in an evolutionary biology curriculum. Emphasis is placed on how these types of research projects conducted as part of in-class activities can help promote diversity, equity, and inclusion in the biological sciences.

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### Morphological and Genetic Analysis of *Rostroraja* in the Gulf of Mexico

*Kaitlyn Cisz, Lindsey Nelson, Christian Jones, Eric Hilton, Jan McDowell, Christian Jones*

Two of the eight species in the genus *Rostroraja* are known to occur within the Gulf of Mexico: *Clearnose Skate*, *Rostroraja eglantaria* (Bosc 1800) and *Roundel Skate*, *Rostroraja texana* (Chandler 1921). Samples of *R. texana* were collected in 2019 from the Southeast Area Monitoring and Assessment Program (SEAMAP) trawl surveys in the northern Gulf of Mexico. Preliminary assessment of these specimens suggests that a third species of *Rostroraja* is also being caught in the Gulf of Mexico, tentatively identified as the *Ocellate Skate*, *Rostroraja ackleyi* (Garman 1881). The geographic range of *R. ackleyi* is currently described as being limited to the area stretching from Southern Florida to Cuba, and the far northwestern edge of the Yucatan Peninsula, and not overlapping with that of *R. texana*. Since *R. ackleyi* and *R. texana* share many morphological characteristics, including similar body form and pectoral ocelli, it is possible that if *R. ackleyi* is present in the Gulf of Mexico, it is being mistaken as *R. texana*. The purpose of this project is to use morphological and genetic data to determine if *R. ackleyi* is present in the Gulf of Mexico. Misidentification of species can have serious implications on the exploitation of both species. It is therefore important to determine which species occur in the region to better inform management decisions.

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### Metabolic Costs of Losing Microbial and Immune Defenses in Coqui Frogs (*Eleutherodactylus coqui*)

*Natalie Claunch, Zuania Colón-Piñero, Arik Hartmann, Anja Julian, María Torres-Sánchez, Ana Longo*

Procedures to obtain skin secretions in frogs may induce stress from handling or injection with stress-associated hormones (norepinephrine). We investigated the metabolic costs of procedures used to assess amphibian antimicrobial capacity and skin microbiome. We randomly assigned 48 adult coqui frogs (*Eleutherodactylus coqui*) to four treatments: microbiome depletion, antimicrobial peptide (AMP) depletion, microbiome and AMP depletion, and an unmanipulated control group. Microbiome depletion was achieved by soaking frogs in an antibiotic cocktail bath, whereas the AMP depletion was done by injection of norepinephrine followed by a buffer bath. We used a flow-through Sable Systems Field Metabolic System to collect respirometry data following a 30-minute acclimation period to respirometry chambers. Respirometry data were collected at three timepoints: (1) Baseline at 2-3 weeks prior to treatment; (2) Post-treatment representing 2 days after AMP depletion and 4 days after microbiome depletion; and (3) Final data at 6 weeks post-treatment. Then, to assess the effects of norepinephrine injection at a shorter timescale, a subset of 24 frogs that had not previously experienced AMP depletion were assigned to either AMP depletion or a buffer bath-only control group. Respirometry data collection began without acclimation to respirometry chambers, immediately after removing frogs from buffer baths. Over the 6-week period, we found no consistent treatment effects on metabolism in coqui frogs. At the shorter timescale, metabolism increased with

time-since-handling and after norepinephrine injection. Our results show that standard procedures predicted to increase stress at the individual level do not have a lasting effect on whole-frog metabolism.

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### What happens when you bring the seasons back: food-web and fish responses to restoration of variable flows.

*Justin Clause, Matthew Young, Nicholas Buckmaster, Veronica Violette, Steve Parmenter*

Flow variability cues fish migrations, maintains natural geomorphic processes, and mobilizes sediments in riverine systems. The reduction or removal of seasonally variability in flow can impair ecosystem function and lead to community-level changes. In the Owens River Gorge, California, decades of competing anthropogenic water demands have led to significant flow alterations and a complete removal of seasonal flow variability. Mandated variable restoration flows are currently being used to benefit fish populations and particularly Brown Trout (*Salmo trutta*), a non-native species that is locally important to recreational anglers. We used a combination of tools to examine the effects of these restoration flows on food-web structure and Brown Trout within the river. We observed substantial geomorphic alterations to the river that were paired with reductions of invasive invertebrates and increases in invertebrate prey species for Brown Trout. Using stable isotope analysis ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of muscle tissue and eye lenses, we identified longitudinal and ontogenetic changes in food-web structure and trophic support. Using otolith aging, we demonstrated marked differences in fish growth and development trajectories, suggesting flow-mediated variability in life histories. The maximum size of Brown Trout increased post-restoration flows suggesting more favorable growth conditions. Our results demonstrate the effects of flow restoration and suggest that restoring seasonal flow patterns can achieve targeted outcomes.

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### Correlating Environmental DNA and Physical Surveys for Atlantic Shark Species

*Paul Clerkin, Jan McDowell, Ellen Biesack*

Environmental DNA (eDNA) is a relatively new technology that collects DNA shed by organisms into their surrounding environments to study those organisms. Originally developed for rare freshwater species in enclosed systems, there has been recent interest in using eDNA methods for open ocean environments. However, before surveys can rely on eDNA methods to survey species, methods must be validated alongside traditional physical surveys, something that has been done in only a few studies in open marine environments, and even fewer in the offshore deep-sea. During 2022 we collected water samples from Chesapeake Bay and the Virginian coast with the Virginia Institute of Marine Science's (VIMS) R/V Bay Eagle (COASTSPAN long-line survey) and from North Carolina to Texas with the NOAA R/V Oregon II during their southeastern longline survey. A total of 241 water samples was collected using 1.7L Niskin bottles and a 1L subsample was passed through a 0.45 $\mu\text{m}$  nitrocellulose filter, preserved in 95% ethanol and held at -20 °C until eDNA was extracted. A short segment of the mitochondrial 12S rRNA region was amplified from each sample, indexed, pooled into a single library, and sequenced on an Illumina MiSeq System. Sequenced reads were clustered and assigned to a reference library including sequences from all barcoded shark species. Species detected by eDNA were compared to the composition of the physical survey to address the correlation between the two methods. Results from this research could inform future eDNA methods for deepwater surveys.

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### On the Distribution of Oral Sensory Papillae in Snakes

*Matthew Close, Kirsty de Wit*

Oral sensory papillae are conspicuous sense organs in the oral cavity in snakes, and relatively sparse studies of these structures offer conflicting conclusions as to the roles the papillae play. Histological and ultrastructural studies of snake oral papillae have described these structures as having features of vertebrate tactile and/or gustatory sense organs, but the functions that oral sensory papillae perform as well as variation in their distribution across major snake families remain poorly studied. We describe some of the gross functions these sense organs play in prey capture and swallowing and

compare distribution pattern, number and density of sensory papillae from representatives of several snake families within the context of jaw and fang evolution in snakes. Our preliminary results suggest that while there is little apparent variation in the histological structure of the oral sensory papillae, the distribution patterns across taxa vary considerably. The behavioral and anatomical data suggest that while the papillae may be useful for the positioning of prey items during swallowing, they may also provide a role in placement of the maxilla during prey capture. The evolution of the venom delivery system may thus have required a concomitant rearrangement of the sensory structures associated with the fang sheath for fang placement. Future anatomical studies should focus on the ultrastructure of the papillae, and well-controlled behavioral studies may elucidate the role these structures play in both prey capture and swallowing.

### Swim-glide Behavior and Optimal Dive Geometry Reduces Energy Expenditure in an Undulatory Rajiform

*Daniel Coffey, Carl Meyer, Yuuki Watanabe, Nicholas Payne*

Animals that travel through three-dimensional space may exploit potential energy from gravity or buoyancy to minimize the energy they need to expend in locomotion while maximizing their movement. For example, negatively buoyant elasmobranch species may utilize energy savings from vertical movement through intermittent gliding, alternating between active swimming and passive gliding to reduce the energy required for traveling a given distance. In particular, dorso-ventrally flattened stingrays (Rajiformes) have high lift-to-drag ratios, which optimizes glide performance with shallow pitch angles. Here, we used multi-sensor data-loggers to examine the fine-scale swimming behavior and activity (overall dynamic body acceleration as a proxy for energy expenditure) of free-ranging brown stingrays (*Bathytoshia lata*). During ascent from the seafloor, stingrays increased their pitch angle to reduce the energy required to move upwards against the force of gravity and decreased their pitch angle during descent to increase speed (terminal velocity during passive glides) and locomotor efficiency. The horizontal cost of transport for brown stingrays is minimized by ascending at an angle of 18-30° and descending at shallow angles near 5°, which closely matches the observed dive geometry. Though, descent pitch angles observed during passive glides varied depending on the vertical and horizontal distance traveled. These results demonstrate the oscillatory vertical movements of brown stingrays reduce energy expenditure relative to swimming at a level depth along the seafloor.

### Sandbar shark (*Carcharhinus plumbeus*) Preliminary Demographics, Habitat Use, and Movements in Nantucket Sound, MA

*Caroline Collatos, Jeff Kneebone, Greg Skomal, Nick Whitney*

Overfishing throughout the 1980s to 90s caused sandbar shark (*Carcharhinus plumbeus*) populations from MA to FL to drastically decline, prompting them to be listed as a prohibited species. Recently, sandbar sharks have shown signs of recovery and are becoming more common in regions where they were once scarce, including Nantucket Sound, MA. To better understand the importance of Nantucket Sound to their continued recovery, we used acoustic telemetry and catch data to describe sandbar shark demographics, habitat use, and movements. From July to September 2020 to 2022, 111 sandbar sharks [64% female, 36% male; 72% juveniles, 28% adults; 119 – 184 cm fork length (mean  $\pm$  SD: 145.8 cm  $\pm$  13 cm)] were caught. In 2021 and 2022, a subset of 41 sharks was tagged with coded acoustic transmitters and monitored with an array of 12 to 14 acoustic receivers deployed around Nantucket Island. Thirty-eight of the 41 sandbar sharks acoustically tagged were detected from July to September 2021 and 2022. Minimum residence time ranged from 1 to 44 days ( $n = 39$ , mean  $\pm$  SD = 12.7  $\pm$  10.2). Sandbar sharks were detected for an average of 29 minutes, and the duration of visits varied between locations. Individuals showed high site fidelity to tagging sites. These preliminary data suggest that Nantucket Sound is a recurring habitat for adult and juvenile sandbar sharks from June to September and may be an important area for their species' recovery.

### Ten-year update on the status of the worlds' tunas, mackerels, and billfishes

*Bruce Collette, Beth Polidoro*

Updated IUCN Red List assessments have recently been completed for the worlds' 61 species of tunas, mackerels and billfishes. The purpose of this 10-year reassessment was to determine if the global status of these species has improved or worsened, since their first comprehensive assessment in 2011. Both the updated assessments and the original 2011 assessments classified approximately 20% of species in threatened or Near Threatened categories. However, 17 of the 61 species were recently reclassified into an increased or decreased threat category. Five commercial tunas (*Thunnus*) have improved in recent years, partly due to better fishing methods, better regulations, and better enforcement of existing regulations by the several regional tuna FMOs (Fishery Management Agencies). However, the threat status of several smaller scombrids that are under the control of individual countries, including two of the three species of Indian mackerels (*Rastrelliger*) and two species of Spanish Mackerels (*Scomberomorus*) has gotten worse, primarily due to overfishing from increased numbers of fishing boats and the use of small mesh gill nets that catch young fish before they are old enough to reproduce. These results provide a broad overview on the global risk of extinction of species, compared to populations. Examples of populations of species that may be severely overfished in one region, while other populations of the same species may be stable or increasing, are presented to highlight the need for improved regional management in some areas, regardless of global IUCN Red List status.

### Genomic Powerhouse: de novo Assembly and Phylogenetic Analyses of Anolis Mitochondrial Genomes

*L. Caden Comşa, Anthony J. Geneva*

The lizard genus *Anolis* is an important model clade for the fields of evolutionary biology, ecology, and their intersection. However, despite extensive study of the genus, very little genomic data are available for the over 425 species in the genus. We set out to improve availability of genomic data among anoles by assembling mitochondrial (mito-)genomes of several anole species, as well as to test for the conservation of mitochondrial structure and genetic elements. We created a pipeline to process short-read sequencing files and generate mito-genome assemblies. We implemented the pipeline on whole genome sequencing (WGS) reads from our own datasets, as well as on WGS and Target Capture sequencing (TC) data publicly available on the Sequence Read Archive from NCBI. We successfully de novo assembled and annotated 18 complete mito-genomes using our pipeline, including one from off-target reads from a TC dataset. The structure of the mito-genomes was conserved across all analyzed species. Protein coding and rRNA genes from the mito-genomes were used to perform single-gene or concatenated phylogenetic analyses. Our phylogenetic analyses showed strong support for the monophyly of *Anolis* and for several relationships between the eight subgenera of anoles that have remained unresolved based on previous phylogenetic analysis of smaller sequence datasets. We show that mito-genomic data such as these are relatively easy to generate and can improve scientific understanding of *Anolis* by opening up new avenues for hypothesis-driven research, such as investigating potential differences in mitochondrial evolutionary rates between different *Anolis* lineages.

### Contributions to the developmental biology of the terrestrial red-backed salamander *Plethodon cinereus*

*Thais Condez, Tetsuto Miyashita, Hillary Maddin*

Within Plethodontidae, the most speciose group of salamanders, the terrestrial species *Plethodon cinereus* is one of the best-studied amphibians in the world. Besides being abundant in wooded areas from eastern North America, widely distributed throughout its range, and fairly represented in herpetological collections, information on its developmental biology is incentive. These haven't averted the target as one of the promising non-conventional model organisms for Developmental Biology. This lungless species trespasses the aquatic larval phase observed in metamorphosing amphibians. As a small-bodied direct developing species, it nests hidden in cavities or crevices, with clutches comprised of few eggs often laid underground. These characteristics

prevented earlier advances in studies of the reproductive biology and development of their eggs and embryos. However, studies have addressed the maintenance of aquatic larvae characteristics in *P. cinereus* embryos, which brings relevant contribution to our understanding of the evolutionary history of the larval phase in terrestrial and semi-aquatic plethodontid salamanders, as well as general amphibian evolution. The impressive background knowledge of its biology, the availability of a staging table, and current experiments of husbandry, supply new research venues with the development of this species. In this study, we aim to contribute to the knowledge of the internal anatomy development of *Plethodon cinereus* based on conventional osteological clearing and staining techniques and computer imaging, such as those derived from standard and contrast-enhanced microCT data. Besides providing new insights into the analysis of its developmental series, our findings might subsidize further studies with this species.

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### **Latitudinal differences in summer flounder sex ratio possible evidence for temperature-induced sex-reversal**

*Hailey Conrad, Holly Kindsvater*

Summer flounder, *Paralichthys dentatus*, have exhibited temperature-induced sex-reversal in experimental aquaculture settings, but little is known about how temperature may impact the sex determination in the wild population. Additionally, male and female summer flounder exhibit different water temperature and depth preferences at local scales, resulting in spatial sex-segregation. We used geographically weighted regressions and generalized linear models to identify whether patterns of spatial sex-segregation could be found in fishery-independent data across the entire range of summer flounder, using data collected by the NEFSC fall trawl survey. In accordance with previous studies, we found relationships between sex ratio and temperature and depth. We also identified latitudinal differences in the sex ratio of the adult population, with female-biased sex ratios in the northern half of the range and male-biased sex ratios in the southern half of the range. These latitudinal differences in sex ratio are consistent with trends between sex ratio and temperature found in experimental studies of temperature-induced sex-reversal in the species.

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### **Spiny-rayed fishes have not succeeded in the deep-pelagic ocean? The use of large midwater trawls challenges this paradigm.**

*April Cook, Tracey Sutton*

The standard dogma regarding fish assemblages in the deep-pelagic ocean (water column below 200 m depth) is that they are comprised largely of basal, soft-rayed fishes such as Myctophiformes, Stomiiformes, and Aulopiformes. While the diversity of soft-rayed fishes in the deep sea is certainly much greater than the derived, spiny-rayed fishes, it is the abundance and biomass of the latter that has been historically underestimated. Our view of the deep sea has been framed by what we can catch with relatively small, research-sized, rectangular midwater trawls (RMTs) towed from single-warp oceanographic vessels. Large, dual-warp midwater trawls (LMTs) have only been used repeatedly for research in the last two decades. The most extensive large-trawl sampling of meso- and bathypelagic fish assemblages was conducted in 2011 in the Gulf of Mexico as part of the Deepwater Horizon disaster damage assessment. During this sampling we found that spiny-rayed fishes were collected in much higher ratios than those of RMTs, and that this fauna represented a large proportion of fish biomass below 200 m. This program, dubbed ONSAP, both RMTs and LMTs were used to collect deep-pelagic fauna in the northern Gulf of Mexico between 0-1500 m depth. Data will be presented comparing the catches of basal, soft-rayed fishes to the more derived, spiny-rayed fishes using these two gear types. The resulting synthesis suggests that while soft-rayed fishes do indeed numerically dominate the deep-pelagic domain, the notion that spiny-rayed fishes are not successful in this habitat is likely a function of historical gear bias rather than evolutionary processes.

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### **Comparative Osteology of Western Australian Toadlets (Myobatrachidae; Uperoleia)**

*Ryan Cook*

Australian toadlets (genus *Uperoleia*) are the most species-rich frogs in the family Myobatrachidae, itself the most diverse anuran family in Australia. The genus includes 30 currently recognized species, and relatively little is known about phenotypic variation across this genus. External morphology of *Uperoleia*s largely conservative, and most species are not easily identifiable based on traditional characters. Other sources of phenotypic variation, however, may be more informative. My study uses micro-computed tomography (micro-CT) scans, bone-by-bone analyses, geometric morphometrics, and phylogenetic comparative methods to analyze variation in the osteology of Western Australian *Uperoleia* frogs. I predict that (1) miniaturization of the genus may have imposed a superficial similarity across congeners that has previously obscured functional and phylogenetic differences and (2) careful examination of *Uperoleia* osteology will reveal diagnostic characters that reflect molecular phylogenies of the genus. I find that bone-by-bone and geometric morphometric analyses, combined with phylogenetic comparative methods, reveal diagnostic characters in the genus and provide clarity on the uncertain identity of the type species *U. marmorata*. This study follows up on previous preliminary work with quantitative analyses, and it is the first to employ multiple phenotypic traits in an integrative taxonomic context for this group of frogs and could provide a model for studying the phenotype of other traditionally “difficult” taxonomic groups.

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### **Determining impact of two decades of nursery habitat change on juvenile lemon shark survival with satellite remote sensing and mark-recapture.**

*Emily Cormier, Emmanuel Devred, Derek Tittensor, Matthew Smukall, Kristen Wilson, Heike Lotze*

Many shark species are important for the structure and functioning of marine ecosystems. Currently, shark species worldwide are facing high extinction risk and habitat loss may play an important role in population declines, especially for species which require specific habitats for critical life history stages. In Bimini, the Bahamas, juvenile lemon sharks (*Negaprion brevirostris*) show philopatry to specific mangrove and shallow seagrass nursery habitats and juvenile sharks remain in these nurseries for the first several years of life. However, over the past two decades, dredging and development have removed large areas of mangrove and seagrass, potentially impacting lemon shark's use of this habitat. My project uses satellite remote sensing to create habitat maps from the late 1990s to 2020, to quantify change in seagrass and mangrove distribution; these maps have been paired with annual standardized juvenile lemon shark mark-recapture datasets to understand the long-term impacts of change in habitat availability on juvenile lemon shark survival. Use of random forest and automated adaptive signature generalization algorithms has allowed for the standardized mapping of benthic habitat over long time scales and this project provides a framework with which to assess past habitat distributions with high accuracy.

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### **Deep on Runnin’ – Jack Musick’s 50-year Journey of Deepwater Chondrichthyan Discoveries Continues to Inspire New Generations of Scientists**

*Charles F. Cotton, R. Dean Grubbs, Brian J. Moe*

John A. (Jack) Musick has directed or influenced studies of deepwater chondrichthyan for over a half-century (and counting). Beginning with an incidental catch of a bramble shark while trawling off Virginia in 1968, Musick developed a strong interest in deepwater chondrichthyan during the following decades. As a preeminent shark researcher, Jack was recruited to participate in a landmark expedition in the mid-1980's (MARE) to determine why sharks were biting prototype fiber optical cables in the deep Eastern Atlantic Ocean. He would go on to forge many lasting international collaborations focused on deepwater chondrichthyan, largely fueled by a renewed global interest in valuable squalene. Toward the end of his career, Jack became more focused on promoting policies that offered protection for vulnerable deepwater chondrichthyan, e.g. attending the novel Workshop on



Conservation and Management of Deepwater Chondrichthyan Fishes at the FAO's "Deep Sea 2003" meeting. Jack's influence is pervasive throughout AES and abroad, with academic "children" and "grandchildren" joining or leading several consequential modern projects, including: an international expedition in 2004 to characterize mid-Atlantic Ridge fauna (MAR-ECO); the refinement and development of novel methods for modeling age and growth of deepwater chondrichthyans; a long-term study of northern Gulf of Mexico chondrichthyans affected by the Deepwater Horizon oil spill (Deep-C); an AES symposium (2012) and Deep-Sea Research journal issue (2015) dedicated to the "Biology of Deepwater Chondrichthyans"; and a recent book chapter that highlights new developments in deepwater chondrichthyan research and provides regional summaries of global deepwater chondrichthyan fisheries.

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## The Decoy Hypothesis in Juvenile *Plestiodon multivirgatus*

*Toby Covill, William Rosenthal, Catherine Wagner, Sean Harrington*

Most skinks of the genus *Plestiodon* possess blue tails as juveniles that change to match body color as they mature. Blue tails have been shown to serve as a predator diversion tactic in *P. fasciatus* and *P. laticeps*, but it is unclear if this function is conserved throughout the genus. To expand our knowledge of the function of blue tails in the genus *Plestiodon*, we used a clay model predation experiment to examine the effects of the trait on avian predation in the many-lined skink, *P. multivirgatus*, in southeast Wyoming. We found that the presence of a blue tail significantly increased the average distance of avian attack away from the head towards the tail of the model and altered the distribution of attacks on the body and tail. Blue-tailed models were also found to be attacked less than black-tailed ones. These results suggest that the blue tails do serve to aid in evading predation, and additionally suggest a potential function in crypsis as well. The second conclusion contradicts previous experiments examining similar traits, opening new avenues for future research into this understudied species.

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## Assessing the Physiological Response of Sand Tiger Sharks (*Carcharias taurus*) to Longline Capture

*Chestina Craig, Christine Bedore, Kady Lyons, Johanne Lewis*

In sharks, capture and handling can elicit a physiological stress response, often quantified by changes in blood chemistry associated with mobilization of fuel (increased glucose and ketones) and shifts in metabolic state (increased lactate). Stress responses are variable across species, sexes, and ontogeny, and can be influenced by other factors such as temperature, dissolved oxygen, and hook time. *Carcharias taurus* is a nearshore, migratory shark, popular in aquaria, and targeted by scientists for research. The objectives of this study are to characterize the physiological response of *C. taurus* to longline capture and identify factors that may influence plasma glucose, lactate, and ketone concentrations at the time of capture. Sharks were sampled off St. Helena Sound, South Carolina, via longline, reeled to the boat, and a blood sample was taken via caudal venipuncture. Shark sex and length was recorded. Environmental factors and soak time were noted for each longline set. Plasma was analyzed for glucose lactate and ketone levels. Sex and length did not have a significant effect on glucose, lactate, or ketones, nor did environmental factors. However, soak time did have a significant effect on plasma lactate levels; as soak time increased so did plasma lactate concentrations. This preliminary data suggests that there are no sex differences in *C. taurus*'s response to longline capture. Additionally, *C. taurus* appears to be a relatively robust species and not easily perturbed by most variables explored here.

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## Hypothesized Morphological Correlates of Slug-eating in *Contia*, Compared with Related Dipsadine Snakes

*Lauren A. Craige, Alan H. Savitzky, Helen Bond Plylar*

Worldwide, at least 10 genera of snakes include species known or believed to rely primarily on a diet of mollusks. Two lineages, the Asian Pareidae and the Neotropical Dipsadini, include species that are highly adapted to extract the soft parts of snails from their shells, including asymmetry of mandibular dentition in some Pareidae. Despite also consuming mollusks, *Contia* spp., which are slug specialists, exhibit neither the mandibular asymmetry nor the exaggerated

cephalic features of snail-eating snakes. Instead, they have mandibles with unusually long, slender teeth, the functional significance of which is not understood. For this study, we compared *Contia tenuis* and *C. longicauda* to *Carphophis amoenus*, and *Diadophis punctatus*, to examine the morphological correlates involved in the consumption of slugs. All three genera are basal members of Dipsadinae and are similar in body size. However, *Carphophis* primarily consumes worms, and *Diadophis* feeds on a variety of prey, including small vertebrates. High-resolution X-ray micro-computed tomography (micro CT) with diffusible iodine contrast-enhanced scanning (diceCT) was used to image the cranial anatomy and cephalic musculature. Our findings revealed substantial cranial and muscular differences among the three genera, some of which we interpret as adaptations for consuming different classes of soft-bodied prey. Comparisons are complicated by the fossorial adaptations of the skull of *Carphophis*. Detailed observations of prey handling in *Contia* and *Carphophis* may inform a functional interpretation of their morphological differences.

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## Mercury Bioaccumulation is Linked with Immunosuppression in the Northwest Atlantic White Shark (*Carcharodon carcharias*)

*Lisa Crawford, Nolwenn Dheilly, Charles Edelson, Anne McElroy*

As apex predators, white sharks (*Carcharodon carcharias*) are susceptible to bioaccumulation of contaminants like mercury; however, the potential molecular effects of mercury exposure are understudied, particularly in wild-caught animals. We measured total mercury (THg) and extracted RNA from white shark muscle tissue from 29 sharks sampled during 2018-2021 OCEARCH expeditions along the U.S. East Coast. Muscle THg averaged 11.3 mg/kg d.w. (range 4.30-25.3 mg/kg d.w.). RNA-sequencing was performed and transcripts were annotated using the white shark genome. Differential expression analysis comparing transcription between the sexes, sampling locations, and maturity stages revealed only one differentially expressed gene between males and females that may be associated with sex organ development. Weighted gene co-expression network analysis (WGCNA) was conducted to investigate relationships between gene transcription and muscle THg. Three gene modules were significantly correlated with THg levels. Using functional enrichment analysis, THg bioaccumulation was found to influence genes involved in immune system function, response to viruses, and cell signaling, suggesting that mercury may cause immunosuppression. This study provided novel evidence to begin to answer long-standing questions about the consequences of mercury bioaccumulation in sharks and responded to calls to integrate next generation sequencing technology with traditional ecology and toxicology to study the susceptibility of elasmobranchs to anthropogenic stressors.

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## An application of habitat modeling to fisheries spatial management

*Dan Crear, Steve Durkee, Tobey Curtis, Tom Warren, John Carlson*

Multiple large spatial management areas throughout the U.S. east coast and Gulf of Mexico are closed to various longline fisheries. Since their implementation 20 years ago, the majority of these areas have had very little fishery-dependent data collected from them making it difficult to assess their effectiveness at protecting bycatch species, such as shortfin mako shark, dusky shark, and leatherback sea turtles. NOAA Fisheries developed a modeling tool that uses fisheries observer data, environmental data, and gear information to predict the interaction probability of bycatch species in longline fisheries within these closed areas. This model, Highly Migratory Species Predictive Spatial Modeling (PRISM), then generates metrics to assess the effectiveness of the closed areas using the predicted bycatch interaction probabilities. This information is also used to help make spatial and temporal modifications to the closed areas to improve the effectiveness in bycatch protection and guide careful fishery-dependent data collection efforts inside the areas. This approach demonstrates how science based efforts like habitat modeling can be applied to the design, assessment, and adjustment of fisheries spatial management.

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## Beyond the Sea(snake): Reviving Historical Collections for New Lessons in Collaboration and Beyond

Hayley Crowell, Samuel Borstein, Jenna Crowe-Riddell, Alison Davis Rabosky, Haley Martens, Ramon Nagesan, Randy Singer

Sea snakes are the most diverse group of living marine reptiles (>60 species) and they operate as mesopredators within an array of aquatic ecosystems. Sea snakes are found in coral reefs, estuaries, deep-sea plains, and can even inhabit areas spanning entire oceanic basins. By studying sea snake feeding ecology, we can also indirectly study the unique communities that sea snakes occupy, as well as create new opportunities for cross-institutional collaboration between herpetologists and ichthyologists. As active foraging predators of fish, sea snakes frequently access new or complex habitats, such as deep-ocean, that biologists would otherwise have difficulties sampling. Museum collections can greatly enhance our natural history knowledge of such animals by providing access to large numbers of sea snake specimens collected across space and time. For this project, we x-rayed and CT-scanned over 220 preserved sea snake specimens from multiple museums to identify new diet, reproductive, and other undocumented biological records from across the globe. We dissected all snakes with visible stomach contents or other objects of interest (i.e. embryos, internal parasites, etc.) and identified the prey items in collaboration with scientists from both the University of Michigan Museum of Zoology Herpetology and Ichthyology divisions. We discovered multiple new prey species records, reproductive information, and novel insights into sea snake feeding ecology. Our results also demonstrate how non-invasive sampling can maximize the results of natural history research while minimizing the impacts of destructive sampling techniques.

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## Plasticity in Sex Determination of Painted Turtles (*Chrysemys picta*)

Claudia Crowther, Luke Hoekstra, Fredric Janzen, Lisa Schwanz

In order to maintain stable sex ratios, and avoid population collapse, reptiles with temperature-dependent sex determination (TSD) must respond to environmental fluctuations, both natural and anthropogenic. Adaptive plastic responses may balance sex ratios, however the presence and mechanisms of this plasticity are not well established. Evidence from species with TSD suggests that maternal physiological traits can adaptively influence offspring sex. We investigated evidence for inter-annual variation in the pivotal temperature ( $T_{piv}$ ) and transitional range of temperatures (TRT) in a wild population of painted turtles (*C. picta*). Additionally, we tested for correlation between clutch sex ratios and two potential mechanisms of maternal control over offspring sex: egg size and maternal age. We observed significant inter-annual covariation between  $T_{piv}$  and mean nest temperatures, in line with previous work. However, we did not find evidence of adaptive plasticity in  $T_{piv}$  or TRT, when we accounted for fluctuating incubation temperature. Similarly, variation in clutch sex ratios was not significantly related to egg size or maternal age, though there was a nonsignificant trend for older females to produce female-biased clutches. The differing results from two measures of nest temperature may be explained by the feminising effects of fluctuating nest temperatures, or maternal behavioural responses to climate variability. The lack of evidence for adaptive plasticity in *C. picta* may be a cause for concern under current rapid climate change.

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## Feeding and Trophic Ecology of Invasive Blue Catfish in the Nanticoke River, MD/DE

Zach Crum, Noah Bressman

Invasive species can cause dramatic ecological impacts in aquatic systems by altering community and ecosystem structure. Blue Catfish (*Ictalurus furcatus*) were introduced into the Chesapeake Bay in the 1970s to provide fishing opportunities and have since expanded in geographic distribution and population size throughout the Bay. Now classified as an invasive species, diet studies from the Chesapeake Bay's Western Shore indicate that Blue Catfish consume native species of economic and conservation concern with diet composition varying by tributary. To address tributary specific management goals, we assessed Blue Catfish diet and trophic ecology in an Eastern Shore tributary of the Chesapeake Bay. We analyzed stomach contents from 1,017

Blue Catfish collected throughout the Nanticoke River watershed in Maryland and Delaware. Prey items were identified visually and using molecular analysis. Additionally, we collected white muscle tissue samples from Blue Catfish and species that make up their diet to analyze stable isotopic ratios of carbon, nitrogen, and sulfur as a secondary method of investigating feeding and trophic ecology. Our results indicate patterns in diet composition that vary spatiotemporally and by size of Blue Catfish. Large Blue Catfish (>550mm) pose the greatest threat to species of economic and conservation concern in the Nanticoke River as they prey heavily on White Perch (*Morone americana*), river herring (*Alosa* spp.), Atlantic Menhaden (*Brevoortia tyrannus*), and Blue Crab (*Callinectes sapidus*). Fisheries managers should continue to monitor populations of these particularly impacted prey species of concern in the Nanticoke River and implement further conservation measures as necessary.

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## Effects of anthropogenic landscape features on population connectivity of a small-bodied, benthic, headwater specialist fish.

Alexis Culley, River Watson, Catherine Haase, Matthew Thomas, Stephanie Brandt, Michael Floyd, Rebecca Blanton

The imperiled Kentucky Arrow Darter (KAD), *Etheostoma spilotum*, is endemic to first and second order streams in the upper Kentucky River system and has experienced substantial declines in occurrence and population size over the past several decades. Declines are attributed to extensive habitat degradation, particularly from mining activities and deforestation. To evaluate the potential effects of surface mining and other landscape variables on population connectivity, we performed isolation by resistance and distance models using genetic distance calculated from eleven microsatellite loci and resistance mapping of multiple landscape variables. We created multiple resistance surfaces from landscape variables that are known to influence KAD occurrence, including elevation, presence of dams, stream order, and percent landcover of agriculture, urbanization, forest cover, and surface mining. We created a cumulative resistance surface from the individual surface layers with resistance values assigned from the literature to calculate cumulative resistance among populations using the R package *gdistance*. Cumulative resistance was correlated with FST values using a linear mixed effects model in *ResistanceGA*. FST values indicated a high level of genetic fixation between KAD populations. Initial analysis of single surface resistance modeling showed that decreased forest cover is linked to high genetic fixation, suggesting that land use practices leading to deforestation, such as surface mining, agriculture, and urbanization, contribute to genetic isolation of KAD populations. Results of the cumulative impacts of extensive mining activities and other landscape variables on population connectivity of the Kentucky Arrow Darter will be discussed.

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## Emerging Satellite Telemetry Applications in Elasmobranch Fisheries Management

Tobey Curtis

Satellite telemetry has become a common tool in elasmobranch research for addressing a wide variety of ecological, physiological, and conservation questions. The numbers of telemetry studies on species subject to fisheries catch is growing at a rapid pace, often with support of governmental fisheries management agencies. Results can be very informative in supporting and evaluating management strategies. This is especially true for spatial management applications such as Essential Fish Habitat, stock identification, and closed area assessment. However, comparatively few studies have generated data that can more directly inform the stock assessment process that serves as a foundation for U.S. fisheries management. I will review recent advancements with illustrative case studies demonstrating how satellite telemetry can be used to estimate bycatch risk, catchability, post-release mortality, and natural (M) and fishing (F) mortality rates. Additionally, species distribution models generated from telemetry data can be used to improve indices of abundance, and help predict climate-influenced shifts in distribution that may have ramifications for fisheries. Based on these examples, telemetry users may be able to adjust analytical approaches to tangibly increase their impact on the fisheries management process.

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## Mercury and selenium concentrations in muscle of the scalloped hammerhead *Sphyrna lewini* from western North Atlantic waters

*Tatyana Dacostagomez, R. Dean Grubbs, Bryan Frazier, Gretchen Bielmyer-Fraser, Brooke Discher, Jim Gelsleichter*

The scalloped hammerhead *Sphyrna lewini* is a large, coastal-pelagic/semi-oceanic shark species common on U.S. east coast waters, although it has declined in abundance over the past 50 years due to overexploitation and bycatch in commercial fisheries. In addition to risks from fishing-related activities, scalloped hammerheads accumulate elevated quantities of environmental toxicants, putting them at risk from coastal pollution. However, to date, only a small number of studies have examined pollutant uptake in northwest Atlantic populations of this species. In this study, we examined muscle concentrations of the toxic metal mercury (Hg) and the Hg antagonist selenium (Se) in newborn to adult scalloped hammerheads from sites along the U.S. east coast, ranging from North Carolina south to the Florida Keys. Total Hg (THg) concentrations in scalloped hammerhead muscle ranged from 0.12 to 3.20 mg/kg wet weight (mean  $\pm$ SD = 1.12  $\pm$ 0.90) with >40% of samples exceeding global thresholds for human dietary purposes as well as fish thresholds for toxicological responses (1.0 mg/kg ww.). Muscle THg concentrations were significantly correlated with animal size, demonstrating Hg bioaccumulation in this species. The Se:Hg molar ratio declined below protective levels of 1:1 at fork lengths above ~100 cm, suggesting the potential for toxic effects in sharks above this size as well as human consumers of scalloped hammerhead meat. The results of this study show that Hg may pose toxicological risks to scalloped hammerhead populations on the U.S. east coast, necessitating the need for further research on this topic.

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## A Multi-Tissue Stable Isotope Analysis of Juvenile Lemon Sharks, *Negaprion brevirostris*, From Distinct Nurseries.

*Jack Dales, Jeremy Stalker, Matthew Smukall, Bryan Franks*

Shark nurseries are important habitats that provide protection from predators and an abundance of prey items. Lemon sharks (*Negaprion brevirostris*) often use shallow lagoons and coastal inlets as nursery habitats, exhibiting fidelity to pupping grounds. Neonate lemon sharks near Bimini the Bahamas remain within their primary nursery habitats year-round, and in some cases for many years before dispersing. Around the Florida Keys, lemon sharks have a faster growth rate and may disperse from nurseries younger, but still reside year-round. Conversely, lemon sharks born within coastal waters of the Carolinas and Georgia make long migrations to and from an overwintering site in Cape Canaveral, Florida. The objective of this study is to assess diet patterns in juvenile lemon sharks using stable isotope analysis from individuals using disparate nurseries that vary both in biotic and abiotic factors, and in timing of use by these sharks. Stable isotope analysis (carbon and nitrogen) of blood plasma and muscle tissue from juvenile lemon sharks captured in each study location are being compared to determine differences in foraging habitat and relative trophic position. In addition, stable isotope analysis may yield information regarding how differential use of nurseries may affect diet and growth for young sharks. As these geographically separate nursery sites are subject to differences in temperature range, habitat type, and predator and prey availability, this variability may have ecological implications for the ecology of juvenile lemon sharks within these regions.

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## A Global Review of the Conservation Status of Microgobies

*Cameron Davis, Samuel Davis, Christi Linardich, Kent E. Carpenter*

Amongst the world's vertebrates, both the smallest and the shortest lifespans are represented by microgoby fishes. Microgobies constitute 340 species in 13 genera with 70% in *Eviota* and *Trimma*. These tiny, cryptobenthic reef fishes primarily occur in shallow tropical waters and play a key role in reef ecology as prey. To identify global marine conservation priorities, the IUCN Marine Biodiversity Unit is assessing the extinction risk of all marine fishes for inclusion in the IUCN Red List. The first phase of an initiative to assess all 2,000+ gobies will focus on the 110 microgobies not currently published. An analysis of the conservation status of the 230 previously assessed microgobies identified 6 threatened and Near Threatened species. These restricted

range species have areas of occupancies ranging from 8-3,000 km<sup>2</sup> and are experiencing major threats from coastal development, coral reef degradation (e.g., *Eviota raja*) and/or estuarine degradation (e.g., *Eviota ocellifer*). Forty-nine microgobies are listed as Data Deficient (DD) primarily due to poorly understood distributions. Three-quarters of the DD microgobies are known from 1-2 collection localities and an average of 13 specimens. Seven DD microgobies have major threats that could justify a threatened category upon confirmation of range restriction. This highlights the need for sampling of microgobies to confirm range size. Ongoing work to assess the remaining 110 species may reveal additional threatened species. These results will be used to inform conservation for these fishes that are sensitive to nearshore degradation and are frequently overlooked in management plans.

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## Diversity and Occurrence of Introduced *Hemidactylus* in the Rio Grande Valley of south Texas, USA

*Drew R. Davis*

Currently, two species of introduced *Hemidactylus* are recognized to occur in south Texas, USA. *Hemidactylus turcicus* (Mediterranean Gecko) was first recognized and documented in Brownsville, Cameron County in the early 1950s, and has since spread across much of the state and throughout the southern USA. More recently, a single population of *H. mabouia* (Wood Slave) was documented from a small area (ca. 3.1 ha) in Brownsville in 2013. Here, I document the first records of two new species of *Hemidactylus*: *H. aff. parvimaculatus* (Sri Lankan Spotted House Gecko) from Cameron, Hidalgo, and Willacy, as well as *H. garnotii* (Common House Gecko) from Cameron County. Here, I discuss the distribution of *Hemidactylus* across the region, provide baseline inventories that allowing for future comparisons to monitor the spread of these species across the region, and comment on perceived introduction routes, cold tolerance, and species interactions.

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## Phylogenetic Analyses Reveal New Potential Species Within *Syncope*, A Subgenus Of *Chiasmocleis*

*Grace Davis, Molly Wierman, João Tonini, Rafael de Sá*

In the past decade, attention was focused towards describing the diversity of amphibians, with emphasis on species-rich groups within Microhylidae. The subgenus *Syncope* (genus *Chiasmocleis*) contains the smallest species of Gastrophrynine Microhylid frogs. They have bone fusions, the loss of two vertebrae, and reduction with loss of digits. *Syncope* as a species are bromeliad dwellers, making it challenging to detect them in biological surveys. Thus, genetic information for *Syncope* is limited to a few populations. Our primary goal is to build a comprehensive phylogenetic tree including samples from all species in the *Chiasmocleis* genus and from new populations sampled in Colombia to expand our understanding of the evolutionary history within subgenus *Syncope*. In this analysis, we utilized DNA extracted from various tissue samples across South America using two mitochondrial (16S, 12S) and three nuclear markers (BDNF, SIA, TYR). We generated a phylogenetic tree using Maximum Likelihood and our results showed two major clades of *Syncope*. The first clade contained 5 species including: *C. (Syncope) bassleri*, *C. (Syncope) supercilialba*, *C. (Syncope) haddadi*, *C. (Syncope) hudsoni*, and *C. (Syncope) jimi*. The second clade contained 4 species including: *C. (Syncope) tridactyla*, *C. (Syncope) carvalhoi*, *(Syncope) antenori*, and *C. (Syncope) parkeri*. Our sampling from populations in Colombia suggests two new species within *C. (Syncope) bassleri* and one new species within *C. (Syncope) antenori* and *C. (Syncope) tridactyla* respectively. Future work will focus on examining morphology of the new taxa identified by the molecular phylogeny, to differentiate them from the current described species of *Syncope*.

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## Systematics of Dragonfishes and the Evolution of Bioluminescent Structures

*Matthew Davis, Emily DeArmon, W. Leo Smith*

Dragonfishes are a species-rich lineage of deep-sea pelagic fishes (~319 species) that have a worldwide distribution and captivating adaptations that have evolved for living in their environment. In this study, the evolutionary relationships among the barbeled dragonfishes are inferred from a combination of

ultraconserved elements (UCEs) and protein-coding gene fragments providing new insights into their evolutionary history and classification. Prior studies have hypothesized that marine lineages living in open-ocean habitats may exhibit lower species richness due to a reduction in genetic isolation among populations. The observed high species richness of dragonfishes indicates that this lineage is undergoing genetic isolation in the deep sea, and previous studies have indicated that the barbeled dragonfishes are diversifying at a rate that is significantly higher than expected given their clade age. This increase in diversification has been attributed to their anatomically species-specific bioluminescent barbels, which are sometimes sexually dimorphic and exhibit tremendous anatomical variation among the various species of dragonfishes. This research focuses on investigating the evolution of dragonfishes and the evolution of their bioluminescent structures. Character evolution of features associated with bioluminescence are investigated across the dragonfish radiation.

### **Movement Behavior of Large Juvenile and Adult Smalltooth Sawfish (*Pristis pectinata*) in the Florida Keys and Everglades National Park**

*Ashley Dawdy, Dean Grubbs*

The smalltooth sawfish (*Pristis pectinata*) became the first marine fish to be listed as Endangered under the United States Endangered Species Act (ESA) in 2003. This listing was the result of a drastic population decline and subsequent range contraction attributed to high bycatch rates in commercial fisheries, loss of mangrove habitat due to coastal urbanization, and poaching, and the species has since been listed as Critically Endangered by the IUCN. While critical habitat has been designated for juvenile *P. pectinata* in the United States, it remains undesignated for adults. The Grubbs lab and collaborators have internally tagged over 70 large juvenile and adult *P. pectinata* with passive acoustic transmitters since 2016 that have been detected on over 500 acoustic receivers. On the 20th anniversary of its ESA listing, we discuss trends in movement behavior, including migratory behavior and space use, of large juvenile and adult smalltooth sawfish in the Florida Keys and Everglades National Park.

### **Tracking Four Years in the Life of a Female Whale Shark Reveals Consistent Migrations in the Gulf of Mexico**

*Daniel Daye, Julian Garrison, Rafael de la Parra, Jeremy Vaudo, Guy Harvey, Jessica Harvey, Mahmood Shivji, Bradley Wetherbee*

Concerns about sustainability of whale shark (*Rhincodon typus*) populations and their interactions with humans have generated a high level of interest in the movements and migration of the largest fish in the ocean. We tracked Rio Lady – a mature female – along with 25 other sharks from the male-dominated aggregation near Isla Mujeres, Mexico via SPOT (Smart Position and Temperature) tags. Rio Lady has been tracked for nearly 1,500 days and reported over 1,400 locations, travelling at least 40,000 km. During a period of frequent reporting from 2018–2021, Rio Lady exhibited a repeated pattern of seasonal movements between three regions within the Gulf of Mexico (GOM). This included intense use of a small area off Isla Mujeres, Mexico during summer months among a large aggregation of whale sharks. The other sharks that were tracked long enough similarly returned to this area. Tagging whale sharks with SPOT tags and acquiring relatively accurate locations over long time periods enabled the use of state-space modeling (SSM), move persistence modeling (MPM), and machine learning to identify areas of concentrated movement and construct predictive habitat suitability models for the GOM. Habitat suitability models allowed the prediction of areas of high use based on move persistence values and the associated environmental variables. The combination of these analytical methods and high resolution, Argos-derived location data is promising for revealing long-term movement patterns of whale sharks and for identifying areas of high use outside of known feeding aggregation sites.

### **DNA Barcoding Sheds Light on Deep-Sea Fish Diversity in the Southern Atlantic**

*Heloísa Caixeta, Claudio Oliveira, Marcelo Melo*

The deep ocean, the largest and most extreme environment on Earth, holds approximately 15% of fish biodiversity, including some of the rarest and highly adapted species. Recent technologies, like DNA barcoding and metagenomics, lead to the elucidation of taxonomic problems, including the discovery of new species; however, studies focusing the deep-sea species remain challenging, especially in the South Atlantic. Herein, we assessed such diversity in the Brazilian EEZ using DNA barcoding. About 1,027 tissue samples from 120 species were collected during the cruises conducted onboard R/V Alpha Crucis, as part of the DEEP-OCEAN Project. The DNA was extracted, and the COI gene was amplified using universal primers. The PCR products were sequenced bi-directionally, and the sequences were compared with the available databases confronting our preliminary morphological identification. Maximum Likelihood and Bayesian phylogenetic trees were performed including all sequences. We generated 2 sequences from one hagfish species, as well as 208 COI sequences from 104 actinopterygian species, belonging to 50 families, and 22 orders. The chondrichthyans will be our next target. The phylogenetic trees clustered species according to their taxonomic classifications. Most of the sequences were the first contribution from the South Atlantic, and for some species, they represented the first sequence of the genus. The DNA barcoding revealed a high phylogenetic diversity of deep-sea fishes, supporting our preliminary identifications and even the discovery of new species.

### **Polymixia (Polymixiiformes: Polymixiidae) in the Western South Atlantic: a New Chapter on the Taxonomy of this Enigmatic Group of Fishes**

*Heloísa Caixeta, Claudio Oliveira, Marcelo Melo*

*Polymixia* is a deep-sea fish genus, currently including 11 valid, extant species and one valid, fossil species. The specimens were collected by the R/V Alpha Crucis, as part of the DEEP-OCEAN project, on the southern Brazilian continental slope. An integrative taxonomic study revealed a new species of *Polymixia* endemic to the western South Atlantic, which had been previously confused with *P. lowei*. Herein, we introduce the new species with a discussion about its phylogenetic relationships. A total of 56 specimens were examined, including the museum specimens. Muscle samples were obtained from fresh specimens and fixed in 95% ethanol. The mitochondrial genes 12S, 16S, and COI were sequenced and the sequences of eight additional species were obtained from GenBank. *Polymixia* sp. n. can be diagnosed by soft dorsal-fin rays 22–28, gill rakers 19–24, posteroventral edge of preopercle rounded, and ctenii arranged in vertical rows. *Polymixia* sp. n. has a unique plasticity in the number of pyloric caeca, which may be absent or present, numbering 46–71. The phylogenetic trees recovered *Polymixia* sp. n. sister to *P. lowei*, with bootstrap support of 83% and posterior probabilities of 0.99. The delimitation models (GMYC/PTP/ABGD) corroborated the topologies of the trees. The K2P distance between *Polymixia* sp. n. and *P. lowei* is 3%, corroborating the two distinct species. The new species is distributed between the state of Bahia, Brazil, and Uruguay, in depths between 400–600 meters.

### **Using vertebrate species distributions to identify freshwater conservation priority areas for Guyana**

*Lesley de Souza, Nigel Pitman, Nicholas Kotlinski, Anna Ormiston, Jake Bicknell, Andrew Snyder, Jonathan Armbruster, Donald Taphorn, Michelle Thompson, Matt Hallett, Burton Lim, Doug Stotz*

Guyana has committed to protecting 17% of its land area by 2025 and 30% by 2030. Meeting these targets in a way that effectively protects the country's spectacular biodiversity requires spatial planning to complement existing conservation areas with currently unprotected priority areas. As is common worldwide, such analyses to date in Guyana have focused on terrestrial species. To ensure that new conservation lands also protect freshwater environments and biodiversity, we quantified the diversity and distribution of freshwater vertebrates in Guyana and the degree to which they are protected by existing protected areas. Spatial distribution models of freshwater vertebrates were



used to determine conservation priority areas while minimizing opportunity costs for forestry, mining, agriculture and urban areas. We documented 861 freshwater-dependent vertebrate species in Guyana, 82 of which are endemic to the country. Marxan analysis of 672 of these species revealed that Guyana's five existing protected areas provide effective protection for only 17.9% of the country's freshwater vertebrate biodiversity. Marxan analysis also identified 19,420 km<sup>2</sup>, or 9.2% of Guyana's land area, as new priority areas for freshwater conservation. We found significant overlap between these freshwater priority areas and those determined by terrestrial analyses, including large agreements regarding the Rupununi wetlands and the Pakaraima highlands west of Kaieteur National Park, including the endemic-rich upper Mazaruni River. Our analysis also identified previously unprioritized areas that are vital for protecting Guyana's freshwater assets, especially the lower Mazaruni, upper Cuyuni, Rewa, and Barima rivers. Together, the combined map of terrestrial and freshwater priorities represents an important tool for achieving Guyana's commitments by 2025. The unexplored area of the New River basin and other tributaries of the Corantijn River were not taken into account, and represent an opportunity for binational cooperation between Suriname and Guyana in the disputed region.

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### Characterizing ontogenetic dietary shifts in young-of-year Atlantic sharpnose, *Rhizoprionodon terraenovae*

Jessica Dehn, Dean Grubbs

Shark stock assessments examine population dynamics of adult life stages because this stage is most susceptible to fisheries. As a result, most early life-history stages are understudied, although it is crucial to investigate all facets of shark stages to understand if adults can be replaced by younger generations at the same rate that they are fished. The Atlantic sharpnose, *Rhizoprionodon terraenovae*, is one of the most abundant coastal shark species found in the Gulf of Mexico and St. George Sound, Florida. Studies have been conducted on the feeding ecology of the three main life stages (young-of-year (YOY), juvenile, adult) of Atlantic sharpnose, but little is known of YOY's ontogenetic shifts within their first five months of life. I will be investigating the diet of YOY Atlantic sharpnose in St. George Sound with gillnet sampling beginning in May 2023 and continuing into October 2023, repeating this process in 2024. Individuals that meet the criteria of a YOY (length and presence of umbilical scar) will be measured, sexed, and undergo a gastric lavage. Qualitative and quantitative analysis will be conducted on gut contents. Groups will be created within YOY stage based on size, and gut contents will be compared to acknowledge a possible correlation between group size and quality/quantity of diet

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### Exposure to silver nanoparticles negatively affects growth and survival of *Ambystoma mexicanum* (Axolotls)

Jennifer Deitloff, Kristen Cherry, Kyle Root

Nanoparticles (NPs) are gradually becoming more common in commercial products and manufacturing. Nanoparticles enter the natural environment through various sources. The effect of nanoparticles on biological systems is largely unknown, and scientific studies that have examined these effects have had contradictory results. Relatively few studies have examined developmental effects in animals. With this study, we examined whether growth and mortality rates of axolotls (*Ambystoma mexicanum*) were affected by prolonged exposure to silver nanoparticles (AgNPs). We predicted that the growth of axolotls would be hindered, and that the axolotl mortality would increase when exposed to higher concentrations of AgNP. In our experiments, groups of axolotls were consistently exposed to a variety of AgNP concentrations (low, medium or high) dispersed in their tanks. We measured axolotl developmental progress weekly (length and weight) weekly and recorded mortality as it occurred. In general, all treatment groups had high mortality rates. For the various AgNP treatment groups, statistical results were ambiguous among response variables and between the experimental groups. However, results presented graphically demonstrate a striking effect on all 3 response variables (length, weight, and mortality) in the medium and high treatment groups. We suggest that the high mortality in our experiments negatively impacted our ability to detect differences among treatment groups, and future studies should focus on creating ideal environments for survival before

testing the effects of AgNPs. Inferences about the effects of environmental toxins on the health of individuals relies critically on the survival of control groups.

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### Effects of paper mill effluent on the reproductive axis of the Bullhead minnow, *Pimephales vigilax*

Allison DeLoache, Chris Murray, Kyle Piller

The United States has the highest concentration of paper mills in the world, with roughly 450 mills currently in operation. Paper mills are a known source of environmental pollutants in proximal ecosystems. These mills produce millions of gallons of liquid waste (effluent) that is treated and released into waterways. The xenobiotic chemicals and natural compounds present in these effluents vary greatly based on the paper product produced and the treatment protocol employed by the mill. Some of the observed effects seen in exposed fish are delayed or altered secondary sex characteristics, partial or complete sex reversal, slowed gonad development, downregulation of genes that control metabolism, and upregulation of genes responsible for reproductive processes. These effects can cause larger impacts within a population by skewing sex ratios or potentially decreasing population size. In this study, we examined the effects of effluent from two kraft mills on a wild population of the Bullhead Minnow (Leuciscidae: *Pimephales vigilax*), from the Pearl River, in Louisiana and Mississippi, as well as the Bogue Chitto River, a tributary of the Pearl River, which served as a control. Both traditional histological and modern gonadal transcriptomic approaches (QuantSeq) were used to assess the impacts on reproductive parameters. Preliminary gonadosomatic index (GSI) analysis suggests that minnows from the Pearl River are allocating energy away from reproductive development. A detailed presentation of the results from the histological and transcriptomic analyses will be discussed.

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### Migratory Patterns of Sand Tiger Sharks (*Carcharias taurus*) Along the US East Coast

Bethany DeLoof, Dewayne Fox, Danielle Haulsee, Jeff Kneebone, Greg Skomal, Matt Oliver, George Madave, Joseph Barney, Brad Wetherbee

Sand tigers (*Carcharias taurus*) are often captured by recreational anglers and are among the most commonly displayed species of sharks in aquaria. Sand tigers also have one of the lowest rates of reproduction among elasmobranchs, producing two offspring every two or three years. This combination of traits has generated a great deal of concern about the sustainability of sand tiger populations along the US East Coast and interest in characterizing movements and essential habitat for this species. However, knowledge of their movements in the wild is fragmentary. We used acoustic telemetry to investigate the seasonal migratory patterns of sand tigers along the US East Coast. A total of 384 sand tigers were tagged with acoustic transmitters from 2007-2016, primarily in Duxbury Bay, Massachusetts and Delaware Bay. These sharks were detected nearly three million times from 2008-2020 on acoustic receivers between Florida and Massachusetts. Different demographic groups of sand tigers exhibited distinct and consistent spatial and temporal migrations along the US East Coast, moving seasonally between winter and summer habitats. Sharks primarily wintered in North Carolina waters. In summer, one- and two-year-olds migrated north to small bays and estuaries, as far north as Massachusetts, while older individuals occupied Delaware Bay; a small proportion of mature females remained in North Carolina waters in summer. Our results provide a more complete picture of sand tiger movements along the US East Coast, including distinct patterns of movements among demographics. This representation provides insight for protecting habitat most frequently utilized by this highly migratory species.

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### Examining the motivation for repetitive barrier interactions in bearded dragons (*Pogona vitticeps*).

Melanie Denomme

In captivity, the presence of repetitive behaviours may indicate chronic stress or frustration when housed in inadequate environments; however, the exact motivation of these behaviours is often unclear. In captive reptiles, repetitively interacting with the barriers of an enclosure (hereafter, IWB) has been

documented in a variety of species. As IWB tends to become more common in barren environments, and because it is directed at the barriers of enclosure, many assume this behaviour indicates a motivation to escape. However, this assumption has never been directly examined. If IWB in captive reptiles is driven by a motivation to escape, then it should be directed preferentially towards barriers that allow escape. To examine this, we observed captive bearded dragons (*Pogona vitticeps*) in their home cages over one and a half years. Lizards were housed in naturalistic and standard enclosures, which differed in the enrichments they provided: Naturalistic enclosures contained two hides, a loose substrate, and a propped-up piece of cork bark, whereas standard enclosures contained a single paper hide and paper substrate. Lizards were randomly assigned to one of the two enclosure styles and swapped into the opposite style after 8 months. They were observed twice, after approximately 2 and then 25 weeks in each enclosure style. Lizards were moved in and out only from the front of their enclosure, which was also the only transparent barrier; therefore, only this side should be associated with escape. These results will be used to draw conclusions about the potential underlying motivation of IWB.

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### **A New Species of *Sphaerodactylus* from the Northwest Limestone Region of Puerto Rico**

*Alondra Diaz-Lameiro*

Advances in both morphological and molecular techniques have uncovered many lineages across the tree of life, and Neotropical vertebrates are no exception. *Sphaerodactylus* geckos (*Sphaerodactylidae*) are abundant and important components of the Neotropical herpetofauna, but few studies have thoroughly investigated them using a combination of morphology and modern molecular genetic methods. Here, we combine morphological and genetic data to describe a new species of *Sphaerodactylus* from the northwestern karst region of Puerto Rico. The new species is compared to other closely related and sympatric species of *Sphaerodactylus*. Morphological analysis shows that the combination of small body size (median SVL=21.5 mm), lepidosis, skull morphology, and coloration of the head differentiates the new species from its closest relatives, including the related species, *Sphaerodactylus klauberi*. Comparing sequences of the mitochondrial 16S rRNA gene showed a genetic distance between *S. klauberi* and the new species of 5.1–5.6%, which is similar to genetic distances among other recognized gecko species. This is the first new species of *Sphaerodactylus* to be described from Puerto Rico in nearly a century, highlighting the continued need to evaluate and chronicle biological diversity even in well-studied regions.

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### **Using Environmental DNA (eDNA) to assess functional diversity indices in three Louisiana Rivers**

*Thomas Dodson, Kyle Piller*

The southeastern United States is incredibly rich in freshwater fish diversity. However, habitat degradation and anthropogenic modifications have impacted fish assemblages over the past century. Assessing the current status of many species in these systems requires the use of a robust sampling approach which is challenging in large rivers. Environmental DNA (eDNA) metabarcoding is an increasingly popular and efficient means of obtaining presence/absence data for habitats that are otherwise difficult to sample. Recovery of eDNA data is typically analyzed using standard alpha diversity metrics, however, the presence/absence data is also amenable to other types of analyses including functional diversity. Functional diversity typically refers to aspects of biodiversity that influence how an ecosystem functions and indices of functional diversity have been widely used in studies of biodiversity and ecology. In particular, they have become increasingly popular in evaluating changes in freshwater fish assemblages. The goal of this study was to combine eDNA surveys and functional diversity analyses as there is a paucity of functional index/eDNA studies in the literature. We focused on three large river systems in Louisiana. We compared representation efficiency of functional indices via eDNA to known community structures from historical surveys across the Red, Sabine, and Ouachita Rivers. Water samples were collected and analyzed via eDNA metabarcoding and multiple diversity indices were

calculated. The coverage of eDNA functional diversity indices compared to historic surveys of the rivers show the capability of using eDNA metabarcoding as an efficient means of calculating and comparing functional diversity indices.

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### **The Effects of Alkaloid Sequestration on Poison Frog Metabolism**

*Caitlin Doughton, Ralph Saporito, Vincent Farallo*

Members of the frog family *Dendrobatidae* consume invertebrates that contain alkaloids which allow the frogs to sequester and secrete alkaloids as a chemical defense mechanism. In nature, many alkaloids exist, and some *Dendrobatidae* species modify alkaloid structures prior to secretion. Thus, different poisonous frog species likely sequester different types and quantities of alkaloids. Although various physiological mechanisms by which *Dendrobatidae* secrete alkaloids have been studied, to our knowledge no studies have quantified how alkaloid sequestration affects metabolism. Any change to metabolism will impact other aspects of their ecology, such as how they can respond to environmental changes. The black-legged poison dart frog (*Phylllobates bicolor*), is a member of the family *Dendrobatidae* that secretes alkaloid toxins through skin glands. Our experiment will analyze the effects of alkaloid secretion on *P. bicolor* metabolism. This study will serve as a framework that can be repeated with other members of *Dendrobatidae*. We hypothesized that the metabolic rates and energy consumption of *P. bicolor* will increase with alkaloid sequestration. We also hypothesized that *P. bicolor* metabolic rates will proportionally increase with the quantity of alkaloids sequestered. To test our hypotheses, experimental *P. bicolor* were fed 1% decahydroquinoline (DHQ) alkaloid-vitamin dusted fruit flies, and a control group was fed vitamin-dusted fruit flies (n=24). The metabolic rates (O<sub>2</sub> consumption and CO<sub>2</sub> expulsion) of the experimental and control groups were measured with a respirometer once every 30-day for five months. Trends in the control and experimental groups' metabolic rates will be analyzed and statistically compared.

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### **Frogs with a Southern Drawl: Maintenance of Species Boundaries between the Northern and Southern Cricket Frog**

*Julia Drennan, James Tumulty*

What is a species? How do they form? And how do they remain distinct from one another? These questions are central to the concept of speciation: the evolution and subsequent maintenance of new species through the divergence of populations. Research on speciation usually concentrates on how female mate choice prevents interspecies matings. Male-male competition also plays an important role in reproduction but is relatively understudied as a mechanism of speciation. I plan to study how sexual selection via male-male competition contributes to speciation in two local "sibling" species of frogs: northern and southern cricket frogs. I propose a field playback experiment to discern whether male northern cricket frogs (*Acris crepitans*) can discriminate between male calls of their own species versus those of the southern cricket frog (*Acris gryllus*) and modulate their aggressive responses accordingly. This research will provide insight into the interplay between speciation and competition in two closely-related species.

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### **Relating Habitat Parameters to Population Abundances in the Eastern Massasauga**

*Michael Dreslik, Sarah Baker, John Crawford, Andrew Stites, Ethan Kessler*

Before conservation measures can be taken, three questions about a species must be addressed: 1) where are they distributed; 2) what habitats do they use; and 3) how many do we have on the landscape? Habitat suitability index (HSI) models are primarily used to predict habitat quality on the landscape for a given species, but they also provide a method to answer the second question with the supposition that the higher the suitability, the greater the abundance. The link between suitability and abundance estimations is often not made, even though both are critical for measuring conservation success. Here we examined several habitat parameters to determine their efficacy at predicting population abundances of the Eastern Massasauga at their

southern range limit in Illinois. Specifically, we tested whether parameters in an existing HSI model provided range-wide applicability or if proximate point-based or remote-sensed lidar-based measures of vegetation cover were better predictors.

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## The Continued Decline of the Eastern Massasauga in Illinois, from Distribution to Population Scales

*Michael Dreslik, Tyler Stewart, Tom Beauvais, John Crawford*

Marked changes have occurred to the earth's biomes through the Anthropocene due to human activity altering landscape-level processes. Many species reliant on ecosystems that have been heavily impacted have suffered massive range contractions and population declines. Given most prairies have been converted for agricultural purposes, species occupying those systems have exhibited precipitous declines. Here we examined the decline of Eastern Massasauga in Illinois using approaches at different scales, including g historical and contemporary distributions using Species Distribution Models and element-of-occurrence records to specific population trends using demographic monitoring. Unfortunately, the prognosis for the continued presence of the species on Illinois' landscape is not promising.

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## Case Studies for Involving Stakeholders in the Management of Shark Depredation

*Marcus Drymon, Amanda Jargowsky, Matthew Ajemian, Alena Anderson, Angela Collins, Danielle McAree, Ana Osowski, Evan Prasky, Steven Scyphers, Michael Sipos, Matthew Streich*

Shark depredation, defined as the partial or complete removal of captured species by non-target species, is a growing human-wildlife conflict in recreational fisheries worldwide. Depredation negatively impacts fishermen through lost catch and gear while reducing the quality of the fishing experience. Depredation can also negatively impact target fish species through increased mortality, and non-target species via increased bycatch and changes in behavior. Characterizing the nature and extent of shark depredation, as well as identifying solutions to mitigate and manage depredation, are therefore urgent priorities among fisheries researchers and managers. Here, we explore two case studies wherein citizen scientists have contributed local ecological knowledge and partnered in a data collection effort to advance depredation management in the Gulf of Mexico. The first case study involves a collaborative workshop designed to identify depredation knowledge gaps and encourage co-production of knowledge and reciprocal learning among researchers, resource managers, and stakeholders. These objectives were accomplished using fuzzy-logic cognitive maps known as mental models, or visual representations of fishermen's beliefs about the social and ecological impacts of depredation. The second case study involves a research project where charter captain collaborators are engaged in camera-based data collection to ultimately address depredation-related barriers to the adoption of best handling and release practices for reef fishes. This project provides an opportunity to educate captains directly, who can in turn share information with their clients. Collectively, these case studies offer unique insight into effective stakeholder education and engagement, critical components for understanding and managing complex social-ecological issues like depredation.

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## Modular partitioning of the Weberian apparatus in siluriform fishes

*Kevin Duclos, Terry Grande*

Amongst development mechanisms, modularity, wherein modules constitute sets of highly correlated traits which vary semi-autonomously from other traits, is a crucial component facilitating the evolvability of complex systems. One such complex system, the Weberian apparatus, is a mechanical linkage (modified anterior centra, neural arches, supraneurals, and pleural ribs) transmitting motion of the gasbladder wall to the inner ear. This linkage, diagnostic of Otophysi, enables audition of frequencies up to several thousand hertz and increases sensitivity throughout the frequency range. Siluriformes display significant variation in the presence and the shape of Weberian ossicles,

suggesting the Weberian apparatus is highly modular within that order. Here, we employ micro-CT technology and 3-D geometric morphometrics to address two objectives. First, we evaluate modularity of the anterior vertebrae with respect to other skeletal systems across Siluriformes. Second, we assess Weberian apparatus modularity and variation in *Ictalurus punctatus* (Channel Catfish). The breadth of developmental research and materials associated with *I. punctatus* and the presence of all Weberian ossicles make it a good model species for Weberian apparatus modularity within Siluriformes. Our results support the anterior vertebrae as a module across Siluriformes, but also suggest some covariation with the dorsal fin complex. The Weberian apparatus of *I. punctatus* shows strong allometric and modular signals, with a seven-module partition being supported. Most notably, the tripus, intercalarium, scaphium, and rib 4, which play a concomitant functional role, constitute an integrated module. Developmental and functional factors appear to drive Weberian apparatus modularity within Siluriformes, warranting further investigation of evolutionary trajectories.

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## Chelonian community surveys following a mortality event of Florida softshell turtles (*Apalone ferox*) in an isolated wetland

*Faith Dunlap, Cory McKinstry, Arik Hartmann, Kuttichantran Subramaniam, Ana Longo*

Turtles are amongst the most threatened vertebrate groups, primarily attributed to overexploitation and habitat loss. Emerging pathogens also threaten turtle biodiversity. Outbreaks of ranavirus have impacted freshwater turtle populations across North America, and a newly described pathogen, turtle fraservirus 1 (TFV1), was isolated from mass mortality events of wild freshwater turtles in Florida. Following a mortality event that killed at least 22 Florida softshell turtles (*Apalone ferox*) in a small, isolated wetland in 2022, we conducted mark-capture-recapture surveys of the impacted turtle community to identify possible causes, describe impacts, and establish associations with pathogen infections. We collected oral and cloacal swabs to test for ranavirus and TFV1[*H1*], and assessed turtles for gross signs of disease, such as lesions or mucosal discharge. So far, we have only screened for ranavirus, which we did not detect in any of the samples, suggesting TFV1 may be the causative agent of these die-offs. The majority (>50%) of turtles captured as part of our surveys were Florida mud turtles (*Kinosternon steindachneri*). We captured very few softshell turtles (*Apalone ferox*), which may indicate that the population at this site is in decline. As chelonian pathogens become more prevalent, it is important to identify drivers of infection and geographic ranges to inform conservation strategies.

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## Conservation Genomics of the Proserpine Shiner, *Cyprinella proserpina*.

*Kayla Dye, Kevin Conway, Megan Bean, Amanda Pinion, Sarah Robertson, Christopher Hollenbeck, David Portnoy*

The Proserpine shiner *Cyprinella proserpina* is found in spring-fed tributaries of the lower Rio Grande within Texas and is considered threatened by the Texas Parks and Wildlife Department. *C. proserpina* has undergone recent range contraction and fragmentation in response to numerous anthropogenic stressors, including water extraction, reservoir formation, and the introduction of non-native species. However, conservation programs are limited due to a lack of information about the species, including an understanding of genetic population structure. Therefore, patterns of genetic variation within and among geographic samples of *C. proserpina* were assessed using double digest restriction-site associated DNA sequencing (ddRAD) to provide a better understanding of connectivity and short- and long-term population viability. In addition, landscape genomic approaches were conducted to disentangle the influence of geographic position, environment, and their interactions on patterns of genomic variation. These data will allow resource managers to implement tailored conservation measures for the identified genetic units and provide a means to evolutionarily enlightened management.

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## Effect of Leaf Litter Diversity on Treefrog Oviposition Site Selection

*Julia E. Earl, Daniel J. Edwards, Joseph Aubert*

Female treefrogs use pond characteristics to decide where to oviposit to provide their offspring with the best opportunity to survive. Previous work shows that oviposition preference is affected by the species of leaf litter in ponds. Most ponds have a mixture of different species of leaves, so we were interested in whether leaf diversity affected treefrog oviposition. To examine this, we established 45 aquatic mesocosms as potential oviposition sites for Cope's Gray Treefrogs (*Hyla chrysoscelis*). Mixtures of leaves were randomly established containing 0, 3, 5, 7 or 9 species of leaves. We had 5 pools with 0 leaves, and all other treatments had 10 replicates. Functional diversity was estimated for each pool using leaf tannins, C:N, C:P, and N:P. Pools were monitored for oviposition during May and June. We further measured water tannins, dissolved oxygen, pH, conductivity, turbidity, and water color. 49,778 eggs were deposited on 8 nights, representing approximately 33 clutches. We found no effect of leaf species richness or functional diversity on the number of eggs deposited. However, more eggs were deposited in pools with higher water tannin concentrations despite the potentially toxic effect of high tannin concentrations, consistent with previous results. Water tannins were highly correlated with water color, so we cannot separate these variables. There was also a negative relationship between the number of eggs deposited and the N:P ratio of the leaves. Treefrogs appear not to be influenced by leaf diversity in ponds, but they are affected by leaf traits.

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## Discovery of Potential Novel Biomarkers of Mercury Bioaccumulation using Supervised Machine Learning in a Keystone Predator

*Charles Edelson, Lisa Crawford*

Advances in next-generation sequencing technology have made obtaining feature-rich gene expression profiles accessible to answer an abundance of biological questions. However, their high-dimensional nature often makes it difficult to identify which genes are associated with a particular physiological process. We used machine learning techniques to explore functional relationships between gene expression and muscle total mercury (THg) concentrations in 29 Northwest Atlantic white sharks (*Carcharodon carcharias*). First, we found that tree ensemble regression models were able to predict THg from gene expression better than linear regression models trained on shark demographic data exclusively, with the best performing ensemble method explaining twice the variance of the linear model. Next, we leveraged supervised learning's natural ability to control for confounding variables and showed how a tree ensemble classification model could classify sharks into high- and low-THg classes while controlling for sex and body length. Finally, we performed feature importance estimation for our ensemble models and identified a group of genes which are potential novel biomarkers of THg bioaccumulation and warrant future investigation. These methods are applicable to many other biological systems and research disciplines, and may be particularly useful when studying rare and endangered species for which samples are limited.

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## Phylogenomic uncertainty and its implications for understanding the evolutionary history of Middle American Cichlids

*Diego J. Elías, Fernando Alda, Prosanta Chakrabarty, Caleb D. McMahan*

The tribe Heroini (Cichlidae) is the only lineage of Neotropical cichlids that has colonized and diversified within Middle America. With ~158 species, heroine cichlids are one of the dominant components of the freshwater fish diversity in the region. Heroine diversity is distributed across five main clades (Astatheroines, Amphilophines, Caquetaines, Herichthyines, and Mesonautines), and some genera are not consistently nested within any clade (e.g., *Australoheros*, *Nandopsis*). In recent years genomic-scale data have improved our understanding of the phylogenetic relationships of this group. But equally likely evolutionary hypotheses have been proposed based on analytical approaches (i.e., concatenation or coalescent) or markers utilized (e.g., exons, ultraconserved elements - UCEs). Despite this, the implications

of the use of alternative topologies in the inference of evolutionary history for heroine cichlids have not been fully explored. In this study we inferred evolutionary relationships of the Heroini using UCEs and a complete taxonomic sampling of Heroini at the generic level. Phylogenomic inference was performed using concatenation and coalescent approaches. We calculated gene and site concordance factors and normalized quartet scores to estimate the frequency of gene trees that support alternative topological hypotheses among major clades of Heroini. Finally, we investigated biogeographic patterns of the colonization of Middle America by heroine cichlids using the recovered alternative topologies. Our results support the monophyly of the five clades of Heroini but we observed high uncertainty in the placement of herichthyines. We recovered contrasting biogeographic patterns using alternative topologies highlighting the importance of exploring gene tree heterogeneity when performing phylogenomic inference.

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## Investigating the Relationship Between the Microbial Environment and Predation Stress in a Larval Amphibian.

*Kyle Emerson, Sarah Woodley*

The gut microbiota influences many aspects of vertebrate physiological development, including neurodevelopment and behavior, through the microbiota-gut-brain (MGB) axis. In amphibians, the composition and structure of the gut microbiota is shaped by environmental factors, including the presence of colonizing environmental microorganisms. One major question in microbiome research is the relationship between these microbial communities and environmental stressors. As most microbiome research uses mammalian models for biomedical applications, host-microbe relationships in non-mammalian wildlife are comparatively understudied. Here, I manipulated the composition of the gut microbiota in newly hatched Northern Leopard Frog tadpoles (*Lithobates pipiens*) by raising them in either natural pond water or sterilized pond water; previous studies show that raising tadpoles in sterilized pond water reduces the diversity of their gut microbiota by reducing the availability of colonizing microorganisms in their housing water. Additionally, I manipulated tadpole stress levels in both water treatments by exposing them to predator cues, exogenous corticosterone (CORT; stress hormone), or a vehicle control. Initial results found that regardless of pond water treatment, tadpoles exposed to exogenous CORT developed wider tail muscles. Further, tadpoles raised in sterilized pond water were larger, developed faster, and exhibited increased locomotory and exploratory activity in response to predator cues during behavior analysis compared to tadpoles raised in natural pond water. These results demonstrate a potential association between the microbial environment that amphibians develop in and their responses to environmental stressors, which can have implications on tadpole fitness and survival.

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## Tracking the decline and recovery of salamanders in Appalachian forests for 30 years following timber harvest

*Tori Engler, Carola Haas, Nicholas Caruso, George Brooks*

Silvicultural practices can drastically alter the abiotic conditions of forest floor environments. Elevated soil temperatures, reduced humidity, and diminishing leaf litter are all expected following canopy removal. As a result, intensive silvicultural practices are assumed to be detrimental to salamanders that rely on cool, moist environments to survive and deep leaf litter to forage. Although many studies have shown declines in salamander abundance immediately following timber harvest, the ability for populations to recover or recolonize harvested areas once they have returned to a forested state is largely unknown. Moreover, recovery may depend on the silvicultural practice employed (e.g., clearcut vs group selection). Here we analyze 15-30 years of *Plethodon cinereus* relative abundance data across seven silvicultural treatments on six research sites in southwest Virginia and West Virginia. We measured relative abundance by conducting night-time area constrained searches only during or after rain events when the ground and leaf-litter were saturated and temperatures were within normal activity levels for salamanders. We found that regardless of silvicultural treatment, salamander relative abundance decreased in the first 1-3 years following any canopy removal. (Populations in the midstory herbicide treatment and the untreated control varied among years but did not decline in the years post-treatment.) However, in all cases populations began to show signs of recovery 15-20



years post-harvest. After 30 years, relative abundance in all treatments had reached or exceeded pretreatment levels. Our findings illustrate the resilience of plethodontid salamander populations at least to small (2 ha) harvests in Appalachian hardwood forests.

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### Population Analysis of Geckos of the Genus *Bavayia* (Diplodactylidae) Using Rapidly Evolving Long Exon Capture (RELEC) Datasets

*Mustafa Erkaya, Aaron M. Bauer, Todd R. Jackman*

New Caledonian diplodactylid geckos of the genus *Bavayia* comprise 41 described species, most of which appear to have arisen via allopatric speciation following habitat fragmentation. Most of these species are microendemics, but two species, *B. septuiclavis* and *B. geitaina*, have broad distributions across the ultramafic substrates in the south of New Caledonia. Sampling in these species is adequate to investigate patterns of population structure. In addition to existing mitochondrial ND2 data and data from several widely used nuclear markers, we used RELEC (Rapidly Evolving Long Exon Capture) loci, particularly PKDREJA and PKDREJC, to conduct expanded phylogeographic studies and evaluate population structure within these two taxa. Both species exhibit substructuring, but *B. geitaina* shows more well-supported geographical patterning than does its congener. This may relate to the greater reliance of *B. geitaina* on forest habitats than the more thoroughly terrestrial *B. septuiclavis*. This work represents the first use of the rapidly evolving PKDREJA and PKDREJC genes in a phylogeographic study of squamates.

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### Comparative anatomy review of the gill arch muscles in the Anguilliformes

*Vinicius Espindola, G. David Johnson*

Eels are among the most diverse and distinct groups of teleosts, with over 1,000 species distributed in 20 families. The variability of the anguilliforms can be observed in many habitats encompassing freshwater, brackish water, reef, benthic, pelagic, and abyssal environments. The evolutionary adaptation to different habitats led to the eels' having an equal representation in feeding behavior, comprising durophagous, piscivorous, molluscivorous, invertivorous, and scavenger activities. All eel taxa have a specific gill arch muscle arrangement for exceptional miscellaneous feeding activities. For this study, we dissected 110 specimens in 19 families of Anguilliformes, including the recently described Protanguillidae. We reviewed 21 muscles and the sections and subsections of the gill arches of the elopomorphs, and we evaluated the homology and nomenclature of these structures. In this analysis, we suggest synonymizing the terminology of the rectus ventralis 4 communis (present in all anguilliforms) and subpharyngealis (in moringuids, muraenids, and synphobranchids) into different arrangements of the rectus communis. We are providing a new synapomorphy based on the configuration of the rectus communis for all anguilliforms except Protanguilla palau. Additionally, the transversus ventralis 4 is present in all eels, except in nemichthyids and sacco-pharyngoids, concluding that these clades are sister groups.

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### Comparative Evolutionary Genomics of a Novel Strain of *Anolis Adenovirus*

*Cleo Falvey, Anthony Geneva*

Whole-genome sequencing technology can be harnessed to glean insights into the co-evolution of viruses and their hosts. Adenoviruses are a clade of DNA viruses that primarily infect squamates. Species-specific strains of Adenovirus have been identified across a diverse array of lizards and snakes including bearded dragons, worm lizards, and *Anolis* lizards, but the virus has also clearly undergone some host switching. Most analyses of Adenovirus rely on a 300 base-pair segment of the DNA polymerase gene. Only two strains of reptile-infecting Adenoviruses have full genome sequences available, one from a corn snake and one from a bearded dragon. Here, I present the whole-genome assembly of a strain of Adenovirus, *Anolis Adenovirus 2*, which were extracted from a wild-caught *Anolis distichus ravitrum* individual. We performed metagenomic sequencing of gastrointestinal extracts and bioinformatically extracted Adenovirus scaffolds from de novo assembly

of metagenomic reads. Previous phylogenetic analyses suggested that *Anolis Adenovirus 2* was closely related to other anole infecting strains. However, due to the potential for viral recombination, individual genes may not accurately represent the true phylogeny. To address this, we present multilocus phylogenetic analyses of these strains along with previously published Adenovirus genomes to infer the evolutionary history of this group and assess the degree of co-evolution vs host switching among Adenoviruses.

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### Using Biomechanical Modeling and Fish Survey Data to Predict Microhabitat Usage in Flatfish Species

*Stacy Farina*

The ability of organisms to interact with their physical environment changes throughout development of an individual and can vary on a microhabitat scale. Biomechanical models have the potential to generate hypotheses about microhabitat usage of organisms at critical life history stages. In this lightning talk, I will briefly discuss how our lab is using biomechanical modeling of flatfish burial performance to make predictions about flatfish habitat usage across a range of fish size and species. I will then discuss our approach of using fish survey data to test these predictions.

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### Ritualized Male-Male Combat Resulting From Intraspecific Food Competition in Three Species of *Agkistrodon*

*Terence Farrell, Henderson Gull, Frederick Boyce*

Male-male combat has been observed in many snake species and is often thought to be a result of competition for mates. We argue a more thorough assessment of the contexts in which combat occurs is needed for snakes. We made field video observations of *Agkistrodon conanti* in central Florida, *A. piscivorus* in eastern North Carolina, and *A. contortrix* in eastern Tennessee. These observations were made in frequently-used foraging sites; near drying ephemeral ponds in Florida and North Carolina; and in a forested area with abundant emerging cicadas in Tennessee. In all three populations, males engaged in combat. In six instances, a variety of lines of evidence including season, local food resource abundance, local female abundance, and post-combat behaviors all indicated that access to food resources, rather than mating opportunities, was the catalyst for agonistic behavior. We will present videos of these interactions that demonstrate food resource-induced combat appears behaviorally identical to combat for access to mates. Simplistic assumptions about the environmental contexts that induce combat may lead researchers to make inaccurate conclusions concerning the duration of the breeding season and the frequency of intraspecific competition for food in snake species. We outline a rigorous set of criteria that should be met to determine the context for bouts of male-male combat.

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### Movement Patterns of Fishes Associated with Qatar Oil Platforms in the Arabian Gulf

*Lauren Faulkner, Christopher Lowe, Radhouane Hamadou, Felipe Torquato, Johan Sorensen, Mathieu Carrara, Alexei Godina*

Some man-made structures such as offshore petroleum platforms may function as ecologically successful artificial reefs, programs like Rigs-to-Reef aim to keep the platform structures in place after production ceases. The offshore platforms at Al-Khaleej Oil Fields in the Arabian Gulf are reaching the end of production life, yet knowledge about their ecological importance is unknown. This field consists of six platforms in the deepest depths of the gulf (~65 m) ranging between 5-10 km apart, with few natural reefs nearby. Gulf's conditions are extreme with high temperatures ranging from 22°C to 35°C and a salinity range of 36 to 40.31 psu. Passive acoustic telemetry was used to quantify the residency and movement patterns of pelagic and benthic fishes associated with three offshore platforms in the field. Twenty-five benthic fishes, thirty-one pelagic fishes ranging from 29 to 79 cm SL have been tagged and monitored. Some individuals showed differences in depth use at the platforms, with pelagic fishes using shallower areas of the platforms while benthic fishes using deeper parts of platforms ( $D = 0.99$ ,  $p < 0.001$ ). Overall, tagged fishes showed moderate mean and variable ( $\pm$ SD) residency index (RI) of  $53 \pm 40\%$  (days detected/ days monitored;) at platforms, with benthic species showing

similar RI (54±56%) as the pelagic species (54±34%). Over 14% of tagged fish moved between monitored platforms during a 3-month period. Understanding fish residency and movements will help reveal the ecological importance of the offshore platforms in the extreme environment of the Arabian Gulf.

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### Assessing the status of urban populations of the Atlantic coast leopard frog, *Rana (Lithobates) kauffeldi*, following hurricane Sandy.

*Jeremy Feinberg, Erik Kiviat, Matthew Schlesinger, Joanna Burger*

Extreme weather and climate events can have negative consequences for species. In some cases, it may be helpful to examine species-specific responses and consider potential conservation implications, especially where taxa may be further constrained by limited vagility and isolation within highly fragmented landscapes. The Atlantic coast leopard frog, *Rana (Lithobates) kauffeldi*, was discovered in 2012 and later described from New York City (NYC) in 2014. In October 2012, only months after the species' discovery, hurricane Sandy struck the mid-Atlantic US, presenting uncertain risk to the majority of populations known at that time. We assessed the status of five remnant populations in the NYC area and documented the distribution, size, and intensity of breeding choruses for pre-storm comparisons. We confirmed survival of adult males within each population, found apparent demographic changes at most chorus sites and at least one distributional shift, and heard calls at four previously undocumented locations. We also compared water chemistry to pre-storm conditions at local wetlands with and without leopard frogs and found significant increases in salinity when considering all sites (207%) and only those with frogs (186%). Salinity was correlated non-significantly with estuary proximity. Turbidity varied most among sites and dissolved oxygen varied least. We also found significant correlations between changes in chorus size and post-storm salinity (positive) and turbidity (negative). Overall, *R. kauffeldi* appears capable of surviving large-scale storms, flooding, and salinity increases, but not necessarily without tolerance limits or costs to other life stages or total populations.

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### Observational Study on Gravid Timber Rattlesnakes

*Divia Feinstein, Mary O'Donnell*

The Timber Rattlesnake (*Crotalus horridus*) is a North American venomous pit viper. The Pennsylvania Wildlife Action Plan lists Timber Rattlesnakes as a species of greatest conservation need. They are currently threatened by habitat loss, organized hunting, and fear motivated killing. In July 2022, camera traps were set up at potential basking sites at a community park in Lycoming County, Pennsylvania using historical data, habitat evaluation, and current snake sightings. Using time-lapse photography, we documented snake presence or absence at the sites, sex, reproductive condition, and behavior. Two females were observed through the end stages of their pregnancies and parturition. Both gave birth to litters of average size. Duration of maternal care and occupancy of the maternity sites by females and their young varied, and deviated from published records. Data collected from the camera traps is being analyzed as part of a pilot study using camera trapping to collect information on daily activity patterns, basking times and durations for gravid females, as well as maternal care behavior. We found that the gravid females typically emerged between eight and nine AM during the summer months, and basked for one to two hours in the late morning and early afternoon. Juvenile basking times were longer and varied from adult activity patterns. Better information on rattlesnake habitat preferences and activity patterns may reduce human-wildlife conflict to more effectively protect recreational hikers and imperiled snakes.

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### Comparative Skull Morphology and Miniaturization in Mainland African Plated Lizards

*Kelsey Fenner, Aaron Bauer*

Plated lizards in the subfamily Gerrhosaurinae are a group of heavily armored reptiles native to sub-Saharan Africa. Within the five genera, there is a bimodal size distribution, with three genera (*Broadleysaurus*, *Matobosaurus*, and *Gerrhosaurus*) exhibiting a large-body size (up to 300mm SVL) and two

genera (*Tetradactylus* and *Cordylus*) exhibiting a small body size (up to 80mm SVL). We hypothesized that the divergence in body size between the large and small-bodied genera is driven by miniaturization of the two small-bodied genera, rather than gigantism in the three larger genera, which have body sizes more consistent with their closest relatives, the Madagascan plated lizards and the cordylids. To investigate this, we used micro-CT scans to digitally dissect the skulls of seventeen gerrhosaurid species. We performed a comprehensive review of the primary literature to identify morphological traits associated with miniaturization in other squamate lineages, and employed 3D geometric morphometrics and ancestral state reconstruction to investigate the relationship between skull size, shape, and phylogeny. Both qualitative and statistical approaches support the hypothesis that the two small genera have undergone miniaturization. *Cordylus* and *Tetradactylus* exhibited many of the cranial morphological traits associated with miniaturization in other squamate groups including an increase in the amount of space associated with sensory organs in relation to the rest of the skull, and greater fusion in the lower jaw bones. The PCA plot showed clear separation between the large and small-bodied species in the morphospace, and the ancestral state reconstruction indicated that these genera likely evolved from large-bodied ancestors.

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### Herpetological Research and Teacher Education: An Example Using Mediterranean Geckos (*Hemidactylus turcicus*) in Central Oklahoma

*Allison Fenwick, Beth Allan, Liz Lane-Harvard, Mike Nelson*

Many reptile and amphibian systems adapt well to outreach via citizen science, especially via the activities of natural history collections. In contrast, we are aware of few examples of herpetological research in collaboration with K-12 teachers. A group of biologists, mathematicians and education researchers at the University of Central Oklahoma recently won a Robert Noyce Teacher Scholarship grant from the National Science Foundation to train teacher leaders in STEM practices. The recruited Master Teaching Fellows conduct short independent research projects in STEM in their first year of a M.S. program, conduct education research in their classrooms in the second year, and participate in ongoing leadership development and community building in years 3–6. Research in the Fenwick lab leverages charismatic, easy-to-study lizards in working with Master Teaching Fellows to answer questions related to evolution and ecology, key areas of high school biology content. The purpose of this poster is to facilitate discussion on more effective ways to work in partnership with K-12 teachers to benefit both research and education.

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### Evidence for independent breeding groups in the highly vagile spinner shark found in putatively adaptive loci

*Andrew T. Fields, William B. Driggers III, Bryan S. Frazier, Elizabeth A. Vinyard, James Gelsleichter, Jill M. Hendon, Jeremy M. Higgs, David S. Portnoy*

Highly migratory species (HMS) fisheries management can be difficult due to the possibility of straddling stocks and mixing of individuals from independent stocks. Spinner sharks are a cosmopolitan, large coastal, highly migratory species for which there are few studies focused on the genetic population structure. For spinner sharks in the western North Atlantic Ocean, life history differences exist between the Gulf of Mexico (Gulf) and US Atlantic Seaboard (Atlantic) which could indicate population level differences; however, tagging data suggest movement between the regions. Therefore, genetic variation was assessed within and among immature spinner sharks collected from both the Gulf and Atlantic regions using double digest restriction-site associated DNA sequencing. The only heterogeneity found in the data was driven by a small number of loci significantly associated with long-term mean oceanic silicate concentration. Analysis of these loci clustered individuals into two distinct genetic units, with juveniles from one group dominant in the Atlantic and juveniles from the other group dominant in the Gulf. However, this pattern was absent in adult spinner sharks. These results suggest that two genetic units of spinner sharks are present in the western North Atlantic Ocean but also that those units mix due to the species' highly vagile nature. Further, this work demonstrates an approach that may be tailorable for delineating stocks and mixing rates in other HMS species.

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## Evaluating the Effects of Drought on Yellowcheek (*Nothonotus moorei*) and Yoke Darter (*Nothonotus juliae*) survival, growth, and behavior

*Kearstin Findley, Daniel Magoulick*

Drying conditions are impacting aquatic systems at greater frequency and intensity across the globe and these trends are expected to be exacerbated with climate projections and higher anthropogenic water demands. Riverine systems often lose critical habitat during droughts that can reduce or eliminate species that are unable to respond to the physiological stress water reduction can cause. For many species, there is a knowledge gap regarding how they respond to changes in abiotic and biotic interactions that occur during droughts. Our study utilized laboratory stream mesocosms to simulate drought with endangered Yellowcheek Darters (*Nothonotus moorei*) and imperiled Yoke Darters (*Nothonotus juliae*) to evaluate how survival, growth, and behavior are impacted. Passive integrated transponder (PIT) tags were utilized to identify individuals and observe their location in different habitats (riffle, run, pool) created in each mesocosm. Yellowcheek Darters had lower tag mortality (17%) than Yoke Darters (30%). Survival overall was low, however, more individuals for both species survived in the drought treatments than the control treatments. The only significant growth difference was Yoke Darters in control treatments gained more length (p-value 0.037) than drought treatment fish throughout the 60-day experiment. Habitat utilization varied between treatment and species. This study provides laboratory drought simulation information for Yellowcheek and Yoke Darters, and also evaluated the use of PIT tags for small-bodied stream fishes. The information gathered here will provide conservation managers with valuable information to help preserve these two declining species, as well as many other stream fishes living in drought-stricken environments.

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## The next generation of Red Listing

*Brittany Finucci, Cassandra L. Rigby, Rima W. Jabado*

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species is the world's most comprehensive inventory of the global conservation status of animal, fungi, and plant species. In response to the growing awareness and concern of fisheries impacts on chondrichthyans (sharks, rays, and chimaeras), the IUCN Species Survival Commission Shark Specialist Group (SSG) was established in 1991 and is now recognized as the leading authority on the status of chondrichthyan fishes. All 1200+ species of sharks, rays, and chimaeras have now undergone at least one round of the Red List assessment process. Red List assessments are living documents and are updated at least every ten years. Some species are now being globally reassessed for the third time, shedding light on the increasing extinction risk of this group over recent decades. Over the course of this process, Red List assessments have been designed to provide updated and evidence-based data on the global status of each species. Continued advancements in knowledge and expertise needs to be gathered from the SSG and wider global research community to update assessments and ensure this knowledge accurately captures the status of a species throughout its entire range. This can lead to conservation action for priority threatened species groups. Despite progress in our knowledge of chondrichthyan biology, population trends, and threats, crucial species-specific knowledge gaps still exist. Without this information, data must be inferred from alternative sources. Here, the most needed and useful information often missing for Red List assessments will be provided to guide future research efforts.

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## Efficacy of eDNA Methods for Elasmobranch Detection in a Large Estuary Using Three Novel eDNA Primers

*Kristina Flanigan, Ryan Lowndes, Erin J. Burge, Abigail Cavaris, Shae Schreckengost, Dan C. Abel*

Environmental DNA (eDNA) analysis is an emerging, non-invasive community monitoring tool. This study aimed to determine if eDNA methods can be reliably used in a large brackish, partially-mixed estuary by developing and testing three novel eDNA primers, for Sandbar Sharks (*Carcharhinus plumbeus*), Blacknose Sharks (*Carcharhinus acronotus*), and Bonnetheads (*Sphyrna tiburo*). These primers were designed to target 109, 156, and 120 base pair (bp)

fragments, respectively, of the highly conserved NAD2 gene in the mitochondrial genome of each species. Primer function was validated through testing against 102 known genomic source samples and 25 filtered water samples from aquaria in which the species were exhibited. A total of 198 water samples were collected alongside active longlines in Winyah Bay, South Carolina, and extracted for eDNA analysis. We created three species-specific eDNA primers for the target species, and validated them against 8+ target genomic samples and 14 other local elasmobranch species as negative controls. Detection was successful when applied to aquarium samples from five separate institutions. Large, naturally-occurring organic compounds in Winyah Bay consistently inhibited gene expression from the raw water samples, thereby limiting the utility of eDNA in the study system. This study provided evidence that species-specific primers of closely related Carcharhinid species can be developed and utilized, while also showcasing the challenges of eDNA detection in a highly productive marine environment. Further study in waters of lower organic content, or with advanced techniques, is needed to demonstrate the full functionality of developed assays.

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## Density Dependence in Space Use of Eastern Red-Backed Salamanders (*Plethodon cinereus*)

*Jill Fleming, David Miller, Evan Grant*

There is wide variation in the densities of red-backed salamander (*Plethodon cinereus*) populations throughout the species range. Variation in population density may have implications for life history and behaviors related to space use. Partners within the Salamander Population and Adaptation Research Collaboration Network (SPARCnet) are distributed across the range of the red-backed salamander and collect mark-recapture data using standardized methods. We performed spatial capture-recapture analyses to estimate population density and space use of red-backed salamanders and examined geographic variation in these parameters. Our analysis found some evidence of density-dependence in space-use, whereby the area of space use is negatively correlated with population density. However, this relationship is highly variable by site and season. Our findings of density-dependence in space use are consistent with well-studied life history characteristics of the species such as territoriality. Examining density-dependence in space use may further illuminate the role of such life history characteristics in determining population density. Further, with many monitoring sites distributed across the range, SPARCnet may investigate site-level environmental variables and how they may moderate the overall relationship between space use and density.

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## Does the Bayou Topminnow, *Fundulus notti*, occur in Florida?

*Adania Flemming, Larry Page*

The Bayou Topminnow, *Fundulus notti*, is recorded as occurring in several river drainages in the panhandle of Florida. However, our recent re-examinations of the specimens on which these records were based appear to be, with perhaps only one exception, the morphologically similar Russetfin Topminnow, *Fundulus escambiae*, which is common throughout the panhandle of Florida. The two species are extremely similar morphologically and are separated using color pattern (which is seasonally variable) and the arrangement of scales on the head. The distribution of this *Fundulus notti* in Florida may be much smaller than currently thought. Given the extreme similarity of the two species, *Fundulus notti* may not occur at all in Florida. If it occurs in only one stream in Florida as recent examinations suggest, it should be considered for protection. We will utilize computerized tomography (CT) scans and photogrammetry to expand the traits that can be used to separate these two species as well as molecular data to test morphological identifications given the difficulty of using morphological traits only.

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## Discovering Ichthyology and Herpetology through a course model to intentionally increase the diversity of students in STEM

*Adania Flemming*

Being immersed in the processes of research can develop undergraduate students' science identities and support their persistence in pursuing science careers. However, it can be difficult for students to find opportunities for engaging in authentic scientific research. The professional staff and scientific objects in natural history museums provide such an opportunity that can create relationships through which both undergraduate students and museums benefit. Students require authentic practical experiences to better understand their academic fields and career trajectories, and museums require assistance curating and managing collections. This can be accomplished through mentorship, training, and research experience in a formal course. At the University of Florida, I developed and taught a course titled Introduction to Natural History that engaged students in projects in museum collections. Discussion sessions replaced lectures by introducing topics such as the concept of research, distinction between predictions and hypotheses, understanding of the nature of science, and how to conduct literature reviews. In 2019 and 2020, students completed a pre- and post-course survey to gauge their understanding of science and their anticipated career trajectory. My results demonstrate that mentorship and authentic experiential science opportunities using museum collections enable students to realize a passion, sense of purpose, and better understanding of science and careers in science.

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## Avian Predator Occurrence Tracks Lizard Prey Diversity Across Island Communities

*Edita Folfas, Luke Mahler, Luke Frishkoff*

Ecological theory suggests that high predator occurrence may diminish prey community diversity and abundance signifying strong top-down ecosystem control. Alternatively, predator presence may track areas of high prey diversity and abundance if bottom-up ecosystem controls predominate. Past research across islands of various sizes has suggested an important role of predators in regulating insular lizard populations and communities, implicating strong top-down control. If top-down control predominates, we should see similar patterns within islands—areas of greater predator occurrence should have fewer species and lower abundances of lizards than expected based on environmental conditions. Using eBird data spanning 10 years, we inferred landscape-level presence across all bird species that prey on anole lizards across both Jamaica and Hispaniola. By summing occurrence probability of anole-predator birds, we estimated total avian predation pressure and combined these with anole community data from a mark-recapture study spanning geographic space and climate gradients on both islands. Both anoles and avian predators were affected by climate, with both total predation pressure, as well as anole abundance and species richness increasing with annual temperature. We found that while lizard abundance was uncorrelated with predator occurrence, predation did have a positive relationship with anole species richness. These findings suggest that while islands with more predators tend to have fewer species and lower abundances of prey, within islands, top-down forces of predation may not be strong determinants of lizard community structure. Instead, bottom-up forces linked with climate may be more important drivers of diversity in both lizards and their avian predators.

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## That changed a tad(pole): differing ecological effects of toad tadpoles with inter-annual variation.

*Jessica Ford, David Green*

A species' ecological effect is often thought to be static, with species having fixed roles within an ecosystem. However, static ecosystems are often the exception rather than the rule. The effects of a species in an ecosystem can change due to year effects such as temperature, precipitation, droughts, and frost dates. The impact of year effects is expected to increase with climate change as extreme weather events become more common. Despite this, studies of species ecological roles and interactions are rarely repeated between years, resulting in an incomplete understanding of these complexities. To gain a better understanding of species dynamics in small pond

ecosystems, we replicated an outdoor mesocosm experiment assessing the effect of toad tadpoles on their environment over three years, using Fowler's toad and American toad tadpoles in Long Point, Ontario. Even in semi-controlled mesocosm environments, the effect of toad tadpoles on their surrounding ecosystem varied significantly between years. Everything from the effect of toad tadpoles on their algal food sources to the outcome of interspecific competition differed between years. Although each year was different, the results were not random, with trends being consistent between mesocosms in the same experimental group within a given year. These results demonstrate the dynamic relationship that toad tadpoles have with their environment, and the impact that inter-annual variation can have on their ecological effect. These results also highlight the need for more field experiments to be replicated across years in order to capture the full scope of a species' ecological impact.

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## Beyond Science: Jack Musick's Exemplary Role in Shark Conservation

*Sonja Fordham*

Notwithstanding contributions to many other disciplines, Jack Musick's beneficial effect on the field of shark conservation was monumental. He stood out for his active engagement in fisheries management, tirelessly making the case for science-based fishing limits before government officials and a range of stakeholders. His remarkable expertise stretched beyond ecology and fisheries science to the practical aspects of fishing and regulatory processes. He began advising fishery managers of Virginia and the greater Mid-Atlantic region in the 1980s and, as various shark management plans developed, expanded his influence to the Atlantic States Marine Fisheries Commission and the National Marine Fisheries Service. To environmentalists just delving into fish issues in the 1990s, he gave support, legitimacy, and confidence. His highest profile Atlantic shark conservation achievement may have been sandbar and dusky shark protection, but his gravitas and straight talk were also integral in reversing the decline of spiny dogfish. Two decades ago, he helped facilitate the listing of smalltooth sawfish under the Endangered Species Act, an event that has since significantly improved the species' status. Dr. Musick also famously led and coordinated conservation policy positions for the AES and ASIH, and had global reach as Co-Chair of the IUCN Shark Specialist Group. With a unique style and an extraordinary commitment that continued long after his retirement, he blazed trails that remain vital to conservationists today. His legacy in this field is unparalleled in breadth and impact.

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## Phylogenetics and Species Discovery of *Rhinella* in Colombia

*Isabella Foust, João F. R. Tonini, Mariela Osorno Muñoz, Rafael O. de Sá*

Colombian amphibian fauna is among the richest in the world and new species are continuously described. The genus *Rhinella* is one of the most diverse groups of bufonid toads that occurs throughout Central and South America. The genus currently comprises 93 valid species of which 23 are reported in Colombia. Widespread amazonian species within *Rhinella* have been commonly confused and misidentified. The discovery of new populations helps us identify new species by using genetic markers to build phylogenetic hypotheses and to understand their evolutionary relationships. In this study, we present a phylogenetic tree including samples from all described *Rhinella* species available from Genbank and from newly discovered populations in Colombia. Phylogenetic analyses were performed based on the mitochondrial markers 12S and 16S using Maximum Likelihood in IQTree. Populations in Colombia correspond to potential new lineages related to *Rhinella proboscidea*, *R. margaritifera*, and *R. castaneotica*. The current phylogeny includes 14 taxa out of those 23 species that are reported in Colombia. Future goals include increasing the genetic data by including cytochrome c oxidase (COI) and Tyrosinase and collecting phenotypic data. Additionally, we hope to conduct fieldwork to collect the species in Colombia that are not represented on Genbank or in our current lab index.



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## Factors affecting migration timing and periodicity for white sharks (*Carcharodon carcharias*) in the western North Atlantic Ocean over multi-year scales

*Bryan Franks, John Tyminski, Nigel Hussey, Camrin Braun, Alisa Newton, Simon Thorrold, George C Fischer, Brett McBride, Robert Hueter*

A fine-scale analysis of spatiotemporal movement patterns for migratory species is critical to understanding the biology and ecology of these animals. Identifying cues for migration over multi-year time scales can provide insight into the physiological and ecological drivers of behavior at the individual and population level. To understand individual migration timing and identify biotic and abiotic factors associated with migratory behavior in white sharks in the western North Atlantic, we used data from 65 sharks tagged with a combination of satellite and acoustic tags. Tag-based horizontal and vertical movement data from 2012-2023 were used to discern temporal patterns of space use for individual sharks. Dates of departure from and arrival to residency areas were compared to a suite of environmental factors (e.g. absolute temperature, delta temperature over time, photoperiod, moon phase) to determine their influence on migration timing. While there was substantial individual variability in migratory routes and timing, abiotic factors including temperature appear to be significant drivers of departure timing from residency areas. Ongoing analyses are exploring the presence and scale of intra-annual variability among individuals along with inter-annual variability within individuals in migration cues and route fidelity. By providing information on multiple individuals over a broad spatiotemporal scale, these findings contribute key information on white shark migration and space use in the region. As ocean conditions change, identifying the effects of these alterations on migratory predators will be fundamental to understanding the comprehensive impacts to marine ecosystems.

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## Acoustic Telemetry as a Method to Quantify Post-Release Mortality: a Review of Past Results with Considerations for Future Applications

*Bryan Frazier, Ashley Galloway, James Kilfoil*

Release of captured sharks by commercial and recreational fisherman has become increasingly common; however, post-release mortality (PRM) may still occur as a result of physiological stress or physical injury due to capture. Post-release mortality in large migratory fishes has primarily been assessed through the application of archival pop-off satellite tags (PSATs), and more recently, accelerometer data loggers (ADLs); a lesser utilized technology is acoustic telemetry. All three methods have potential biases and drawbacks including expense, duration of data collection, tag non-reporting/non-recovery, and ability to determine precise fate of the tagged individual. Acoustic telemetry has been used to assess the fate of a variety of sharks including sand tiger sharks, blacktip sharks, and bonnetheads. It is relatively inexpensive, allowing larger study sizes, and greater duration of tracking of tagged animals, however this comes at a cost. Data are only collected within the presence of an acoustic receiver or by short-term active tracking, and only location/time data are collected. The precise fate of a tagged animal must be inferred, and predation of study animals may be difficult to determine through movement data alone. In this study we examine the feasibility of using acoustic telemetry to quantify PRM, and the conditions necessary to utilize this technology. We will also present results of field testing of Innovasea predation tags in estuarine environments to determine feasibility of using this technology to assess predation of sharks in the field.

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## Ecological limits of diversity across *Anolis* lizard communities

*Luke Frishkoff*

How important is competition for structuring ecological communities? Two alternative hypotheses have been proposed to explain how competition might result in contrasting patterns of local versus regional species diversity. The first posits that competition places a “cap” on the number of species that can co-occur, meaning that when comparing communities across multiple regions, the number of species found locally should be unrelated to the number of species in the region as a whole. The second contends that competition is weak, and therefore local diversity is effectively unbounded—limited solely

by the species diversity of the region. If this were the case then regions with greater numbers of species should also contain richer local communities. The preponderance of past research across a variety of taxa tends to support the unbounded model, suggesting competition is relatively unimportant. We test these alternative hypotheses using mark-recapture data of *Anolis* lizards from over 100 small plots from four regions with vastly different anole diversity—spanning from Jamaica, with only six species of native anoles, to Puerto Rico, Hispaniola, and finally Ecuador, with many dozens of species. Competition is frequently invoked to explain the predictability and repeatability of anole evolution, leading to the expectation that this might be a rare group to buck the trend of unbounded community diversity. Indeed, our findings demonstrate little change in local community diversity regardless of the region in which communities occur, implicating an important role for competition in generating community structure of these lizards.

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## Distribution patterns of Species Richness of Freshwater Fishes in the Usumacinta River Basin: Is there a Mid-Domain?

*César Fuentes-Montejo, Diego Elías, Pavel García, Caleb McMahan*

The Mid-Domain Effect hypothesis has been used as a general model to explain the distribution of species richness along gradients, by testing for relationships between species richness and variables such as elevation or latitude, among others. We compared the distribution patterns of freshwater fish species richness of the families Cichlidae and Poeciliidae along the Usumacinta river basin, in northern Middle America, with different geographical pattern drivers. We estimated the species richness of the two families by performing systematic and exhaustive review of records in scientific collections available through online aggregators. We generated species distribution models for all species of cichlids and poeciliids distributed in the basin using Maxent and a curated database of geographic records. We tested for a relationship between our predicted species richness and driving variables (elevation, sub-basin available area, and river order). We found a total of 23 species of cichlids and 22 poeciliids within the basin, with contrasting distributional patterns. The highest species richness of cichlids was predicted in the middle section of the basin, while the predicted poeciliids species richness was recovered in two non-adjacent areas. We found trends similar to the Mid-Domain Effect when species richness was compared with river order. Contrarily, we did not find a pattern reflecting a Mid-Domain Effect when compared with elevation and sub-basin area. The present-day distribution of species richness for freshwater fishes in the Usumacinta river basin responds to both ecological and historical factors. We provide an example of the Mid-Domain Effect in freshwater organisms.

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## Amphibian Vision: The Roles of Ecology and Ontogeny in the Evolution of a Key Sensory System

*Matthew Fujita, Rayna Bell, David Gower, Ellis Loew, Ryan Schott, Samuel Shrimpton, Jeffrey Streicher, Kate Smith*

Amphibians exhibit striking variation in all aspects of their vision biology that correlates with incredible diversity in life-history characteristics and behavior. From having eyes reduced and covered in skin (and sometimes bone) in fossorial caecilians to some of the largest eyes relative to body mass in frogs, and from the evolution of novel green rods to the loss of rhodopsin 2, it is clear that amphibians provide rich and exceptional opportunities to understand vision at multiple scales. Here I highlight three studies that exemplify how new research informs our understanding of amphibian vision biology. First, using measurements of eye and cornea diameters in 220 species of frogs for all 55 families, we found that adult habitat and activity pattern were correlated with eye size, providing strong evidence for the role of ecology in the evolution of anuran visual characteristics. Second, we extended these measurements to tadpoles and found an allometric shift in eye-body size scaling that indicates prioritized eye growth in tadpoles, patterns that may be explained by both larval and adult ecologies. Finally, we found extensive shifts in expression of vision-related genes during metamorphosis in the Southern Leopard Frog, *Lithobates sphenoccephala*, including genes related to spectral sensitivity and lens composition. Our discovery indicates that the decoupling of gene expression might act to mediate selective pressures during alternate stages

of complex life cycles. This symposium showcases additional examples of how the unique characteristics of amphibians are shining new light on understanding the evolution and ecology of vision.

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### **Taxonomic status of *Lissonanchus luserae* and *Briggsia hastingsi* (Gobiesocidae), with notes on their osteological characters revealed by micro-CT scans**

*Kyoji Fujiwara, Gento Shinohara, Hiroyuki Motomura*

Two gobiesocid species, *Lissonanchus luserae* Smith, 1966 and *Briggsia hastingsi* Craig & Randall, 2009, each originally described as a new genus and species on the basis of a single specimen, are known only from their original descriptions. During our ongoing revisional study on the Indo-Pacific clingfishes, the holotypes of *L. luserae* and *B. hastingsi* plus a newly collected specimen of the former were examined and detail osteological characters were revealed by micro-CT scans. Although *Lissonanchus* and *Briggsia* have previously been assigned to the subfamily *Diademichthyinae* (sensu Conway et al., 2020), they did not share any diagnostic features of the subfamily (e.g., not having complex articulation of posterior tip of basipterygium and ventral postcleithrum). In addition, there are no generic-level morphological differences in the two species. In contrast, we confirmed that *L. luserae* and *B. hastingsi* can be distinguished from each other with species level by some characters (e.g., head size and body coloration). Thus, *Briggsia* is regarded here as a junior synonym of *Lissonanchus* and the latter can be distinguished from all other gobiesocid genera by having 29 or 30 vertebrae, poorly developed cephalic lateral-line canal pores, including 2 nasal, 2 lacrimal and 1 postorbital pores, flattened papillae on the disc region D, and no spines formed by ossified subopercle. The genus *Lissonanchus* is regarded here to contain two valid species, *L. luserae* and *L. hastingsi*, new combination, although its subfamily assignment remains equivocal like other incertae sedis genera of Gobiesocidae (e.g., *Conidens* and *Creoceles*).

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### **Biogeography and body size evolution inferred from mitogenomic phylogeny of *Kalophrynus* (Amphibia: Anura: Microhylidae) in Southeast Asia**

*Ibuki Fukuyama, Masafumi Matsui, Misbahul Munir, Koshiro Eto, Yazid Hossman, Amir Hamidy, Daicus Belabut, Norhayati Ahmad, Ahmad Sudin, Mohamed Maryati, Paul Yambun, Kunio Araya, Hoi-Sen Yong, Jarujin Nabhitabhata, Panupong Thammachoti, Tomohiko Shimada, Kanto Nishikawa*

*Kalophrynus* is a subfossorial Microhylids that inhabits leaf litter in Southeast Asia. It is most diverse in the tropical regions, with multiple species occurring sympatrically. The species of this genus are all very similar morphologically and ecologically, and are thought to utilize the same environment and coexist. A detailed study of this group may reveal how they are able to speciate and coexist in a homogeneous and spatially limited environment. We conducted a molecular phylogenetic and morphological study to elucidate the mechanisms that create species diversity and multispecies coexistence in *Kalophrynus*. Phylogenetic analysis suggested that several species are not monophyletic and that there are multiple cryptic species, indicating that the diversity of this genus has been underestimated. In addition, each lineage was not grouped by region, suggesting that a complex geological history may have facilitated the diversification of this species. Divergence time estimation and biogeographical reconstruction indicate that the genus originated from Sundaland and diversified into many lineages during the Miocene, dispersing to various regions. Ancestral trait reconstructions for body size revealed that the increase of body size and decrease of body size from a medium-sized ancestor occurred in parallel in multiple lineages. Most of the body size increases and decreases occurred in the lineages distributed in Borneo. The fact that Borneo is the only island where more than three species of this genus occur sympatrically suggests that coexistence may be possible in Borneo by displacement of body size.

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### **Use of AI Derived Electronic Monitoring Data for Pacific Sleeper Shark Stock Assessment**

*Keith Fuller, Cindy Tribuzio*

Pacific sleeper sharks (*Somniosus pacificus*) are a data limited species commonly caught as bycatch in Alaskan fisheries. Due to their large size, accurate length and weight estimates are often difficult to obtain, which creates difficulties in performing accurate catch-based stock assessments. The spreading use of electronic monitoring (EM) in Alaskan longline fisheries provides an opportunity to gather data which would otherwise be difficult or impossible to obtain. Use of machine learning approaches have allowed us to develop algorithms capable of automatically detecting and classifying bycatch, which has served as a necessary precursor for an algorithm process capable of assessing lengths from individual *S. pacificus* which are not brought on board. While we found a “bin” approach most useful for our stock of interest, the precision of measurements possible to obtain from this method will vary widely by system and species; for a species as data poor as *S. pacificus*, bin-level measurement represents a data source which simply did not exist previously. The resulting data output, when integrated into stock assessment systems, will serve to provide a valuable and formerly unavailable look into the population status of *S. pacificus* in Alaskan waters.

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### **Evaluating Population Genetic Structure of Mediterranean Geckos (*Hemidactylus turcicus*) with Increased Sampling Across the University of Central Oklahoma**

*Viannka Galarza, Dani Bright, Mari Nguyen, Meryem Assaoui, Cass Condray, Kai Williams, Lauren Branham, Allyson Fenwick*

The Mediterranean gecko (*Hemidactylus turcicus*) is native to the Middle East and has been introduced to many urban areas worldwide. The Mediterranean gecko was introduced to the University of Central Oklahoma during 1963-1965 and 1985-1997. Dr. Fenwick's lab has been sampling since 2014, collecting over 1500 tail tissue samples. Hundreds of samples have not been able to be genetically analyzed because lab work was limited during the pandemic. We genotyped 16 previously published microsatellite loci for 88 individuals, including samples from eight buildings in Edmond. We also checked the entire dataset for potential errors and revised multiple allele calls. We analyzed the data using STRUCTURE and ARLEQUIN. Analyses found low genetic diversity and low genetic differentiation among buildings. We also found different genetic clusters between geckos sampled from distant buildings and those near the original introduction site of Howell Hall. Going forward, this project will continue to perform genotyping and analyses on the approximately 200 samples that have not yet been extracted.

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### **Damming evidence for morphological consequences of artificial barriers in riverine steelhead trout**

*Jacquelyn Galvez, Christian Lizaso, Jack Tseng*

Artificial damming of freshwater rivers influences many aspects of stream ecology. Human-made dams can create barriers to upstream breeding and rearing habitats of anadromous fishes and can limit gene flow between such populations. Studying fish populations impacted by damming provides a unique opportunity to study the downstream effects of these dispersal limitations, and has potential to reveal how damming influences the evolutionary trajectory of populations—possibly in exposure to different selective pressures and/or buildup of reproductive isolation—and how endangered fishes cope with anthropogenic environmental changes. In this study, we conducted a morphological comparison of above- and below-dam samples of anadromous steelhead trout, *Oncorhynchus mykiss*, using 2D geometric morphometrics analysis of lateral body shape. Preliminary results demonstrate that above- and below-dam specimens have minimal overlap in morphospace. Principal components (PC) analysis of shape data suggests that above-dam individuals are wider in the snout and head region with a shorter body, whereas below-dam individuals are narrower in the head region with a wider, longer body. Overall, below-dam individuals appear to have a more streamlined body shape; discriminant function analysis statistically supports clusters apparent in the PC morphospace. These results lead us to propose functional hypotheses

for how muscle mass and movement may differ between above-dam (traditionally freshwater resident) and below-dam (traditionally anadromous/migratory) trout populations.

## You're Gonna Need a Bigger Boat: Sharks as a Holotype of the Sociological Model for Conservation

*Taylor Gamble*

The necessity of humanities in conservation is that the issues transcend natural sciences' quantitative boundaries. Sociology offers a unique paradigmatic position that acts as a bridge to connect conservation-minded scientists to conservation efforts in other disciplines and society. The theoretical frameworks of sociology meld research across fields such as marine biology, public policy, geography, meteorology, epidemiology, political science, psychology, and history. I discuss the efficacy of a sociologically founded model to aid in the following ways: One, communicating scientifically accurate information to stakeholders and the public. Two, assess the perspective, attitudes, and epistemologies of groups interacting with chondrichthyans. Three, evaluate what factors influence differing inclinations toward conservation issues and what affects social support for public policy related to chondrichthyans.

## Short-read genome assembly for spatiotemporal genetic analysis of contemporary and century-old museum fishes from the epicenter of marine biodiversity

*Eric Garcia, Roy Roberts, Brendan Reid, Rene Clark, Jemelyn Baldesimo, John Whalen, Abner Bucol, Ivan Lopez, Malin Pinsky, Kent Carpenter, Chris Bird*

Many population genetic analyses require or are improved by the presence of a reference genome for the focal organism. However, given that producing the long-read data that is traditionally used by assembling software remains expensive, we still lack genomic information for the great majority of non-model species. As part of the Philippines PIRE Project, we test the performance of four commonly used tools (ABYSS, HipMer, SOAPdenovo, and SPAdes) in generating short-read de novo assemblies from several marine fish species of diverse ecologies with contemporary and historical samples (collected over 100 ya) available. We demonstrate that assemblies with relatively high values for BUSCO Complete and Single Copy and N50 are possible using only short-read data, and highlight case studies where this type of assemblies are later used in combination with capture or low coverage whole genome sequencing (lcWGS) to investigate spatiotemporal anthropogenic impacts (such as fishing and habitat degradation) on the genetic composition of marine fish populations from the Philippines. Overall, successful genome assembly from short reads allows for a large suite of downstream genetic analyses from contemporary or museum specimens, and represents a new paradigm for studying non-model organisms, especially for large-scale projects or studies with limiting resources.

## Venom Gland Transcriptomics of the Mexican horned pitvipers (*Ophryacus* and *Mixcoatlus*)

*Ramses Rosales Garcia, Christopher Parkinson, Rhett Rautsaw*

Mexico is a diversity hotspot for pitvipers containing ten genera, including two endemics (*Ophryacus* and *Mixcoatlus*). These genera each contain three species, inhabiting the eastern and southern Sierra Madre mountains. Little is known about the natural history and ecology of these snakes. However, the venom of *Ophryacus* species and *M. melanurus* was recently described using preteomics, revealing a crotoxin-like neurotoxin in *O. sphenophrys* and *M. melanurus*. The crotoxin-like neurotoxins have been identified in other New World genera (*Bothriechis*, *Crotalus*, and *Sistrurus*) and one Asian taxon (*Gloydus intermedius*). To address the lack of genomic information, we used venom gland transcriptomics to describe the content of toxin genes in all species of *Ophryacus* and *Mixcoatlus*. We found that the venom of *M. browni* is composed mainly of PLA2s, followed by BPPs and SVMPs, with a small quantity of SVMPs, which is not very common in pitvipers. We also found crotoxin-like neurotoxins in all individuals of this species. The venom of *M. barbouri* was composed mainly of PLA2s, followed by BPPs and SVMPs,

although the percentage of SVMPs was low compared to other pitvipers. Overall, *Mixcoatlus* species have venom largely composed of PLA2s, and two of the species express crotoxin-like neurotoxins. *Ophryacus* species have a more variable venom that could be largely composed of PLA2s or SVMPs, and only one of the species expresses crotoxin-like neurotoxins.

## Differential responses of juvenile bull and blacktip sharks to tropical cyclones

*Jayne Gardiner, Cecilia Hampton, Victoria Goldner, Tonya Wiley*

Many shark species use coastal areas, which offer abundant food and relative protection from predators, as nurseries. Juvenile sharks have been observed to flee nurseries in response to barometric pressure changes during tropical systems, as the risks of remaining in the nursery are thought to outweigh any increased predation risk. Tampa Bay, on the Gulf coast of Florida, is a well-known shark nursery area. Juvenile blacktip sharks, *Carcharhinus limbatus*, preferring upper polyhaline waters, are seasonally resident in Terra Ceia Bay. Juvenile bull sharks, *Carcharhinus leucas*, preferring oligohaline waters, are resident year-round in the adjacent Manatee River. During a 2022 acoustic telemetry study, two tropical cyclones of differing strength and size impacted the area. In September, 220km-diameter Category 4 Hurricane Ian (240km/h winds) made landfall 90 km south of the study site. In November, 1550km-diameter Category 1 Hurricane Nicole (120km/h winds) made landfall 200km east of the study site. Though the storms caused similar barometric pressure minima in the study area (~995hPa), acoustically-tagged blacktip and bull sharks responded differently to the two events. During Hurricane Ian, all individuals of both species fled the nursery area for deeper waters. For bull sharks, this involved a metabolically-costly change in salinity. During Hurricane Nicole, all blacktip sharks fled for deeper waters, whereas all bull sharks remained in the nursery. These results suggest other environmental cues (in addition to barometric pressure) are involved and that blacktip and bull sharks may weigh the costs-benefits differently in determining when to depart the nursery.

## Residency and philopatry of juvenile great hammerheads, *Sphyrna mokarran*, in Tampa Bay, FL: evidence of a nursery area

*Jayne Gardiner, Susan Lowerre-Barbieri, Kim Bassos-Hull, Krystan Wilkinson, Tonya Wiley*

The great hammerhead, a circumtropical coastal-pelagic and semi-oceanic shark species, is particularly vulnerable to overexploitation. Globally, it is listed as critically endangered by the International Union for Conservation of Nature. The lack of knowledge of habitat use, especially pupping and nursery grounds, is a major management challenge. Large coastal sharks that give birth to relatively small young typically use discrete nursery areas, but evidence for great hammerheads is inconclusive. The presence of young-of-the-year (YOY) and juvenile great hammerheads has been documented in lower Tampa Bay and because YOY sharks have been captured across multiple years of sampling and are not regularly encountered elsewhere in the Gulf of Mexico, we hypothesized that this estuary serves as a great hammerhead nursery. However, designating a nursery area also requires evidence that immature animals use the habitat for extended periods (weeks to months). To examine spatiotemporal patterns of habitat use in the Tampa Bay estuary, juvenile great hammerheads were tagged with surgically-implanted acoustic transmitters. Upon release, movements in Tampa Bay and adjacent Gulf of Mexico waters were detected by arrays of passive acoustic receivers, with data from other areas received through collaborative telemetry networks, the Integrated Tracking of Aquatic Animals in the Gulf of Mexico (iTAG) and the FACT Network (an Ocean Tracking Network node). All individuals were found to use the Tampa Bay estuary for extended periods and to return to the same areas across multiple years. These data provide further evidence of a great hammerhead nursery area in lower Tampa Bay.

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## Do Personalities and Behavioral Syndromes of Eastern Red-backed Salamanders (*Plethodon cinereus*) Explain Historical Range Expansion?

*Kelsey Garner, Cari-Ann Hickerson, Carl Anthony*

Behavioral syndromes are suites of behaviors that are correlated across contexts and are comprised of behaviors which are repeatable within individuals through time. Behavioral repeatability and correlates have potential fitness consequences at both the individual and evolutionary scale. However, few studies have examined these processes in terrestrial amphibians. We examined the repeatability of three commonly studied behaviors (boldness, exploratory tendency, and neophilia) in two genetically distinct groups (Northern Clade and Ohio Clade) of the Eastern Red-backed Salamander (*Plethodon cinereus*). The unique and extensive distribution of the Northern Clade suggests that differences in behavioral traits may contribute to the historical dispersal success of these individuals. We tested the following hypotheses in the laboratory: (1) Both clades would exhibit repeatable behaviors; (2) Boldness, neophilia, and exploratory tendency would be positively correlated, suggestive of a behavioral syndrome; and (3) The Northern Clade would repeatedly exhibit bold, neophilic, and exploratory tendencies consistent with dispersing across a large range. We found strong repeatability of boldness and exploratory tendency but not neophilia. However, we found little evidence to support the presence of a behavioral syndrome. Contrary to our prediction, the Northern Clade was less exploratory, neophilic, and bold than the Ohio Clade. Considering our results, we suggest three alternative hypotheses for differential rates of range expansion: 1) Loss of behavioral syndrome through time; 2) Adaptation to ecological factors; and 3) Behavioral switching due to landscape changes.

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## Modeling shortfin mako shark habitat suitability via machine learning methods

*Julian Garrison, Daniel Daye, Jeremy Vaudo, Mahmood Shivji, Bradley Wetherbee*

Shortfin mako sharks (*Isurus oxyrinchus*) have experienced substantial population declines from direct and indirect fishing pressure, sparking considerable interest in understanding their habitat preferences to better inform conservation efforts. However, knowledge gaps remain owing to these sharks' mobility and the complexity of marine environments across multiple spatiotemporal scales. Machine learning (ML) offers a powerful suite of techniques for analyzing extensive and highly dimensional data, such as large telemetry and remote sensing data sets, but has not been widely applied to shortfin mako movement ecology. We leveraged the predictive power of ML to generate process-based habitat suitability models for shortfin makos that combine nearly a decade of satellite telemetry data analyzed using state-space-derived movement persistence modeling with data for 17 environmental variables derived from remote sensing. We modeled movement persistence as an index of habitat use from telemetry data of 95 shortfin makos in the North Atlantic Ocean and Gulf of Mexico tagged with Smart Position and Temperature (SPOT) tags. We then trained and evaluated a suite of ML algorithms to predict movement persistence from remotely sensed environmental data across the two ecoregions and identify important environmental variables. Among ML algorithms used, tree-based methods such as random forest generally performed the best and generated predictions of shortfin mako movement persistence across a broad geographical range. Our results highlight the potential of ML-based approaches for constructing mechanistic habitat suitability models that incorporate a large number of environmental variables to reveal long-term movement patterns of shortfin makos and their underlying environmental drivers.

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## Comparing Variation in Diet, Parasite Load, and Morphometrics Between Invasive Cane Toad (*Rhinella marina*) Populations in Central and South Florida

*Michael Garvey, Madison Harman, Carrie De Jesus, Samantha Wisely, Christina Romagosa, Jacob LaFond*

The cane toad (*Rhinella marina*) is a large neotropical toad, found throughout Central and South America. Invasive populations of *R. marina* have become

established in multiple countries including Australia, Japan, the Philippines, and the states of Hawaii and Florida in the United States. *Rhinella marina* has had major negative impacts on many of the ecosystems where it has become established and has been described as one of the 100 worst invasive species globally. Despite decades of publications devoted to the ecological impacts of this species in regions like Australia, surprisingly little is known about the ecological impacts this species is having on Florida ecosystems. We collected 244 *R. marina* specimens from 12 populations across their Florida invasive range, including established populations in south Florida and populations at the edge of the invasive range in central Florida. For each specimen we completed a stomach dissection, checked the internal body cavity for acanthocephalan and pentastome parasites, and collected morphometric data. We found *R. marina* most readily preyed on species from the order Coleoptera, with at least 1 Coleopteran species recovered from the stomachs of over 59% of all specimens. We did not recover any pentastome parasites but did recover acanthocephalan parasites in 6/12 populations. We also found populations at the edge of the invasive range appear to be adapting quickly to local environments as demonstrated by significant variation in limb length and mass of organs associated with digestion and respiration compared to established populations.

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## Establishing a Statewide Fish Collection in Missouri: Current Progress and Future Visions

*Aaron Geheber, Robert Hrabik*

Statewide museum collections are a fundamental resource for understanding distributional, ecological, and evolutionary aspects of organismal diversity across generous spatial scales (i.e., across an entire state). The state of Missouri harbors 239 species of fish (including non-natives), however, no statewide repository has been designated or established for the housing and cataloging of fish specimen collections and collection related data. Moreover, an estimated ~10,000 lots of historical collections exist in the state, but the housing and management needed for collection use and accessibility is non-existent. Historical collections include specimens linked to The Fishes of Missouri text by W. L. Pflieger (collections made prior to 1995) as well as specimen collections referenced in the upcoming Fishes of Missouri text by R. A. Hrabik. Our aim was to establish a statewide fish collection in Missouri to house and manage historical state collections and act as the state repository for future scientific fish collections. In March 2023, historical collections were transported to the University of Central Missouri as a first step towards establishing the Missouri statewide fish collections. Up to date efforts related to collection organization and management will be outlined, and our visions for the enhancement and long-term use and accessibility of the collections will be discussed.

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## Are sharks on the U.S. east coast exposed to harmful levels of the “forever chemicals?”

*Jim Gelsleichter, Bryan Frazier, R. Dean Grubbs, John Bowden*

Per- and polyfluorinated alkyl substances (PFAS) are a large family of potentially toxic anthropogenic chemicals used in a variety of commercial products such as stain-resistant fabrics, non-stick cookware, fast food containers, and fire-fighting foams because of their water- and oil-repelling properties and high heat resistance. There has been growing concern about the occurrence of PFAS in the marine environment because of their high stability and environmental persistence, features that have led many to refer to them as the “forever chemicals.” There is special concern about PFAS accumulation in top marine predators; however, few studies have examined PFAS uptake in sharks despite their tendency to accumulate higher levels of environmental pollutants than other fish. We are currently examining concentrations of multiple PFAS in plasma of sharks occurring along the southeast U.S. coast. This presentation describes the preliminary results of this work, focusing on PFAS levels in sandbar sharks from Florida rivers and estuaries, as well as black-nose, finetooth, and bonnethead sharks from coastal sites from South Carolina to south Florida. Overall, PFAS concentrations were low in most sharks from these sites in comparison to levels observed in other large marine vertebrates. However, we observed greater concentrations of some currently used PFAS that have increased in use and production in recent years as certain legacy



PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been phased out of use because of their environmental persistence. This suggests that these chemicals may be accumulating in marine food chains, warranting further study.

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### LED light baits fail to improve aquatic turtle capture success

*Benjamin W. Genter, Aaron R. Krochmal, Travis J. LaDuc, Timothy C. Roth II, Drew R. Davis*

Aquatic turtles are an imperiled group of organisms facing global declines, and developing efficient means to detect and inventory species is critical. The addition of light baits to traps has been shown to increase capture success of some aquatic organisms, such as amphibians, but recent data with turtles has shown a small but significant increase in trap success with blue lights. Given the influence of red, blue, and yellow light on turtle behavior, we sought to investigate the influence of colored LED light baits on turtle trapping success. We added red, yellow, and blue LED lights to turtle traps as part of ongoing turtle sampling efforts in Maryland and Texas, USA, and recorded the number of turtle captures with each light color. A total of six species of turtles across three families (Chelydridae, Emydidae, and Kinosternidae) were sampled in 2022. We found no effect of LED light baits on turtle captures. Previous studies have suggested that LED light affects turtle behavior and can influence turtle trap success, but our data fail to support these findings. Given the mixed results, studies should continue to investigate the role of LED light baits on trap success to determine if results are context- or species-dependent and if their addition can be a practical means to increase the effectiveness of sampling efforts.

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### Did Early Natural History Museum Collectors in California Neglect the Central Valley?

*Marina Gerson*

The VertNet ([vertnet.org](http://vertnet.org)) database is an invaluable resource; the data can be used to explore patterns of biodiversity, subject to adequate collecting. Nonetheless, while 33,215 amphibian and 32,458 reptile specimens are documented as having been collected from California, USA between 1873-2019, only 6.3% were collected from the seven counties of the San Joaquin Valley that lie south of the San Francisco Bay. I hypothesized that this distribution of data is at least partially the result of collector bias, with natural historians from museums in Northern California focusing resources on collecting expeditions in Southern California and vice-versa, with few having considered the Central Valley a destination for interesting sampling. I expected each California-based collector to have sampled most extensively in the region close to the home institution and in the opposite region of the state. However, preliminary analysis of example collectors did not reveal the expected pattern, nor was there a consistent pattern to the collections they made in California. Analysis of additional collectors and examination of field notes to elucidate patterns of collecting trips will further understanding of the limitations of available data in assessing past patterns of biodiversity in the Central Valley of California; however, the ability to document extirpated species, or those whose ranges have been severely reduced in the face of agricultural expansion, may be long since lost.

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### Anatomy and Evolution of Bioluminescence in the Gulper Eels (Anguilliformes).

*Michael Ghedotti, Kandice Agudo, Flor Gonzalez, Kurt Riggin, Matthew Davis, W. Leo Smith, Benjamin Frable*

Gulper eels in the genera *Eurypharynx* and *Saccopharynx* are deep-water fishes with extremely enlarged jaws, a pronounced black coloration, a caudal light organ that has been suggested to be used in prey luring, and dorsolateral "white lines" identified as putatively bioluminescent. In this study we seek to provide a first description of the histology of the bioluminescent organ in *Eurypharynx*, determine if the "white lines" in both genera are bioluminescent, explore the presence of ultra-black coloration in *Saccopharynx*, and place these structures into an evolutionary context. We used histological sectioning and gross examination to explore the anatomy of museum specimens and used a published DNA-sequence based phylogeny for the anguillid eels

to explore character evolution. The bioluminescent organs in *Eurypharynx* are intrinsic and use partial screening via ultra-black melanosome anatomy, based on histology the white lines in both genera likely are bioluminescent, and both white lines and caudal lure exhibit a muscular opening mechanism. *Saccopharynx* also exhibits ultra-black coloration. The anatomy in the context of the phylogeny suggests that a dermal anatomy associated with ultra-black coloration evolved in the ancestor of the deep-ocean anguillids and that the intrinsic caudal and "white line" bioluminescent organs evolved once in the most recent common ancestor of *Eurypharynx* and *Saccopharynx*.

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### Relationships Between Horizontal and Vertical Movements of Shortfin Mako Sharks (*Isurus oxyrinchus*) in the Western North Atlantic Ocean

*Katie Gheysen, Julian Garrison, Jeremy Vaudo, Mahmood Shivji, Brad Wetherbee*

Highly mobile pelagic predators, such as tuna, swordfish, and sharks often undergo changes in depth over a 24-h period and exhibit diel vertical migration (DVM), presumably to maximize foraging efficiency. Studies on the diving behavior of shortfin mako sharks (*Isurus oxyrinchus*) have been inconclusive as to whether this species consistently exhibits DVM. We investigated relationships between horizontal and vertical movements in shortfin mako sharks by using location and depth data collected from SPLASH tags and pop-up satellite archival tags (PSAT). A state-space model was applied to location data derived from SPLASH tags to generate continuous tracks and to quantify horizontal behavior in terms of "movement persistence", which classifies behavior on a scale of "resident" to "transient", based on speed, angle, and distance between locations. Sharks engaging in resident behavior occupied shallower depths compared to transient behavior. Average depth and range of depths occupied by sharks were at least three times greater during transient than to resident behavior. Depth records from sharks tagged with PSATs indicated that longer-term use of the water column was similar to results obtained from SPLASH tags. Our results suggest that shortfin makos restrict their movements both vertically and horizontally, possibly to concentrate their activity within prey-rich waters. Restricted movements in the upper water column may be strongly associated with prey availability, explaining the lack of consistent DVM behavior exhibited by this species.

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### Putting the energetic-savings hypothesis underground: Fossorial and non-fossorial amphibians have similar metabolic rates

*Danilo Giacometti, Glenn Tattersall*

By living underground, fossorial animals may be challenged by limited gas exchange due to prolonged exposure to low oxygen levels (hypoxia) and carbon dioxide buildup (hypercarbia) in their burrows. The negative effects of hypoxia and hypercarbia generally relate to changes in breathing and energy metabolism. In mammals, reduced metabolic rates are a common adaptation to fossoriality. It is unclear, however, whether fossoriality exerted a similar selection pressure over the metabolism of fossorial ectotherms. In this study, we tested whether fossorial amphibians showed reduced metabolic rates compared to non-fossorial ones in a phylogenetic framework. We found that whole-organism resting metabolic rates varied according to body mass and temperature. However, our analyses did not support the hypothesis of lowered energy expenditure in fossorial species. We suggest that the intrinsically low energetic requirements of amphibians, coupled with their relatively small body sizes, and ability to breathe through both lungs and skin potentially circumvented the energetic and respiratory challenges imposed by fossoriality. This view of energetics in the context of fossoriality integrates central tenets of eco-physiological theory (metabolic scaling) and comparative physiology (control of bimodal breathing), and opens a new avenue for research aiming to understand the physiological correlates of fossoriality in vertebrate ectotherms.

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## Previously Unidentified Biodiversity of Fish Fauna in the Upper Triassic Dockum Group of Western Texas

*Sarah Gibson, Spencer Ott*

The Upper Triassic Colorado City Formation, Dockum Group, of western Texas preserves an abundance of isolated fish remains in a geologic deposit representing ancient ephemeral ponds, streams, and floodplains. Fish remains are preserved from a specific site near Otis Chalk, Texas as disarticulated scales and skull bones, with a few examples exceptionally preserved in three-dimensions. Two described species of fishes from the order *†Redfieldiiformes*, *†Cionichthysgreeni* Schaeffer, 1967 and *†Lasalichthys otischalkensis* Gibson, 2018 are based on articulated, complete skulls and partial bodies. Herein we describe new, previously undocumented biodiversity of fish fauna from the Otis Chalk site, including a nearly complete skull preserved three-dimensionally. The skull includes a skull roof, preoperculum, maxilla and circumorbital series. Based on the shape of the skull bones, paths of sensory canals, and ornamentation, this skull does not belong to either described redfieldiiform taxa from the Dockum Group, and likely represents a new species of actinopterygian fish. We compare it to similar taxa from the Early Mesozoic and discuss its affinities.

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## Evolution of the Rovers, Redbaits, and Bonnetmouths (Emmelichthyidae)

*Matthew Girard, Carole Baldwin, Katherine Bemis*

The family Emmelichthyidae (18 species) is a demersal group of marine fishes found between 100–400 meters depth in tropical and temperate waters. They are predominantly planktivorous, with highly protrusible jaws that have few rudimentary teeth or are completely toothless. Specimens of Emmelichthyidae are rare in museum collections because they are typically collected by rod and reel or found in the stomach contents of large pelagic predators; thus, little is known about their diversity and evolutionary history. Heemstra and Randall (1977) were the last researchers to assess the diversity of the family, although several new species have been described over the past 40+ years, and no hypotheses of relationships have been proposed. In this study, we examine the taxonomic diversity and evolutionary history of the Emmelichthyidae using a combination of osteological characters from  $\mu$ CT-scanned and cleared-and-stained specimens as well as 15 mitochondrial loci from whole mitogenomes. Based on this integrative dataset, we recover a phylogeny for the family and find that the diversity of these fishes has yet to be fully described.

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## New species of Monomitopus (Ophidiidae) from Hawai'i, with the description of a larval coiling behavior

*Matthew Girard, Jacques Carter, David Johnson*

Professor Jack Musick's life-long fascination with sharks, turtles, coelacanths, marine sport and commercial fishes and his beloved fishes of the Chesapeake Bay are well known. But Jack also had a passion for deep-sea demersal fishes that led into a multi-year deep-sea program, with UNOLS/NSF research vessel support, providing VIMS graduate students and scientists with opportunity to explore life in the deep-sea and discover new species of benthic and benthopelagic fish. In 1985, Carter and Cohen noted that there are several yet-to-be described species of *Monomitopus* (Ophidiidae), including one from Hawai'i. Recently, blackwater divers collected a larval fish off Kona, Hawai'i, similar to previously described larvae of *M. kumae*, but DNA sequence data from the larva does not match any of the six previously sequenced species within the genus. Within the USNM Ichthyology Collection, we find a single unidentified adult specimen of *Monomitopus* collected North of Maui, Hawai'i in 1972 whose fin-ray and vertebral/myomere counts overlap those of the larval specimen. We describe this new Hawaiian species of *Monomitopus* based on larval and adult characters. Additionally, blackwater photographs of several species of *Monomitopus* show the larvae coiled into a tight ball, a novel behavior to be observed in cusk-eels. We describe this behavior, highlighting the importance of blackwater photography in advancing our understanding of marine larval fish biology.

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## Allometry of sexual size dimorphism in geckos

*Elizabeth Glynn, Dean Adams*

Deciphering trends in sexual size dimorphism (SSD), and how they change across taxa, is a major interest in evolutionary biology. Many lizard species exhibit SSD, though the degree of SSD and its direction differ among taxa. Here we investigated whether SSD varied systematically with species' body size in geckos, and explored what potential mechanisms may have generated these observed patterns. First, we collected snout-vent-length from ~1,600 individuals comprising 127 gecko species from two families (Sphaerodactylidae and Phyllodactylidae), with at least 3 males and 3 females represented per species. Species means were then calculated for each sex, and various measures of SSD were obtained. We found that both families contained a mix of female-biased and male-biased species, and that the overall allometric trend was one where larger species displayed male-biased SSD, while smaller species displayed female-biased SSD (a.k.a. Rensch's Rule). Furthermore, a phylogenetic ANCOVA revealed significant heterogeneity in slopes between families, and subsequent phylogenetic regressions performed on each family separately found that Sphaerodactylidae displayed a positively allometric trend of SSD, while the evolutionary allometry of SSD across species in Phyllodactylidae did not differ from isometry. Thus, the two clades displayed contrasting allometric patterns of SSD across species, where Sphaerodactylidae exhibited strong evolutionary allometry of SSD consistent with Rensch's rule, while species in Phyllodactylidae largely adhered to isometry. Finally, we found that relative egg size was larger in smaller Sphaerodactylidae species, consistent with the hypothesis that selection on reproduction may be responsible for the observed trends in SSD.

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## What Matters for the Survival of Head-Started Blanding's Turtles?

*Callie Golba, Elizabeth Bach, Gary Glowacki, Bill Graser, Kathryn McCabe, Daniel Thompson, Brian Towey, Richard King*

Head-starting is a popular conservation intervention used for many turtle species that involves collecting eggs from the wild, hatching and rearing the turtles in captivity, and releasing them back into the wild once they have reached a specified age or size. The goal is to protect vulnerable life stages, increase juvenile recruitment, and re-establish self-sustaining populations. Analyses of survival, spatial ecology, and growth of head-started Blanding's Turtles demonstrate the potential utility of head-starting for increasing recruitment. However, replicated studies using consistent head-starting and monitoring methods are lacking, so the generality of single-population studies is uncertain. To determine best practices for head-starting as a management tool, we conducted a replicated study of head-start survival at 7 sites in Northern Illinois from 2020-2023. A total of 304 turtles (up to 35 turtles per site per year) were released and tracked using radio-telemetry for 1-3 active seasons post-release and for 1-3 overwintering periods. Head-starts varied in size and age at release and sites varied in size, predator management, and Blanding's Turtle population status. Initial analysis using Cox regression shows that active-season survival was highly variable among sites, ranging from about 40% – 90%. Overwinter survival, estimated directly from the number of transmitter-equipped turtles that survived, averaged 94% across sites and years. These results will be combined with Population Viability Analysis and estimates of economic costs to develop recommendations of best practices for Blanding's turtle head-starting. This will include site-by-site management considerations, release size or age, follow-up monitoring, and adaptive management guidelines.

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## Disentangling a cryptic salamander group with eDNA: a landscape assessment tool for the *Ambystoma laterale-jeffersonianum* complex

*Harrison Goldspiel, Jacob Kubel, Erin Grey, Noah Charney, Paula Blanco-Ortiz, Alyssa W. Kaganer, Samara Davis, Mark Y. Stoeckle*

*Ambystoma* salamanders in eastern North America include five species of conservation concern and a globally unique unisexual (i.e., all-female) lineage whose ecological interactions and population trends are poorly understood in a rapidly changing landscape. Herein, we examine the potential opportunities

for using environmental DNA (eDNA) to address ecological and conservation uncertainties of members of the Ambystoma complex. The objectives of our research are to (1) develop a working eDNA protocol for this group, outlining best-use practices for other researchers and managers (2) expand on the ecology of the Ambystoma complex, including estimating predictors of breeding habitat and co-occurrence dynamics, (3) identify new populations of pure diploid salamanders (e.g., *A. laterale*) in New England, and (4) explore the potential of this approach for complementing existing state-run and volunteer-run monitoring programs for rare salamanders. Pilot eDNA sampling was conducted in 2021 in New York and Massachusetts to optimize eDNA sampling protocols in vernal pools and estimate fine-scale variation in salamander detection across space and time in breeding sites. Preliminary analyses demonstrate the capacity to detect members of the complex through eDNA collection. We will conduct landscape-scale trapping and eDNA surveys in 2023 across New England to calibrate eDNA with traditional survey data and estimate environmental drivers of salamander co-occurrence. We expect eDNA quantity will be linearly related to animal abundance and that breeding habitat use will vary among unisexual salamanders and their hosts.

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### **An integrative approach to species delimitation of the dwarf goby *Eviota sigillata*, a tiny fish with a giant geographic range**

*Marta Gomez-Buckley, Carmen Pedraza-Marron, Mark Erdmann, Luke Tornabene*

The ability to easily sequence tens of thousands of loci across the genome of non-model organisms, and the development of evolutionary models that allow us to use these data to help delimit species, has led to a new paradigm of integrative taxonomy. The highly specious genus of coral reef fishes *Eviota*, with 129 species described to date, presents a great opportunity for the application of genome-wide species-delimitation techniques. For this study, I concentrated on the *Eviota sigillata* complex, which contains ~10 putative species based on mtDNA, which have yet to be analyzed with morphological or genomic data. Using samples collected throughout the entire range of the *E. sigillata* complex, I generated SNP data from double digest restriction associated sequencing (ddRADseq), coupled this with analysis of morphology and mtDNA, to estimate how many species are present in this complex. A phylogenetic tree generated using maximum likelihood for 660,013 base pairs of sequence data (>4000 SNPs) closely matched a tree from mtDNA, with 8 deeply-divergent clades that closely correspond to geographic regions, supporting a hypothesis of micro-endemic allopatric species groups. Ongoing analyses using multispecies coalescent methods for species delimitation, like BPP and SNAPP+BFD, will help us delimit the final number of species in the *E. sigillata* complex, and analysis of morphology will help us determine whether genomic differences are reflected in their phenotypes. Overall, this study will serve as a species delimitation model in the quest to reveal the full diversity of the entire *Eviota* genus, one complex at a time.

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### **Long-Term Research on Species-, Population-, and Community-Level Effects of Urbanization on Snakes and Lizards**

*Matt Goode, Anthony Pawlicki, Javan Bauder, Ryan Hanscom*

Starting in 2002, we have used a variety of methods (e.g., radio telemetry, mark-recapture, systematic road cruising surveys) to examine responses of snakes and lizards to urbanization at our study site in the foothills of the Tortolita Mountains near Tucson. The study site is a low-density residential golf community with luxury homes situated on relatively large lots with minimal disturbance. Over the course of the study, the development has grown from a handful of homes to more than 350. The golf course, residential yards, and roads are watered by drip irrigation and sprinklers, which has created a lushly vegetated landscape in the otherwise hot, dry desert. The response of herpetofauna to this unique environment has led to some interesting and unique findings that shed light on both ecology and conservation of snakes and lizards. We will draw on a large data set to highlight some of our findings, including spatial ecology of Tiger Rattlesnakes and Gila Monsters, changes in

snake and lizard community structure, and various aspects of single-species responses to urbanization (e.g., individual and population growth, abundance estimates, and fine-scale movement patterns).

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### **Ecology Explains Variation in Lens Transmission and Pupil Shape of Amphibians**

*David J. Gower, Ron H. Douglas, Rayna C. Bell, Jeffrey W. Streicher, Ryan K. Schott, Matthew K. Fujita, H. Christophe Liedtke, Caitlyn Rich, Rachel C. Quock, Célio F. B. Haddad, C. Guilherme Becker, Christian L. Cox, Renato A. Martins, Kate N. Thomas*

Vertebrate vision is one of the best-studied and most richly understood of all biological sensory systems. However, much of what we know comes from detailed studies of mammals, birds, fishes, and, to some extent, reptiles. Despite the great ecomorphological diversity of amphibians, their visual systems have remained relatively little-studied and poorly known. This talk will summarize recent discoveries about how lens spectral transmission and pupil shape vary in amphibians (mostly frogs and toads) in the context of phylogeny and ecology. Pupil shape was characterized for 1,294 species, and lens spectral transmission quantified for 115 species, and variation in these traits was analysed using phylogenetic comparative methods. Constricted pupil shape is evolutionarily and ontogenetically variable, and correlates with habitat, life stage, and eye size, with elongate (slit-like) pupils occurring only in terrestrial adults, especially those with larger eyes. Lens transmission also varies substantially across the phylogeny, with the least-transmissive (most UV-filtering) lenses associated with diurnal and scansorial ecologies, likely as an adaptation to greater visual acuity. These results are consistent with other recent studies of amphibians, indicating that habitat plays a major role in shaping phenotypic variation in amphibian visual systems.

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### **Retinal Diversity in Non-Caenophidian Snakes**

*David J. Gower, Silke Haverkamp, Bruno F. Simões, Pavel Nemec, Einat Haizman*

There has been much debate about the ecological nature of the origin and early evolutionary history of snakes. The unusual nature of various aspects of the eyes of snakes has been cited as evidence to support competing hypotheses about snake origins, from aquatic, terrestrial burrowing and/or nocturnal ancestors. Increasingly strong molecular evidence for scolecophidian paraphyly has recently prompted the hypothesis that the ancestral snake was scolecophidian-like, with small, reduced eyes adapted for fossoriality. This hypothesis has been challenged by the few morphological and molecular genetic studies carried out on scolecophidians thus far, which instead suggest that their reduced visual systems are too derived to serve as accurate models for the eye of the most-recent common ancestor of all living snakes. However, few techniques have been applied to this topic and relevant taxon sampling has been near-minimal thus far. We present new anatomical, gene-expression and molecular genetic data for the eyes of scolecophidians and other non-caenophidian snakes. Our new transcriptomic and visual-opsin immunolabelling data provide evidence of notable visual-system diversity among these mostly burrowing snakes, including surprising patterns of visual-opsin gene co-expression. The new evidence indicates that approaches applied by previous studies gave a false impression of the level of visual-system reduction in some lineages. Our results are preliminary, there are technical challenges, and sampling remains very sparse, but one interpretation of some of the new data is that dedicated fossoriality in the ancestral snake is not so implausible.

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### **Effect of Female Removal on Offspring Survival in a Glass Frog: New Methods, New Terms, New Insights**

*Johana Goyes Vallejos, Jose Sandoval Siles, Viky Calero, Noemi Rodriguez*

In the glass frog family (Centrolenidae), several species exhibit egg attendance as a form of parental care. Experimental evidence has been obtained for a small subset of glass frogs, including a handful of species with “first-night” care, where removing the parent decreased offspring survival. Because first-night care lasts only a few hours, scarcely any studies have explored its

function. In the glass frog, *Espadarana prosoblepon*, females remain with the clutch for 45–85 min after oviposition. Our study combined female-removal experiments in the wild and semi-captivity to examine whether female presence influences hatching success. To measure if females provide hydration to the clutch, we used a novel method to estimate the gelatinous casings' thickness, calculating the nearest neighbor distance (NND) between eggs using the image processing software Fiji. We hypothesized that greater NND values in control clutches suggest that females provide hydration to their offspring. Removal experiments in the wild showed that female presence does not influence hatching success, with removal and control treatments suffering comparable levels of embryonic mortality. We did not find differences in NND values between both treatments. Our results suggest that this post-oviposition behavior might not constitute parental care. We coined the term "post-oviposition quiescence" to describe the inactivity period observed in females after egg-laying. Ultimately, we aim to challenge the status quo in which adult presence equals parental care by describing natural history aspects of *E. prosoblepon* that have not been studied in-depth and assumed to be like the rest of the members of Centrolenidae.

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### Impact of Roundup on Chemoreception in Rough Shiners (*Notropis baileyi*)

*Daniel Gragson, Jenna Crovo, Carol Johnston*

Roundup is the most widely used herbicide in the United States. There are approximately 280 million pounds of glyphosate applied to 298 million acres annually for weed abatement across the country. Despite its widespread use, few studies have investigated the deleterious effects of Roundup on the chemoreception of wild type freshwater fishes. Using the Rough Shiner (*Notropis baileyi*) as a model, we exposed groups to ecologically relevant levels of Roundup (3.0 mg/L), pure glyphosate (3.0 mg/L), the active ingredient in Roundup, and a control (0.0 mg/L) for 96 hours. We measured fish movement patterns in response to a conspecific alarm pheromone (CAS) added to a behavioral test arena. We also collected waterborne cortisol samples from fish before and after CAS exposure. We hypothesized fish exposed to Roundup and glyphosate would have a diminished alarm response and lower cortisol levels relative to the control group. While fishes exposed to glyphosate did not vary significantly from the control, fish in the Roundup group moved less and exhibited fewer schooling behaviors relative to the control. This study suggests that the inactive ingredients of Roundup, such as surfactants, result in more damage to chemoreception rather than pure glyphosate in Rough Shiners. Future studies should utilize the complete herbicide in ecotoxicology projects to fully understand the risks associated with agrochemical use.

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### Analysis of movement patterns of mobile predators in Sarasota Bay in response to Florida Red Tide

*Jasmin Graham, Breanna Degroot, Jayne Gardiner, Ryan Schloesser, Krystan A. Wilkinson, Kim Bassos Hull*

Harmful Algal Blooms are the result of colonies of algae growing out of control. These colonies can produce toxins, decrease dissolved oxygen concentrations, and have other effects on marine ecosystems. Florida Red Tide is a harmful algal bloom caused by *K. brevis* and has become an increasing environmental concern for the southern Gulf Coast of Florida. Though these blooms have occurred throughout recorded history, they are becoming more common and lasting longer than before. The blooms have resulted in massive fish kills, but not much research has been done to understand how animals, especially mobile predators respond to red tide. This study was conducted to understand if, where, and how these predators are finding refuge in Sarasota Bay during red tide events. From 2016 to 2021 the movements of 87 individual animals throughout Sarasota Bay were monitored using a network of acoustic receivers during a series of Florida Red Tide events. Several species were included in this study including *C. leucas* (n=7), *S. mokarran* (n=4), *A. narinari* (n=39), *C. limbatus* (n=6), and *C. undecimalis* (n=31). Each of these animals experienced at least one Florida Red Tide event and six of them encountered multiple red tide events. Using network analysis to compare space-use for each species across various concentrations of *K. brevis*, we found that although there is evidence of avoidance and changes in movement patterns

for each species in response to red tide, these responses vary between species. With this information we were able to model species-specific responses to red tide.

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### An adaptive management approach to amphibian reintroductions in Bd positive world

*Brian Gratwicke, Blake Klocke, Roberto Ibanez*

More than 60 of 100 Harlequin frogs in the genus *Atelopus* are critically endangered and more than 40 of these are extinct or possibly extinct. Despite more than a decade of effort and research to develop tools to experimentally mitigate the disease *Batrachochytrium dendrobatidis*, we do not have a silver bullet to mitigate the primary threat to these animals in nature. However, captive breeding efforts have been established for many species leading to the availability of surplus-bred animals for reintroduction purposes. We conducted 3 release trials with *Atelopus varius* and *Atelopus limosus* in Panama with intensive post-release monitoring to understand their transition from captivity to the wild. We found that the frogs were very difficult to recapture unless they were fitted with radiotransmitters. 30-day survival probability was just 27% for the hard-released animals, but improved to 46% for animals that were held for one month in a mesocosm prior to release (soft release). Frogs experienced very high levels of predation mortality likely because they lacked toxic skin defenses, and they did not regain their skin toxins after 79 days in mesocosms, but the number of potential tetrodotoxin-producing taxa in their skin microbiome did increase. Our findings show that many of the problems associated with release trials are non-Bd related, and that refining post-release monitoring methods and restoring skin defenses are necessary steps. We recommend continuing release trials of captive-bred frogs with post-release monitoring methods, using an adaptive-management framework to advance the emerging field of amphibian reintroduction ecology.

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### An Individual-Based Model of Basking Sharks in Ireland

*Chelsea Gray, Dale Rothman, Erin Peters-Burton, Cynthia Smith, Chris Parsons, Alexis Garrettson*

While basking sharks are largely solitary, they gather in mixed sex aggregations, which can range from two sharks, up to 1,398 individuals. Aggregations may be related to food availability, though some observational evidence suggests feeding is not the sole reason for this behavior. As basking sharks feed on zooplankton at the surface of coastal water, these aggregations can pose a threat to boaters and the sharks themselves.

An individual-based model of basking sharks in Malin, Ireland, was created in Netlogo. The IBM incorporates zooplankton data from the Continuous Plankton Recorder and localized patchiness of zooplankton on a small scale was simulated. Tests of different shark behavior (only seeking food, only seeking other sharks, a combination of both behaviors) were simulated for 1982-2018. The frequency of shark aggregations and average monthly size of aggregations in the model were compared to sightings data collected by the Irish Whale and Dolphin Group (IWDG) and Irish Basking Shark Group (IBSG).

Simulated aggregations were significantly more likely to match observations collected by the IWDG/IBSG when both seeking food, but also, in select cases, when seeking other sharks. When seen in conjunction with other research from Ireland and other areas, this indicates that aggregations may be significant for reproduction, but are also significant for food intake. These findings agree with previous research and indicate the need for protective measures in these areas when basking sharks are likely to congregate. This is the first research to utilize such methods for basking sharks and demonstrates the usefulness of such methods for understanding the behavior of semi-solitary, migratory shark species such as these.



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## Osmotic Trends and Reproductive Condition of Mature Female Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*)

Emily R. Gray, Stacey Leonatti Wilkinson, Mary Cate Miller, Will Wilkinson, Carissa Adams, Sarah Ebert, Casey Hitchens, John Holloway, Shane M. Welch, Jayme L. Waldron

Eastern diamondback rattlesnakes (*Crotalus adamanteus*; EDBs) are imperiled, heavy-bodied pit vipers endemic to the southeastern Coastal Plain. Reproduction is costly for EDBs; they are slow to mature, reproduce infrequently, and may delay reproduction in the absence of adequate resources. Parturition is often associated with dramatic reductions in body condition and survival probability, yet we have a limited understanding of the physiological trade-offs associated with their reproductive biology. Viviparity tends to be associated with increased hydric demands, as mothers must continuously provide water to developing progeny. Thus, the goal of this study was to assess reproduction-induced water stress and to determine how female EDBs respond to altered water budgets in situ using data collected from an island-dwelling population in South Carolina. We assessed blood plasma osmolality of mature females ( $n=24$ ) to characterize hydration states and we used ultrasonography to confirm reproductive condition. We compared candidate generalized regression models to explain trends in hydration associated with rainfall, reproductive status (gravid or non-gravid), body size, and fecundity. Our results indicate osmoregulation may prove challenging for snakes despite the humid and rainy conditions of a subtropical climate. For gravid snakes, plasma osmolality was negatively associated with precipitation; gravid females relied on access to precipitation to compensate for maternal water provisioning. In the Southeast, droughts are expected to increase in frequency and duration, particularly in summer, when water demands are at their highest for this demographic. Our results suggest climate change may further destabilize populations through the under-studied threat of emerging water scarcity.

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## Distance, Time and Toads: Uncovering the Shape of Dispersal

David Green, Nathalie Jreidini

Dispersal is commonly depicted as a “dispersal kernel”, a graph of the probability density distribution of dispersive distances calculated for a population. Dispersal kernels are characteristically left-skewed with a tail to the right that may be long or short, thin or fat. This variation in shape has long been assumed to have biological significance, generally to indicate distinct short distance vs. long distance dispersal phenotypes. Yet this remains unproven, largely because elapsed, or lag-, time ( $\delta t$ ) has rarely been considered. We investigated the influence of  $\delta t$  on dispersive distances moved by Fowler's Toads at Long Point, Ontario, Canada. These toads are easily caught, individually identifiable and move neither very far nor very fast. Our dataset, amassed over 20 years, consisted of 21,925 movement distances calculated from 8,104 GPS geo-located captures of 1,713 individuals, with  $\delta t$  between any two captures of the same individual ranging from one to 96 days. We subdivided this dataset to compare dispersal kernels generated according to  $\delta t$ . The distributions displayed progressively lower peaks and longer tails with increasing  $\delta t$ . A series of stochastic functions, with parameters varying as the inverse of  $\delta t$ , described these animals' movement probabilities as a 3D surface with axes distance,  $\delta t$ , and frequency. We conclude that dispersal can be a purely stochastic process and that perceived dispersal kernel shape can be largely the product of sampling regime with respect to  $\delta t$ . Thus, the importance of dispersal kernel shape as a presumed fundamental characteristic of the dispersal process has been overestimated.

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## Are you still there? Assessing the historic and contemporary conservation status of *Ammocrypta clara* (Percidae) in Louisiana river systems

Gaelyn Gros, Kyle Piller

The imperiled Western Sand Darter (Percidae: *Ammocrypta clara*) is a cryptic fish that occupies the sandy benthos of large rivers lining the Mississippi River basin. Throughout its range, modifications to these large rivers have contributed to habitat fragmentation and a population decline for this species,

particularly within the southern portion of its range. In Louisiana, *A. clara* is historically known from the Sabine, Red, and Ouachita River basins, but has not been detected in the state since 2014. Detecting a small fish in a large river is always a challenge, deeming typical sampling methods ineffective in the deep, turbid waters that flow through these three river basins. Therefore, this project had two goals. First, we summarized and mapped the historic distribution and abundance of *A. clara* in Louisiana using available museum records. Second, we used eDNA and beach seining to perform an assessment of the conservation status of the elusive and imperiled Western Sand Darter in Louisiana. Two-hundred beach seine hauls (5/site) were completed at 40 localities in the Sabine, Ouachita, and Red Rivers and no specimens of *A. clara* were collected from the three river basins. Environmental DNA metabarcoding (12S mtDNA) is on-going, and to date, no *A. clara* have been detected at any sites. These results will be presented and discussed in light of the current conservation status of this imperiled species historically occupying Louisiana river systems.

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## From fisheries to climate change, the use of fishery-independent surveys as tools for monitoring changes in shark populations.

R. Dean Grubbs

In 1973, Jack Musick began a fishery-independent longline survey off Virginia, which became among the longest running surveys monitoring shark populations in existence. At the survey's inception, the U.S.-China trade embargo remained and commercial fisheries for sharks in the region were small. The movie *Jaws* and its effects on recreational shark fisheries were yet to come. By the late 1980's, dramatic increases in recreational and commercial shark fisheries predictably led to dramatic declines in coastal shark stocks in U.S. Atlantic waters. The relative baseline data from the VIMS shark survey Jack started proved instrumental in informing the first U.S. Federal Management Plan for Sharks of the Atlantic Ocean published in 1993. Under Jack's guidance, I directed the VIMS longline survey, which is still used in numerous stock assessments, for many years. In 2007, I relocated to the Gulf coast of Florida and developed paired gillnet and longline surveys that may serve as important relative baselines to examine another potential threat to shark populations, climate change, as the Gulf of Mexico has warmed at twice the global average over the past 50 years. The FSUCML surveys include a fixed station component sampled 12 months/year and spatially-balanced randomized component during summer covering >4,000 km<sup>2</sup> of seagrass dominated habitat. Over the survey's 14 year history, sea surface temperature in this region has warmed ~3°C, indicating it is global warming hotspot. Analyses of changes in the distributions, demographics and relative abundances of coastal shark species will be presented.

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## Plastics in our ponds and aquatic habitats: How are microplastics impacting larval amphibians

Madelene Grullon, Scott Kornfeind, Sara McClelland

Microplastics are a common environmental pollutant found in the aquatic habitats of larval amphibians. Previous research has shown that microplastics can damage amphibians' tissues/organs (gills, liver, etc.) and impact their overall development. We don't have much information on how exactly microplastics are affecting amphibians' well being; there are too few studies analyzing very low concentration microplastic exposures, especially in tadpoles. In this study, I aimed to determine how microplastics impact tadpole body, gut, and brain morphology. Northern Leopard Frog Tadpoles (*Lithobates* [*Rana*] *pipiens*) were exposed to either 0.5 ppb polyethylene microplastics (34-50  $\mu$ m diameter) or a water control group in a blind, controlled laboratory experiment. After the conclusion of the experiment, I measured tadpole body morphology, extracted the gut for gut morphology, and then extracted the brain for brain morphological analysis. The results from this work will help us understand how microplastics, common aquatic pollutants, are impacting larval amphibians. By better understanding these impacts there is the potential of this leading to better conservation practices for amphibians.

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## Temperature Fluctuations Under Cover Boards Along a Gradient of Urbanization

*Elmer Gutierrez, Breanna Putman*

As human populations continue to grow, so does the rate of urbanization. Due to urbanization, animal populations may experience shifts in microclimate, which can affect ectotherms since they can be sensitive to abiotic changes. In this study, we observed microclimates among areas that differed in level of urbanization to understand whether abiotic selective pressures potentially differ for small ectotherms living in human-impacted areas. We did this by placing 24 cover boards (pieces of plywood) across a gradient of urbanization in and around the California State University, San Bernardino campus. A series of abiotic data was collected across all sites. Temperature loggers were placed underneath all boards to record the temperature at hourly intervals for a year. We found the temperature underneath the cover boards depended on the site, month, and hour of the day. On average, the temperatures did not differ under the cover boards among the different sites. However, our natural sites experienced greater temperature fluctuations compared to our most-urban site. Temperatures in our natural sites were highest in the summer months and coolest in the winter months. Urban boards experienced an urban buffer where temperatures were milder throughout the year. Although multiple studies have used cover boards to document herpetofauna diversity, not many have investigated the consequences of urban environments on the microclimates used by herpetofauna. Furthermore, these results highlight the changes in microclimate that could impact herpetofauna living in urban environments.

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## Comparing the Movement Dynamics of Three Small-Bodied Fishes in Relation to Culverts

*Langston Haden, Jake Schaefer, Scott Clark*

Connectivity of populations is a common goal of conservationists in order to retain genetic diversity, ample habitat, and healthy ecosystem function. Within rivers, culverts are the most common type of barrier to connectivity and movement. Despite the exceedingly high biodiversity and concurrent imperilment of small-bodied fishes, the current literature on movement ecology is highly biased towards large-bodied migratory species. Given this, the movement dynamics of the vast majority of small-bodied species remain unknown. The overarching goal of this study is to address the existing knowledge gaps by observing and comparing the movement dynamics of several headwater species in relation to potential dispersal barriers such as culverts. To select sites, we conducted standardized barrier assessments at all accessible road-stream crossings across four tributaries to the Red River. Six sites with varying barrier scores were selected to conduct mark-recapture studies. Mark-recapture surveys resulted in the marking of 6,518 fish across 35 species. Herein, the movement dynamics of the three species (*Fundulus olivaceus*, *Lythrurus umbratilis*, and *Luxilus chrysocephalus*) with the highest recapture percentages are reported and compared. The mean recapture percentage was 14.22%, but varied depending on the site and species. We identified differences and similarities in movement dynamics at both the site and species level. Our results align with previous work on small-bodied fishes, but provide novel information on the movement dynamics of three commonly occurring small-bodied species. Additionally, these results emphasize the need to consider biogeographical context when assessing or restoring fragmentation of riverine networks.

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## Behavioral Ecology of Three-Toed Box Turtles (*Terrapene carolina triunguis*) in an Urban Mississippi Park

*Langston Haden, Grady Leek, Haley Kudson, Will Selman*

Understanding the behavioral ecology of a species can be vital to conservation and management decisions in the face of changing landscapes in the future. One region that is undergoing rapid landscape-level changes is the Southeastern United States, an area that is recognized as a turtle biodiversity hotspot in need of conservation. One species, the Three-Toed Box Turtle (*Terrapene carolina triunguis*), is found in the western portion of this hotspot, yet little is known about the species ecology in the state. Our goal was to further understand their behavioral ecology in an urban/forest mosaic

landscape. We radio-tracked 10 individuals (6 ♂, 4 ♀) at Lefleur's Bluff State Park to document their thermal biology, behavior, overwintering, and micro-habitat use. Observations of the 10 individuals occurred from April 2018 to August 2021 with a total of 1,833 observations. Male and female thermal profiles were nearly identical, with individuals actively thermoregulating in the cooler spring/fall months and thermoconforming in the warmer summer months. During the active season (March – December), turtles were mostly observed active on the surface or buried under leaf litter, and there was little difference by sex. The overwintering period averaged 115 days from early December to mid-March. Turtles appeared to be forest generalists, preferring habitats with high canopy, leaf, and herbaceous coverage. Additional information is needed to assess the home ranges, movements, and landscape-level habitat use of individuals in this fragmented urban landscape.

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## Weather-induced Functional Extirpation of a Meadow Population of Endangered Sierra Nevada Yellow-legged Frogs (*Rana sierrae*)

*Brian Halstead, Patrick Kleeman, Jonathan Rose, Robert Grasso*

Climate change is expected to increase precipitation volatility in the Sierra Nevada of California, USA. Indeed, some of the driest and wettest years on record in the Sierra Nevada have occurred in the early 21st Century. This extreme variation in precipitation likely has important effects on the demography of aquatic organisms. We studied the demography of a meadow population of Sierra Nevada Yellow-legged Frogs (*Rana sierrae*) using capture-mark-recapture methods from 2003 to 2022, when extensive surveys resulted in the capture of only two adult frogs. We analyzed the data using Cormack-Jolly-Seber and reverse-time models to evaluate the influence of winter severity and drought on apparent survival and recruitment, respectively. Both survival and recruitment were affected by precipitation, and two extreme, multi-year droughts reduced survival and resulted in recruitment failure leading to functional extirpation of this population in 2022. Because the study population is isolated, interventions like captive rearing and repatriation (already underway), increasing the hydroperiod of the meadow, and establishing populations in nearby locations to support a functioning meta-population would likely increase the resilience of meadow populations of Sierra Nevada Yellow-legged Frogs to climate change.

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## Variation in Behavior and Physiology of Red-Backed Salamander Populations Across an Elevation Gradient

*Marlena Hamilton, Matthew Lattanzio*

According to the IUCN Red List, 43% of amphibians are threatened with extinction, and climate warming is one of the primary causes (<https://www.iucnredlist.org/>). Thus, understanding how these species may respond to warming conditions is imperative to informing future management and conservation plans. Species- and family-level studies have revealed potential shifts in body size associated with use of geographically (and thus climatically) distinct habitats as well as over time in relation to recent climate change. However, consideration of traits other than body size, and the extent that any responses to warming may differ among distinct populations, are lacking. In this study we capitalize on replicate low and high elevation populations of Eastern Red-Backed Salamanders (*Plethodon cinereus*) to assess the extent that surface activity and locomotor performance (burst speed), two traits associated with susceptibility to climate change, vary in relation to temperature. We also assess temperature preference of our populations to determine whether thermal preference is conserved in *P. cinereus* (a common assumption in climate studies). Currently, we are nearing completion of data collection for our low-elevation populations and have begun collection of data for high-elevation populations. Based on our findings to-date, we expect low elevation salamanders to prefer higher body temperatures than salamanders from high elevations due to the warmer temperatures they experience throughout the year. We also expect low elevation salamanders to remain surface active and exhibit faster burst speeds at higher experimental temperatures. Overall, our findings should be useful for improving models predicting species responses to future climate changes.

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## Revision on taxonomic, zoogeographical, and evolutionary systematics of the genus *Johnius* (Perciformes; Sciaenidae) in the South China Sea.

Norhafiz Hanafi

This study revised and validated the taxonomy, zoogeography, and evolution of the genus *Johnius* in the South China Sea (SCS) basin. Fourteen species of *Johnius* were recognized as valid species encountered in this study. This study also provides the dichotomous key for the species of *Johnius* in the South China Sea. A zoogeographical inference of *Johnius* was proposed with widely distributed (3 species), northern (4 species), and southern (7 species) species found dispersal in this semi-basin, with specific segregation identified across the Ekman and other current barriers. It is likely been facilitated by juveniles' capacity for long-distance dispersal, which enabled them to colonize coastal regions, speciated, and be confined in the north and south SCS. *Johnius* relationships were inferred by combining concatenate sequence data acquired from the three mitochondrial and two nuclear markers genes from each species in the SCS. The phylogeny implies the ancestral *Johnius* likely inhabit SCS, then the subsequent expansion into two pathways: divergence to northern SCS and southern SCS with high diversification. Thus, due to lineages divergences within *Johnius* going speciation during the Pliocene-Pleistocene, especially the cyclical glaciations.

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## Drivers of Invasive Tegu Diet Composition in Two Florida Populations

Madison Harman, Nicole Fuller, Benjamin Baiser, Andrea Currylow, Amy Yackel Adams, Bryan Falk, Christina Romagosa

Invasive species are a widely recognized threat to natural ecosystems, particularly in Florida, where introductions are numerous and frequent. Invasive species may negatively impact native species through predation, competition, or the spread of disease, and it is important to quantify these impacts in order to properly assess risk and allocate management efforts. Argentine giant tegus (*Salvator merianae*) are likely threats via all three of these mechanisms. Here we focus on predation by comparing diet composition of two tegu populations in varying stages of invasion: Miami-Dade County (long-term management) and Charlotte County (containment). We identified gastrointestinal contents from 275 tegus caught between 2016 and 2021. We then used a distance-based redundancy analysis to explore potential drivers of diet composition. We found that habitat, month, tegu size, and sex were all significant predictors, with habitat being the most important. Diet items included chordates, invertebrates, and fruits across 12 classes, 53 orders, and 97 families. Our findings corroborate the generalist nature of tegus through their breadth of prey taxa and ability to survive in both natural and disturbed habitats. This flexibility likely increases the difficulty of extirpation and therefore supports the need for swift removal efforts in response to new introductions.

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## Spatial Analysis of Invasive Pentastomes Infecting Two Populations of Invasive Tegus in Florida

Madison Harman, Jason Blackburn, Melissa Miller, Benjamin Baiser, Andrea Currylow, Amy Yackel Adams, Bryan Falk, Christina Romagosa

Invasion ecology often focuses on the direct effects of invasive species through predation or competition with natives. However, with the rise of One Health initiatives, invaders' influence on native pathogen and parasite systems are receiving more attention. Disruptions to these systems can stem from the co-introduction of non-native parasites that spill over into native hosts, or from invaders serving as reservoirs, amplifiers, or dead-end hosts of existing parasites. In this study, we investigate an interesting combination of spillover and spillback in Florida, USA, where invasive Argentine giant tegus (*Salvator merianae*) from South America are acting as definitive hosts for invasive snake pentastomes (*Raillietiella orientalis*) from Southeast Asia. We analyzed spatial patterns of infection using Kernel Density Estimation and Getis-Ord statistics. We also examined potential drivers of infection intensity using a zero-inflated negative binomial model. We found that pentastome prevalence in Charlotte County (41%) was more than double that in Miami-Dade County (17%), despite being further from the pentastomes' introduction site in Everglades National Park. Infection intensity was significantly associated with habitat, month, and

host sex. Clusters of high infection intensities within the landscape did not overlap with clusters of high tegu captures and supported the importance of habitat type. These findings take us closer to unraveling the transmission cycle of invasive pentastomes in novel hosts and may prove useful in evaluating risk as *R. orientalis* continues to expand its range northward into new areas.

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## Effects of aquaculture-based habitat modification on elasmobranch abundance and predation intensity in a large coastal lagoon

Rose Harman, Paul Bourdeau, Jose Marin Jarrin

Habitat modification from shellfish cultivation has large effects on local communities, increasing the abundance of small benthic invertebrates and fishes. Elasmobranchs (i.e., sharks and rays) that use nearshore habitat to forage and provide top-down control of ecosystems could be negatively affected by the structural complexity of aquaculture, restricting their hunting, and altering their movements. Humboldt Bay aquaculture yields ~70% of California's oysters, but the bay also provides critical habitat for elasmobranchs. We used baited remote underwater video systems (BRUVS) to investigate whether the presence of oyster aquaculture affected the abundance of elasmobranchs, crabs, and bony fishes in two different habitats (mudflat and eelgrass). We found interactive effects of habitat and aquaculture presence on elasmobranch abundance. Two species of sharks and one species of ray were observed including the leopard shark (*Triakis semifasciata*), broadnose sevengill shark (*Notorynchus cepedianus*), and the bat ray (*Myliobatis californica*). Total shark and ray abundance was higher in mudflats with aquaculture compared to mudflats without aquaculture, and lower in eelgrass with aquaculture compared to eelgrass without aquaculture. Abiotic variables (pH, dissolved oxygen, temperature, turbidity) did not vary among habitats, suggesting that the effects of aquaculture on elasmobranch abundance were due to its physical structure and associated human disturbance. These interactive effects did not affect bony fish or crab abundance, indicating that they are more important for higher trophic level predators. Our results suggest that the effects of aquaculture-based habitat modification on top-down control of community structure and dynamics will depend on the type of habitat being modified.

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## Percidae's Picky Eaters: Phylogenetic analyses of darter diet reveal complex patterns of trophic niche evolution.

Richard Harrington, Thomas Near

Darters (Percidae) represent one of the most diverse groups of freshwater fishes of North America, with approximately 250 mostly stream-dwelling species. Aspects of darter ecology are most often studied on a species-by-species basis, and there are no comprehensive analyses that characterize the evolutionary patterns of the entire clade's trophic niche. We have gathered gut content data for more than 100 species of darters. This broad sampling of darter diet data allows a better understanding of the relationship of darter species with the diversity of benthic prey items available in freshwater habitats, including microcrustaceans, aquatic insect larvae, snails, and even fish eggs. By combining these data with a time-calibrated phylogeny and measurements of darter jaw morphology, we describe unique patterns of darter subclade specialization on important axes of prey ecology.

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## Population Genomics of the Common Kingsnake Complex

Sean Harrington, Hannah Van Tassel, Frank Burbrink

The common kingsnake (*Lampropeltis getula*) species complex is widespread through North America, ranging from the Atlantic to Pacific coast across most of the southern US and northern Mexico. We previously demonstrated that the complex is composed of three distinct lineages with boundaries at the great plains ecotone and the Cochise filter barrier. These lineages have gone through multiple rounds of isolation and secondary contact, and genes asymmetrically migrate among lineages. To identify how lineages maintain their identities in the face of recurrent and ongoing introgression, we sequenced whole genomes from 41 individuals across the range. We examined patterns of genomic differentiation within lineages and across pairs of lineages spanning each boundary to identify genomic regions with higher than expected

divergence. These correspond to regions that resist migration across boundaries, and are good candidates for barrier loci. Barrier loci may resist movement among populations due to local adaptation to differential environments or as a result of accumulated genomic incompatibilities at these regions during periods of allopatry. Our next step is to annotate these regions to identify the putative function of loci to investigate potential links between gene function and local adaptation.

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## The Influence of Soil Moisture on the Color Morph of the Eastern Red-backed Salamander, *Plethodon cinereus*

Rachael Harris, Katie Duryea

This project aims to determine whether or not soil moisture influences the presence of different color morphs of the Eastern Red-backed Salamander (*Plethodon cinereus*). Soil temperature and moisture are two variables that comprise a thermal niche. Prior research suggests that different color morphs of this salamander have been known to be found at different substrate temperatures, however, this finding may be dependent on the location and season of sampling. Measuring the soil moisture and conducting a count survey on these salamanders will gather additional data that can be examined to determine if there is a correlation between the different color morphs and different soil moisture levels or thermal niches. Four plots were established in the Southern New Hampshire University Arboretum, each consisting of 12 coverboards to allow for count surveying to take place. Count surveying and soil sampling were conducted each week for 9 weeks. This study will provide data to support the idea that certain color morphs of this salamander have become more dominant in certain thermal niches.

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## Ontogenetic and Pathogen Drivers of Skin microbiome Composition in a Facultatively Paedomorphic Salamander

Arik Hartmann, Zuania Colón-Piñeiro, Sarah Mcgrath-Blaser, Ana Longo

Amphibians rely on microbial symbioses to maintain physiological processes, such as those related to osmotic and immunological function of the skin. Several studies have shown that biotic and abiotic processes influence the composition of the amphibian skin microbiome. However, it is still unclear how associated bacterial communities vary across species with complex developmental pathways, including those in which the microhabitat and behavior changes through ontogeny. This is particularly relevant for species that undergo metamorphosis as it has been shown that host physiology and ecology drastically influence diversity of the skin microbiome. Our objective was to characterize skin bacterial communities of striped newts (*Notophthalmus perstriatus*) across ontogenetic shifts, and under natural infections of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) and Ranavirus (Rv), two pathogens responsible for amphibian declines. Using 16S RNA sequencing of the V4 region of the bacterial genome, we compared alpha and beta diversity metrics across five ontogenetic categories: (larva, paedomorph, metamorphosing paedomorph, adult, and eft), and among four infection types (Bd, Rv, Coinfected, Uninfected) using Pairwise PERMANOVAs and differential abundances of microbes using ANCOMB. We found that, although most stages share the same aquatic habitat, the skin bacterial communities of the striped newt are primarily shaped by the developmental stage of the hos. Infection status was associated with differential abundances of bacterial taxa, including some with antimicrobial properties. Overall, our work shows that metamorphosis is a pivotal process that shapes skin bacterial assembly in a facultatively paedomorphic species, and that pathogen infection may alter microbiota composition abundances.

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## What to do at the Zoo: The Use of Captive Tortoise Populations in Physiological Research

Alyssa Hartzheim, Jennifer Terry, Sinlan Poo, Lorin Neuman-Lee

Commonly considered a place of entertainment, zoos and other like institutions that care for living collections often have significant research potential. Not only do these institutions house animals as common as raccoons to as rare as scimitar oryx, a species extinct in the wild, they offer a more controlled environment to observe physiological processes. Further, many

captive collections contain species that have yet to be the subject of intense research. For example, the innate immune abilities of two species of tortoise (the Indian star tortoise (*Geochelone elegans*) and northern spider tortoise (*Ptyxis arachnoides brygooi*) are completely unknown. Reptilian species are not traditionally viewed as immunological models, yet they offer a unique perspective. In collaboration with the Memphis Zoo, we tracked immune ability of these two tortoise species over 18 months using minimally invasive blood samples. Bacterial killing assays using *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* were optimized for each tortoise species and completed monthly. Each selected bacterium distinctively activates and interacts with immune mechanisms, allowing for different aspects of innate immunity to be examined, forming a more well-rounding view of the immune response. Immune ability of both *G. elegans* and *P. a. brygooi* against *E. coli* varied among months. Further, immune ability of both tortoise species differed depending upon which bacteria was used. Ultimately, this study showcases the mutual benefit of zoos to physiological research through cross-institutional collaborations, providing invaluable knowledge to both research and animal care staff alike.

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## Resurrection and Redescription of Nominal Species Previously Regarded as Synonyms of *Thrissina mystax* (Bloch & Schneider, 1801) (Teleostei: Clupeiformes: Engraulidae)

Harutaka Hata, Sébastien Lavoué

Within the genus *Thrissina* (Clupeiformes: Engraulidae), species with a long maxilla (posterior tip beyond the pectoral-fin insertion), 13–17 lower gill rakers on first gill arch, snout tip at the level of eye center, and anal fin with 29–37 branched rays, have been regarded as synonyms of *Thrissina mystax* (Bloch & Schneider, 1801), a species widely distributed in the Indo-West Pacific region. However, morphological examination and genetic data (from mitochondrial cytochrome b and COI genes) of specimens of *Thrissina mystax* obtained throughout its distribution, revealed that this species comprises at least three species. Additionally, examination of available type specimens, original descriptions, as well as many non-type specimens collected over extensive geographic ranges confirm the applicable names for these three species as: true *Thrissina mystax* [distributed along the coast of India and Sri Lanka; total gill rakers on first gill arch (1TGR): 25–30; pre-dorsal-fin length (PDL) <53% of SL; snout 4.1–4.8% of SL; second supra maxilla (2SMX) 5.3–6.3% of SL], *Thrissina mystacoides* (Bleeker, 1852) [Southeast Asia, from the eastern coast of Malay Peninsula to the Gulf of Thailand and Sarawak; 1TGR: 21–26; PDL > 53% of SL; snout 3.8–4.2% of SL; 2SMX 5.9–6.8% of SL], and *Thrissina valenciennesi* (Bleeker, 1866) [southern Indonesia; 1TGR 26–32; PDL > 53% of SL; snout 3.8–4.2% of SL; 2SMX 4.8–5.7% of SL].

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## Juvenile Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*) Relative Abundance and Body Condition in Two South Carolina Estuaries Varying in Urbanization

Rileigh Hawk, Daniel Abel

Urbanization near estuaries has been shown to affect the growth and survival of juvenile sharks using the system as a nursery. North Inlet and Murrells Inlet, South Carolina, are similarly-sized, tidally-dominated, bar-built estuaries with extensive Sporobolus-lined tidal creeks, but differ in degree of human impact. We previously found that Murrells Inlet had a significantly lower abundance and diversity of large sharks than North Inlet and that Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) use North Inlet as a primary nursery. Fifty-two neonate *R. terraenovae* were captured on hook-and-line gear from May to September 2022. Sharks were measured for length and girth, weighed, sexed, and released. Noise pollution between the two estuaries was investigated using hydrophone recordings. Relative abundance of juvenile *R. terraenovae* was much greater for North Inlet (n=45) than for Murrells Inlet (n=7). However, body condition, weight-length relationships, girth-length relationships, and growth rates of the juvenile sharks did not differ between the estuaries. Sound analyses found no difference in the total loudness of the recordings between estuaries or the sound power of the recordings for shark hearing frequency ranges (p=0.57, p=0.45, respectively). Besides juvenile *R. terraenovae* abundance, the only measured differences between the two



estuaries were water depth for sampling and boat traffic while sampling (both  $p < 0.001$ ). There was no correlation between the sound recordings and depth or boat traffic. Although the difference in urbanization between estuaries did not affect the growth and body condition of *R. terraenovae*, the drivers behind the difference in abundance of neonates are still unclear.

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### Effects of whole-tissue invasive plant exposure (*Melia azedarach*) to native tadpoles

Noah Hawkins, Chris Distel

Amphibians are experiencing drastic global declines, yet the causes of many declines remain enigmatic. We evaluated the toxicity of a globally invasive plant (*Melia azedarach*), which is known to be lethal to diverse invertebrates, on tadpoles native to central Texas, USA (*Lithobates berlandieri* and *L. catesbeianus*) using environmentally realistic whole-tissue exposures. Replicated experiments were conducted in both lab and mesocosm venues. Exposure caused negative effects on tadpoles, but those effects were dependent on community structure and experimental venue. Future studies should evaluate more complex environmental scenarios and the toxicity of additional invasive plant species.

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### Insights from 6 years of Southern Stingray, *Hypanus americanus*, captures in Bimini, The Bahamas

Elise Hayden, Vital Heim, Matthew Smukall

The Southern stingray (*Hypanus americanus*) is a species of Dasyatidae ray native to the Western Atlantic Ocean from the North-Eastern coast of the United States to the Northern coast of Brazil and the Caribbean. Southern stingrays are mesopredators and have been captured year-round in shallow waters surrounding the Bimini Islands, The Bahamas, at the western edge of the Great Bahama Bank. Here, we analyzed data from 483 captures of 301 unique individuals from the Bimini Islands between 2015 - 2020 to advance our understanding of population structure and size distributions with regard to sex and maturity. We also took morphometric measurements and calculated life history parameters such as growth rate and length-width relationships. Our sample population included mostly sexually mature females, which made up more than 70% of all captures. The average disc width of females was  $81.33 \pm 15.77$  cm and the average disc width of males was  $50.36 \pm 4.95$  cm. The average growth rate (cm/year) in this sample population was  $5.02 \pm 6.59$  for females and  $3.28 \pm 2.38$  for males. Our study documents the population structure as well as the repeated residency of Southern stingrays in nearshore habitats around the Bimini Islands. As such, our results can be used as a foundation to build upon for further studies with this species in the Bahamas and as a means to compare between populations. Additionally, we hope it will contribute to future conservation efforts and habitat protection.

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### Combining Skin Microbiome and Immune Metrics to Assess Snake Fungal Disease Risk in Arkansas Snakes

Natalie Haydt, Lorin Neuman-Lee

Snake fungal disease (SFD) is caused by the fungus *Ophidiomyces ophiodiicola* (Oo) and is afflicting various snake populations in North America. On infected individuals, SFD displays as skin lesions and can lead to systemic infections. Declines in a handful of snake populations, especially small, isolated populations, have been associated with SFD. In states where little sampling for SFD has occurred, documenting if and where snakes are infected can help elucidate whether certain habitats or species are susceptible. Infectivity of Oo and susceptibility of snake hosts is likely impacted by the microbial community on snake skin. By analyzing skin microbiota for both Oo and associated microbes in conjunction with quantifying immune function, we can determine to what extent skin microbes play a role in SFD susceptibility. To this end, we swabbed and collected blood from snakes in Northeast Arkansas. Extracted DNA samples from skin swabs were sequenced to detect both bacterial and fungal operational taxonomic units. To quantify SFD susceptibility, we combined Oo detection and observed symptom severity with measures of immune response (bacterial killing ability and leukocyte population quantification). We then analyzed the influence of skin microbiota, species, and site

(and their interactions) on SFD susceptibility. This research provides insight into which species and habitats might be more at risk for SFD and/or population declines related to SFD and can be used to help prioritize management decisions.

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### Should I stay or should I go: differential residency of great hammerheads, *Sphyrna mokarran*, from Andros and Bimini, Bahamas

Vital Heim, Tristan L. Guttridge, Philip Matich, Matthew J. Smukall, Annie E. Guttridge, Simon Dedman

Understanding movement dynamics of highly migratory species is crucial to address conservation challenges. Great hammerheads, *Sphyrna mokarran*, travel long-distances and have suffered population declines due to targeted and incidental fishing pressure. Limited data are available describing their movements in the Northwestern Atlantic, but previous results showed temperature-driven seasonal movements towards lower latitudes in winter, with some sharks displaying seasonal residency to local areas in The Bahamas during colder months. Here, we compared the seasonal movements of 8 great hammerheads tagged around the islands of Bimini ( $n = 4$ , 3 females, 1 male) and Andros ( $n = 4$ , 3 females, 1 male). We used fin-mounted Smart Position and Temperature (SPOT) tags with dynamic Brownian bridge movement models to quantify differences in residency as well as movements between The Bahamas and the USA. The sharks tagged around Bimini emigrated north into the US Atlantic and the Gulf of Mexico after winter – agreeing with previous results – and three of four sharks tagged around Andros showed year-round residency to Bahamian waters with resident sharks presenting a broader thermal tolerance during the summer months. Our results reveal group-specific differences in space use of great hammerheads sharing neighboring islands with similar habitats, with one group displaying permanent residency within the Bahamian EEZ, a designated shark sanctuary. Thus, residential sharks from Andros are protected year-round, whereas the sharks tagged around Bimini face greater risks from fishery interactions in US waters, highlighting the need for improved conservation management strategies during their summer movements.

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### A Phylogeny of the Gekko group Based on Long Exon Data.

Matthew Heinicke, Juan Daza, Cristian Hernandez Morales, Maria Diago-Toro

We generated sequence capture data for 675000 bp of long exons for 100 specimens comprising a representative sample of Gekko and related genera. Included in our data set are eight species in which data were generated from archival formalin-fixed museum specimens. Phylogenetic analyses were performed to identify the relationships of these taxa and assess interspecific divergences that were not fully resolved in published data sets. Our results support some novel relationships between known subgenera or lineages. For example, *Japonigekko* recovered as more closely related to the clade that includes parachuting geckos (*Ptychozoon*) than they are to the subgenus *Gekko*, previously thought to be their closest relatives. *Luperosaurus palawanensis* is recovered as yet another independently evolved lineage of “*Luperosaurus*”. The phylogeny was used to reconstruct ancestral states of external and osteological morphological characteristics. Results of these analyses suggest that the common ancestor of the Gekko group had a generalized morphology, and traits associated with parachuting, including webbing and skin folds, have evolved at least six times across the Gekko group.

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### Conservation Genomics of the Endangered Pallid Sturgeon

Edward Heist, Richard Flamio Jr., Junman Huang

Pallid Sturgeon (*Scaphirhynchus albus*) is a long-lived riverine fish that is endangered due to habitat loss, overharvest, and hybridization with the closely related Shovelnose Sturgeon (*Scaphirhynchus platyrhynchus*). The species is native to the Missouri/Mississippi River drainages from Montana to Louisiana. In the north, there has been no natural recruitment since a series of dams and reservoirs destroyed much of their habitat. In the south, nearly all fish with Pallid Sturgeon-like morphologies are hybrids, including backcrosses and higher order hybrids. There is limited natural recruitment of genetically pure Pallid Sturgeon in the middle part of the range but also considerable

hybridization. Pallid and Shovelnose Sturgeon are genetically very similar making it challenging to distinguish pure Pallid Sturgeon from hybrids and advanced backcrosses. Hatchery supplementation occurs in parts of the range, and hybrids have inadvertently been used as broodstock in the past. We are developing a panel of single nucleotide polymorphism (SNP) markers for species identification and estimation of population structure and effective population size. The polyploid ancestry makes development of disomic markers challenging in sturgeon. Therefore, we produced haploid Shovelnose Sturgeon and performed reduced-representation DNA sequencing to identify thousands of disomic SNP loci that are much more powerful for discriminating the species than the 19 microsatellite loci currently in use. Currently, we are developing a subset of the SNPs into a genotyping in thousands (GTseq) panel that will allow us to efficiently score hundreds of disomic SNP markers for improved conservation and management of this unique long-lived riverine piscivore.

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### Investigating the Potential for Morphological Change in Invasive Snakes

*Kodiak Hengstebeck, Christina Romagosa*

Biological invasions can help elucidate the adaptive capability of species in the face of novel selective pressures, as invasive species are often forced to establish and spread in biogeographic regions that they did not evolve in. These circumstances provide considerable potential for adaptive or plastic responses to occur over relatively short evolutionary timespans. One such species that has been introduced thousands of kilometers away from its native range is the Burmese python (*Python bivittatus*) in southern Florida. Since their introduction, pythons have been implicated in the severe declines of many native mammal populations throughout the Florida Everglades, which have in turn resulted in changes to the python's diet composition in the core of their introduced range. Changes in diet can result in changes to morphology. Skull morphology, in particular, is often under strong feeding-associated selective pressure due to its many ecological functions and influence on an organism's fitness. In this study, we propose to assess changes to the skull morphology of Burmese pythons across their introduced range as a result of temporal shifts in prey species composition. We plan to use a dataset of >200 python skulls, collected through inter-agency and university efforts from 1995 to 2022, combined with nano-CT technology and geometric morphometric analyses to investigate these changes.

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### The Management of *Typha domingensis* and its Effects on Amphibian Assemblages Within Palo Verde National Park, Costa Rica

*Dakotah Henn, Christopher Murray*

Understanding the presence of invasive species within various ecosystems has long been of ecological importance. Particularly, plant invaders found within wetland systems are often successful and play a role in habitat change. These species often have characteristics contributing to habitat alteration and overall decrease in habitat heterogeneity. In the wetlands of Guanacaste, Costa Rica, the invasive cattail *Typha domingensis* has become well established. These established monocultures have altered the local wetland habitat and previous studies have shown that *Typha* has a negative effect on migratory birds by decreasing available habitat. However, other taxonomic groups, such as macroinvertebrates, exhibit positive correlation in species richness and abundance with *Typha* presence. The effects of *Typha* on amphibians, however, has yet to be evaluated within this system, and previous studies have found marked variation in the effects of invasive plants on amphibian assemblages. Here, I elucidate the effects of *Typha domingensis* on the amphibian assemblage present within the wetland at Palo Verde National Park, Costa Rica in contrast to mechanically managed areas of the invasive plant. Results indicate that the presence of *Typha* has no effect on species richness and abundance of adult and recently metamorphic amphibians. Larval amphibians, however, are absent in *Typha* dominated habitat. These data also suggests evidence of competitive exclusion among species across transects in both habitat types for adult and larval amphibians, however no evidence of this

phenomenon within the recent metamorph age class. Additionally, richness and abundance correlates with cumulative precipitation in both unmanaged and mechanically managed habitats.

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### Venom Complexity of Garter Snakes (*Thamnophis*) and the Role of Diet in Toxin Diversification

*Tucker Heptinstall, Ramses Rosales-Garcia, Rhett Rautsaw, Christopher Parkinson*

Numerous studies have been conducted on snake species with medically relevant venoms to better understand venom complexity, diversity of proteins, and evolution of toxicity. However, front-fanged species only account for approximately 15% of known snake species. Recently, studies have discovered toxins in many species considered non-venomous, like garter snakes. Garter snakes (*Thamnophis*) are a relatively diverse (35 recognized species), widespread, and common genus found throughout North America. This genus has been used as a model in many ecological and evolutionary studies. However, one area of garter snake natural history that has not been investigated is their "venome". To explore venom evolution in these non-front fanged snakes, we performed assembly and analysis of the Duvernoy's gland (venom gland) transcriptome from 10 species of the genus *Thamnophis*. We find variation in toxin gene presence and expression, and total toxin expression, suggesting both inter and intra-species venom diversity. We hypothesize phylogenetic dietary breadth is the primary driver of this venom variation, and we are performing a meta-analysis to assemble a large-scale database of *Thamnophis* dietary records. Future directions of this project will address our hypothesis by comparing phylogenetic dietary breadth to venom complexity. This research will help uncover the selection pressures facilitating the evolution of venom and understand how ecology can shape trait evolution. Our results provide the most complete venom characterization of *Thamnophis* to date, and a large, centralized database of dietary records. This will link *Thamnophis* evolution and ecology and significantly increase our knowledge of venom throughout the Serpentes suborder.

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### Morphological Disparity as a Function of Incubation Environment: Species-Specific Trends in Phenotype-Environment Interaction

*Tristan Herod, Christopher Murray*

Climate change is an active threat against Earth's biodiversity and natural resources and threatens to alter species distributions, accelerate extinction and modify phenotypic characteristics. Oviparous species whose clutches experience variation in incubation environment may expect phenotypic changes due to fluctuating incubation environments. Resulting phenotypic diversity among hatchlings is often overlooked in a comparative context across taxa and may represent an important response variable to thermal variation during development. The amount of phenotypic variation among hatchlings within clutches (i.e. clutch morphological disparity) may link incubation temperature variance to phenotype and may benefit an understanding of how future environmental variation can impact organisms. Here, we explored the relationship between incubation temperature variation and clutch morphological disparity between three species of oviparous aquatic reptiles. Within-nest incubation temperatures were recorded in *Apalone spinifera*, *Trachemys scripta* and *Alligator mississippiensis* nests from southern Louisiana and Alabama. Nest temperature variance, range and thermal effect size for all clutches were used as a predictor variable for clutch morphological disparity, a geometric morphometric technique. Results indicate that *A. mississippiensis* and *A. spinifera* clutches displayed higher morphological disparity with increased thermal variance and thermal effect size, while *T. scripta* clutches displayed higher morphological disparity with increased thermal variance but lower disparity with increased thermal effect size. Our study demonstrates that the production of morphological disparity may mechanistically differ among thermal response variables studied and be constrained by evolutionary history.

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## Convergent Specialization of the Suction Feeding Mechanism in Zooplanktivorous Fishes

Marta Hess, Peter Wainwright

Zooplanktivory has convergently evolved multiple times in marine and freshwater teleost fishes. While convergent aspects of body shape and habitat use have been noted, less is known about the feeding novelties associated with making the evolutionary jump to zooplankton feeding. This study compared three lineages of fish (Cichlidae, Haemulidae, and Serranidae) that have evolved zooplanktivory, and found that the ability to protrude their premaxilla beyond their lower jaw is found in each case. Such extreme upper jaw protrusion may be advantageous for suction feeding on prey such as copepods and other plankton. We further investigated specializations in each family by comparing planktivores to non-planktivorous relatives. We analyzed high-speed fish feeding video and used computer digitization to create kinematic plots of various cranial bones. The results indicate that, in addition to extreme jaw protrusion, many zooplanktivorous fishes have greater maxilla rotation and less head elevation than generalist suction-feeders. It appears that zooplanktivores often evolve smaller mouths but higher jaw kinesis, which is adapted for feeding on small but elusive prey that are found in the plankton around coral reefs and some freshwater habitats.

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## The Status and Recovery of the Critically Endangered Dusky Gopher Frog *Rana Sevosa*

Cory Hillard, Joseph Pechmann, Jaime Smith, James Lee, John Tupy, Angie Dedrickson, Scott Hereford, Linda LaClaire, Michael Sisson, Danna Baxley, Allison Bogisich, Sinlan Poo

The dusky gopher frog, *Rana sevosa*, has been plagued by habitat loss and degradation caused by human development and fire suppression. Once ranging throughout south Mississippi, and into southeast Louisiana and southwest Alabama, *R. sevosa* has faced severe population declines. This prompted its listing as endangered in 2001 when the species was only known from a single population, Glen's Pond. Since the species' listing, considerable efforts have been made towards recovery. Along with major upland and wetland habitat restorations, captive rearing and head starting efforts have been instrumental in translocation and reintroduction activities in its historic range. We have translocated head-started *R. sevosa* to 12 sites in Mississippi and observed reproduction at 11 of them. Translocation sites include many aligned with the recovery plan of creating metapopulations. In addition, *R. sevosa* naturally colonized and bred at 2 restored and improved ponds within dispersal distance of Glen's Pond, forming a metapopulation. Glen's Pond, the source and most robust population, is trending upwards since the species' listing and is estimated to currently have around 181 breeding adults, compared to just 42 breeding adults in 2005. This is largely due to consistent captive rearing and head starting for population supplementation, averaging 264 juveniles released at Glen's Pond per year, compared to an average of 135 naturally occurring juveniles per year since species listing in 2001. Captive rearing provides a population buffer and is especially important during dry years because it lessens the effects of failed natural recruitment when the ponds prematurely dry.

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## The Natural History of Jack Musick (1941–2021)

Eric J. Hilton, R. Dean Grubbs, Charles F. Cotton

When John A. "Jack" Musick passed away on February 13, 2021, he left an extensive legacy spanning the fields of ichthyology (ranging from a wide array of teleosts, to coelacanths, to chondrichthyans), sea turtle biology, fisheries science, and marine conservation biology. Jack was an active participant in all disciplines represented by JMIH. He was a lifelong member of ASIH and a founding member of the AES, served on its Board of Directors and served as the President of AES in 1991-1992. Jack spent much of his 40-year academic career as faculty in William & Mary's School of Marine Science at the Virginia Institute of Marine Science (VIMS), where he served as major advisor to an astounding 88 graduate students, including many who remain active in AES and ASIH, and four of whom have also served as either AES or ASIH President. He and his students greatly expanded the ichthyology collection at VIMS, setting the stage for it to become the international resource it is today. He was

author or co-author of 170 scientific papers, served as co-author and editor of 14 scientific books and proceedings, and co-authored eight trade books. In this presentation, we provide an overview of Jack's life, research, and legacy as an introduction to this symposium, which is jointly convened by ASIH and AES and brings together researchers from across all JMIH disciplines to highlight current research that spans the breadth of subjects Jack studied.

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## Extrinsic Factors Influencing Hibernacula Occupancy of the Eastern Massasauga

Blaine Hiner, Michael Dreslik

Determining a species' habitat use and distribution across a landscape is essential for understanding the prevalence of declines due to larger-scale threats. At the landscape level, anthropogenic threats such as climate change and habitat destruction have triggered global population declines; thus, specific data crucial to combat declines must assess their impacts. Collecting landscape-level data has been improved by the advent of occupancy modeling, which can elucidate the impacts anthropogenic factors have on population presence. The Eastern Massasauga is particularly vulnerable to extreme natural events from climate change and habitat loss in the western third of its range, particularly due to its reliance on specific overwintering habitats, which have been largely converted to agriculture. Extrinsic factors affecting overwintering habitat occupancy could include the burning regime, flooding events, aberrant seasonal climate, patch size, and juxtaposition of patches across the landscape. Using a long-term capture-mark-recapture dataset (1999 – present), we determined if extrinsic management and climate factors affected hibernacula occupancy rates. Our results would afford prioritization of site-specific habitat management actions to mitigate factors negatively impacting site occupancy.

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## Little Fish, Big Variety: Incredible Diversity in the Weberian Apparatus of Noturus Catfishes

Jennifer Hoeflich, Juan Liu

While the catfishes of the genus *Noturus* are remarkably similar to one another in terms of ecology and general morphology, they show considerable diversity in the morphology of the ossicle chain of their Weberian apparatus — which is used for conductive hearing. We examined specimens from 17 species of *Noturus* using computed tomography (GE Phoenix Nanotom, voxel size 3.4-16.7µm) with region of interests segmented in Avizo. Qualitative comparisons showed considerable variations among *Noturus* species particularly in the shape of the concha scaphium, size of the scaphium's ascending process, and the robustness of the tripus' anterior process. To explore potential variational drivers, we proceeded with landmark-based 3D geometric morphometrics methods. Ten landmarks were placed on the scaphium and seven on the tripus representing the overall shape and important contact points. Principal component analyses (PCA) using geomorph in R show variations along the PC axis (PC1+PC2 of tripus 46.5% and of scaphium 59.2%), which are in accord with our observations above. The results of morphological disparity analysis showed no significant correlations between the ossicle morphology and stream gradient, region, or venom gland morphology. We also rejected the presence of a strong phylogenetic signal (scaphium:  $k=0.57$ ,  $p=0.374$ ; tripus:  $k=0.565$ ,  $p=0.418$ ). Due to the Weberian apparatus' role in sound conduction, the variation may produce functional differences. As these catfishes are known to possess specialized pectoral spine ridges for stridulation, the highly diversified morphology may be driven by different frequencies of intraspecific communication.

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## Fluorescent fish shed light on the adaptive evolution of cottonmouth venom

Matthew Holding, Hao Hao Pontius, Laura Haynes, David Ginsburg, Jordan Shavit

Snake venoms are complex phenotypes used to incapacitate prey. Deconstructing the functional effects of venom variation and of individual venom components provides an unparalleled window into how complex molecular traits are honed by natural selection. Yet, most work on venom

function is done using in vitro methods such as enzyme assays or in laboratory mouse models, obfuscating the native killing functions of venom as it flows through target prey tissues. Larval, genetically engineered zebrafish function as a living, transparent microfluidic chamber in which many aspects of basic biological function can be directly measured. We have used zebrafish as a model system to test the hypothesis that cottonmouth (*Agkistrodon piscivorus*) venom is adapted to stopping key biological functions of blood flow and heartbeat in fish. Further, we investigate how the venom kills using fish with fluorescently labeled thrombocytes, as well as clotting assays using whole trout plasma. Cottonmouth venom is exceptional in its ability to aggregate fish thrombocytes to form a mobile thrombus in blood vessels. Our results suggest the possibility of fish-adapted paralogs of botrocetin in the venom of fish-eating snakes. Feeding on deeply diverged prey such as fish versus rodents can exert divergent natural selection on venoms, generating the endless variations in venom we observe among snake species.

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### Investigating the potential for the Temperature-Size Rule in *Eurycea cirrigera* and *Plethodon cinereus*

*Charlie Holguin, Nicholas Caruso, Holly Kindsvater*

Amphibian life history, such as growth, development, and reproduction, can vary due to different environmental conditions. For example, at high latitudes and altitudes, some amphibians take a longer time to reach a minimum size for sexual maturity and the age of first reproduction. This phenotypically plastic response of size at different temperatures can in turn produce patterns across environmental gradients (i.e., Temperature-Size Rule; TSR). Resources (food) are another source of variation in body size, and it is unclear how this might interact with temperature. Understanding how temperature and resources interact with different life history strategies can inform other aspects of population ecology and conservation. We collected a total of 120 southern two-lined salamanders (*E. cirrigera*) and 110 eastern red-backed salamanders (*P. cinereus*), which differ in early development (aquatic vs. terrestrial juveniles). We then exposed the salamanders to a factorial design containing two food treatments (High Food and Low Food) crossed with two temperature treatments (Warm and Cool). Once a month, the salamanders were measured for snout-vent length, total length, and mass (g). The data were analyzed in a generalized additive model modeling framework with fixed effects of temperature and food treatments and a random effect for individual identity. By addressing the TSR through laboratory experiments, we will be able to determine and compare how environmental conditions at early life stages influence the growth of these two species with contrasting life histories.

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### Herpetology meets Recreation Ecology: A Tale of Two Reptiles

*Gareth Hopkins, Joshua Kesling, Blake Looney, Ashley D'Antonio*

Recreation ecology aims to understand ecological disturbances caused by outdoor recreation. While the number of recreation ecology studies focused on wildlife has increased recently, studies on reptiles are rare. Reptiles make an excellent model for recreation ecology due to their requirement to bask in open habitats where they may be encountered by park visitors. We examined the potential impacts of recreation on reptiles in two contrasting systems in popular urban-proximate parks in western Oregon: a paved pathway through a grassy area home to six species of squamates; and a paved trail system around a series of ponds and sloughs home to three species of freshwater turtle. In both studies, we quantified the number of reptiles and people recreating through systematic observations. We also examined the responses of the animals to people and environmental variables that might influence their presence. Our two studies differed in their challenges: We encountered squamates infrequently in our path surveys, with an average of <1 animal basking per survey. In contrast, we observed basking turtles much more frequently. Despite these differences, we found a negative relationship between the number of people and the number of reptiles seen in both systems, with animals generally halting basking when people were present. However, our results highlight that invasive red-eared sliders (*Trachemys scripta*) are less sensitive to people than native turtles. Collectively, these studies provide an

important step in understanding the impacts of recreation on reptiles while also highlighting the significant challenges inherent in conducting studies on these cryptic animals.

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### Mating Systems and the Maintenance of Genetic Diversity in a Small Sea Turtle Population

*John Horne, Amy Frey, Alexander Gaos, Summer Martin, Peter Dutton*

In the Hawaiian Islands, hawksbill turtles are rare and the resident population probably consists of less than 100 breeding females, with only 5-26 nesting annually. To better understand the conservation needs of these protected reptiles, we used a 135-locus microhaplotype assay to evaluate genetic diversity, improve censuses of nesting females through parentage analysis (using offspring from nests without observed mothers), infer the number of breeding males by reconstructing paternal genotypes from offspring, and assess patterns of mating and reproduction across the study area. The final data set included 1,002 offspring from 41 nests and 13 nesting females, all from the southern coast of Hawaii Island in 2017. Results showed that every nest without an observed mother was laid by one of the surveyed females, and there were 12 sires for all offspring, revealing a nearly 1:1 breeding sex ratio. Elevated genetic relatedness between breeding pairs was common, suggesting generational inbreeding, but population-level genetic diversity was unexpectedly high with many loci having heterozygosities in excess of 0.5. Across 90 km of coastline, nesting was concentrated into three complexes of beaches that were genetically differentiated from one another, where both sexes appeared to mate preferentially with individuals from their same complex. Patterns of inbreeding at each locus also varied markedly between nesting complexes further indicating demographic discontinuities along the coastline, as well as different genetic loads. Theory predicts that occasional interbreeding between such groups produces heterosis benefits that maintain genetic diversity and stave off the worst effects of inbreeding.

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### Sciaence or Séance? A Legacy of Comparative Sensory Investigations of Sciaenid Fishes and Their Competitors in Chesapeake Bay.

*Andrij Z. Horodysky, Richard W. Brill, John A. Musick*

Jack Musick encouraged and supported our comparative studies of the sensory systems of sciaenid fishes, as early anatomical investigations in his lab demonstrated the rich ecomorphological tapestry of the family and its links to diet, lifestyle, habitat, and behavior. Our electrophysiological investigations described the auditory performance and the visual ecophysiology of sciaenid fishes inhabiting Chesapeake Bay, as well as those of non-sciaenid species that are potential prey, competitors, or predators. To contextualize the relative influences of phylogeny vs. environment on neurosensory performance, we have recently extended our efforts to include reef-associated members of this family. Collectively, we demonstrated that the visual systems of Chesapeake Bay sciaenid fishes are well suited for the dynamic photo-climates in estuaries, and that this group will probably be less disadvantaged by rapid changes in ambient optical conditions during the Anthropocene than will some local competitors and predators. Our studies of reef-associated sciaenid species suggest, however, that they would be disadvantaged by degradation in ambient optical conditions. In contrast, our data on the auditory performance of sciaenid fishes suggest that this group in toto may be acutely sensitive to alterations of the soundscape. Due to their reliance on auditory communication during spawning, the population dynamics of soniferous sciaenid fishes could be impacted by increased anthrophony resulting from coastal human activities (e.g., shipping and naval activities, or the development and operation of offshore energy technologies). We therefore contend that the neurosensory ecology of living marine resources should be considered in modern interpretations of essential fish habitat.



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### Fine-scale analysis of space use by white sharks (*Carcharodon carcharias*) in their overwintering residency area in the western North Atlantic

*Mackenzie Horton, Nigel Hussey, Ashley Johnson, John Tyminski, Chris Fischer, Brett McBride, Robert Hueter, Bryan Franks*

In recent years, the movement and migration of the white shark population in the western North Atlantic has become a subject of increasing interest to better understand areas of importance and habitat use for all life stages, especially as this population continues to rebuild. Recent research on this population is beginning to reveal information regarding movements and habitat use, however, detailed data on population abundance, population dynamics, distribution, movements, and habitat use are needed to develop and ensure sound management strategies. Using a combination of acoustic and satellite tags deployed on white sharks by OCEARCH, this project seeks to better understand the fine-scale movements, potential core habitats, hotspots, and preferred environmental parameters of white sharks while in their overwintering residency area from North Carolina to the Gulf of Mexico. Regularized tracks constructed from acoustic and satellite data are being used to examine site fidelity, habitat preferences, and potentially important areas or habitats within this region. Using multiple tag technologies over multi-year periods allows for a robust analysis of movements and habitat use for individual animals to contribute to the growing knowledge of the western North Atlantic white shark population. This will provide critical information to our understanding of the ecology of these apex predators.

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### Discovering the Detrimental Effects of the Novel Invasive Parasite *Raillietiella orientalis* on Native Florida Snake Populations

*Shannon Horvath, Jamie DeShazo, Haim Flores Cinnamon, Cassie Valenti, Andrew Durso*

We are investigating the negative fitness impacts on Florida's native snakes from the introduction of the novel obligate endoparasite *Raillietiella orientalis*. *R. orientalis* is a pentastome belonging to the subphylum Crustacea and is believed to have been introduced to Florida with the establishment of the Burmese pythons. They have since infected populations of native snakes in southern Florida, with aquatic species appearing to be disproportionately affected. *R. orientalis* is a sexually reproducing organism with several life stages; the adult parasites live their final life stage in the lung cavity of snakes, laying their eggs into the gastrointestinal tract which are then expelled through the feces, but the larval life stage and possible intermediate hosts are not well understood. We present *R. orientalis* infection prevalence and intensity from a plethora of native snakes found killed on roadways in urban and rural parts of southwest Florida. Our aim is to continue identifying affected species and potential intermediate hosts of *R. orientalis* as we attempt to understand more about the host-parasite relationship. We are also monitoring live aquatic snake populations in both rural and urban areas to assess individual fitness and prey choices in known infected populations and uninfected populations through a mark-recapture study using minnow traps in a variety of wetlands.

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### Male Spatial Use Drives Gene Flow in Timber Rattlesnakes (*Crotalus horridus*)

*Christopher Howey, Anne Royer*

Timber rattlesnakes (*Crotalus horridus*) exhibit patterns in spatial use and genetic diversity that are shaped by many aspects of their ecology and environment. These impacts on spatial use and genetic diversity may foster or constrain conservation efforts. We combine spatial use data with nuclear and mitochondrial population genetic data to understand the population structure of snakes in four overwintering sites (hibernacula) in central Pennsylvania. Our analyses support the conclusion that hibernacula separated by only a few kilometers can represent genetically distinct populations. In addition, as the first dataset to compare nuclear and mitochondrial patterns in *C. horridus*, we provide novel evidence for how likely asymmetry in gene flow (with males responsible for inter-hibernaculum matings) shapes timber rattlesnake population genetics.

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### Effects of Vegetation and Historical Land Use on the Occurrence of an Endemic Anuran

*Anna Humphrey, April Atkinson, Jay Cantrell, Will Dillman, Kyle Palmquist, Shane Welch, Jayme Waldron*

The destruction of the longleaf pine (*Pinus palustris*) ecosystem (LPE)—and minimal legal protections afforded to wetlands—has resulted in the alteration or loss of many isolated wetlands in the southeastern US. Efforts to restore and conserve LPE wetlands have been underway for decades to promote the high diversity they support. Most wetland restoration efforts within the LPE focus on vegetative responses to guide management protocols, assuming animals will redistribute across landscapes to occupy the most suitable habitat patches. However, the distributions of many amphibians reflect land use legacies, due to either the inability to redistribute across landscapes or a lag in redistribution. We examined little grass frog (*Pseudacris ocularis*) occupancy as a function of current vegetation structure and historic land use. We used autonomous recording units (ARU's) to sample little grass frog choruses from February to November 2022 at 13 isolated wetlands in the South Carolina Coastal Plain. We developed a recognizer in Raven Pro for chorusing little grass frogs and analyzed wave files from random sampling nights. We conducted vegetation surveys to quantify contemporary vegetation composition and structure, as well as visually inspected wetland ditches, when present. We used historical aerial photographs to assess habitat disturbance from historical land use (e.g., agriculture, silviculture) and the time scale on which it occurred. The results of this study illustrate the importance of historical land use on LPE endemic herpetofauna and can serve to direct land managers to prioritize sites for conservation, restoration, and surveys.

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### Phylogenetic relationships among the New World sea robins (Triglidae: Prionotinae) inferred from UCE loci

*Elizabeth P. Hunt, Kevin W. Conway, David S. Portnoy*

Fishes in the family Triglidae, searobins and allies (order Scorpaeniformes), can be found circumglobally in benthic shelf habitat, across tropical and subtropical seas. Members of this family are well-known for the possession of heavy armor (i.e., dermal cranial bones heavily ossified and often with well-developed spines), large wing-like pectoral fins, the presence of highly modified “free” pectoral-fin rays that play a role in the prey detection, and sound production via sonic muscles associated with the swim bladder. Attempts to reconstruct relationship among species within the family has resulted in conflicting phylogenetic hypothesis at multiple levels due to analytical limitations, such as poor taxon sampling, or the use of only morphological data and/or molecular data sets with few loci. Previous studies have brought to question the monophyly of the New World genus *Prionotus* as well as the validity of two species, *Prionotus alatus* and *P. paralatus*. Here, we use target enrichment of 500 ultra-conserved DNA elements (UCEs) to reassess the phylogenetic relationships of the New World sea robins (*Prionotus* and *Bellator*).

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### Egg binding in amphibians: insight from Canada's most endangered anuran, the Oregon Spotted Frog (*Rana pretiosa*)

*Briar Hunter, David Lesbarrères, Gabriela Mastromonaco*

Post-ovulatory egg retention, or dystocia, is a significant risk to amphibians held ex situ but its documentation, and thus understanding, is minimal. Potential links to age, body condition, amplexus behaviours, and environmental conditions have been suggested but remain primarily anecdotal. Here, we investigated the causes of egg retention in Canada's most endangered amphibian, the Oregon Spotted Frog (*Rana pretiosa*). Using necropsy reports from conservation breeding programs, we classified mortalities related to egg retention as egg binding and compared body condition of conspecifics in zoo and wild populations. We also looked for links between egg binding and age, follicular development, and amplexus behaviours using video and ultrasound technology. While two out of three zoos had significantly higher scaled mass indices (SMIs) than wild conspecifics, the SMIs of egg bound frogs did not significantly differ. However, frogs who became egg bound later in life (> 3 years old) generally had higher body mass than other females of the same age. Higher follicular grades, indicative of mature follicles, were predictive of

egg binding but may have been conflated by altered environmental conditions. Most amplexus contacts lasted less than 8.4 minutes but were not predictive of egg binding. While the causes of egg binding in *R. pretiosa* and amphibians overall remains indistinct, our study plays a critical role in better describing and documenting this issue, aiding amphibian breeding programs worldwide. Further, findings from this study will inform husbandry practices for *R. pretiosa* and improve cohesiveness of recovery actions among partners for this endangered species.

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## **“From Herps to Humans”: How Human Dimensions and Outreach Can Help Shape the Future of Herpetology**

*Lameace Hussain*

The lack of diversity and persistent negative experiences within the field of herpetology can create feelings of isolation among many herpetologists from underrepresented identities. As minority groups and those who identify as women continue to report higher rates of unsafe work environments and harassment in the field, barriers to herpetological careers continue. Further, professionals in the field of herpetology may lack the cultural competency to effectively engage with underserved and marginalized communities through outreach initiatives, often creating animosity and mistrust. Science communication can be a powerful way to engage the public, especially to change negative perceptions of herpetofauna. It also provides an opportunity for our growing discipline to engage with communities that have been traditionally underserved and underrepresented—many of whom may have low awareness about the field of herpetology and experience issues such as access to scientific and management information, access to green spaces, and safe recreation within the outdoors. Therefore, cultural competency, representation, and an understanding of diverse human experiences are all important factors when designing science communication programs and herpetological-based careers. This talk will not only focus on preliminary findings from our ongoing survey of herpetologists within the field, but also highlight some ways to can reach diverse audiences to change the perception of “what a herpetologist looks like” for future generations.

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## **Native Spadefoot Toads (*Spea multiplicata*) in Uranium Mine Containment Ponds: Bioaccumulation and Potential Trophic Transfer of Metals**

*Michael Iacchetta, Bethany K. Kunz, Danielle Cleveland, Jo Ellen Hinck*

In the Grand Canyon watershed, containment ponds associated with breccia pipe uranium mines create surface water habitats in otherwise arid areas. These habitats attract a variety of wildlife, including breeding spadefoot toads (*Spea multiplicata*), which opportunistically use the ponds for oviposition and larval development. Containment pond waters can contain elevated concentrations of uranium and co-occurring metals (e.g., copper, cobalt, nickel, and arsenic), likely due to mineralized groundwater or runoff from surficial mining operations. Thus, *S. multiplicata* are potentially exposed to these metals for the duration of the aquatic larval period. However, it is unknown whether these chronic exposures detrimentally affect the survival of larvae or whether metals bioaccumulated by *S. multiplicata* larvae are transferred into terrestrial food webs to predators of either tadpoles or metamorphs. We conducted several types of exposures with *S. multiplicata* larvae to evaluate these unknowns, including 10-day exposures to field-collected water or sediment from a containment pond at the Pinyon Plain Mine (PPM) (Arizona, USA), and 50-day exposures to copper or cobalt that lasted the duration of the larval period. Exposure to PPM containment pond water and sediment did not cause mortality in tadpoles; however, tadpoles accumulated elevated concentrations of eight selected metals. In the 50-day copper and cobalt exposures, whole-body metal concentrations were up to 30X higher in tadpoles than in metamorphs. This study helps predict the consequences of breccia pipe uranium mining on native amphibian populations and points to the potential for toad-mediated transfer of metals to terrestrial wildlife, particularly predators of *S. multiplicata*.

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## **Fish-Mussel Relationships in the St. Lawrence & Ottawa Rivers: Are Lake Sturgeon the (only) Host Fish for the Endangered Hickorynut Mussel?**

*Katriina Iives, André Martel, Tim Haxton, Noel Alfonso, Annie Paquet, Benjamin Aubrey, Hans-Frédéric Ellefsen*

Understanding the obligate relationship between freshwater unionid mussels and the fish host(s) of their larvae (glochidia) is key to the conservation of this generally threatened fauna. One component of a large collaborative multidisciplinary study of the purported relationship between the Lake Sturgeon (*Acipenser fulvescens*) and Hickorynut Mussel (*Obovaria olivaria*) is to make a definitive link in the wild between mussel species and their host fish. Glochidia were not detected on any of the hundreds of Lake Sturgeon examined in the Lac St. Pierre area of the fluvial St. Lawrence (June, July, August 2021). For the Ottawa River, however, two seasons of fish surveys using gill and seine nets in the Lac Coulonge region of the Ottawa River have yielded evidence of glochidia on the gills of several fish species. Ongoing laboratory work aims to create morphological and genetic standards for determining the species identity of the glochidia found on wild fishes. For morphological identification, we are generating a species-specific multi-dimensional measurement space supplemented with SEM imagery, while genetic confirmation is being conducted by modified DNA extraction and PCR protocols for COI barcoding. Future field work will involve continued surveys in the fluvial St. Lawrence and the Ottawa River watershed to further elucidate fish-mussel relationships. Approximately one-third of all freshwater mussels in Canada are at risk; identifying the life history requirement of which fish species can harbor their developing larvae is critical to the development of conservation plans, not only for the mussel species, but also their host fishes.

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## **Gene Expression Dynamics in Polyploid Unisexual Salamanders**

*Michael Itgen, Robert Denton*

Polyploidization can have diverse effects on gene expression, including gene dosing imbalance, subgenomic dominance, and altered gene expression networks. However, much of our understanding of the relationship between ploidy and gene expression comes from plants, in part due to the relative rarity of polyploidy animals. To bridge this knowledge gap, we investigated gene expression dynamics in the unisexual, allopolyploid lineage of salamanders (Genus: *Ambystoma*). Unlike other polyploid animals, the unisexual salamander lineage is older than many sexual species (~5 million years of mitochondrial divergence), often outnumbers competing sexual, diploid species, and are found across northeastern North America. These salamanders are typically triploid, containing nuclear genomes from two sexual diploid species: *A. laterale* (L subgenome) and *A. jeffersonianum* (J subgenome). The number of chromosome sets from each parental diploid can vary (i.e., LLJ, LJJ), potentially influencing relative subgenomic expression. To better understand the effects of polyploidy on gene expression, we conducted RNA-Seq analyses on unisexuals and the co-occurring sexual species across their range. We broadly tested for ploidy-related differences by identifying differentially expressed genes in the polyploids compared to either sexual diploid parental species. Next, we used a competitive read-mapping method to assess the relative contributions of each subgenome to test the effects of genotype composition on subgenome expression and balance. We contextualize these results with previous work suggesting that subgenomes of polyploid salamanders fit a model of subgenome gene expression balance and suggest that gene expression differences may play a role in mitigating competitive interactions between polyploid and diploid salamanders.

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## **The evolution of the sperm receptor PKDREJ in amphibians and squamates**

*Todd Jackman*

The sperm receptor PKDREJ is a structurally conserved but highly variable intronless gene with over 7,000 bases found in nearly all vertebrates, and its encoded protein is located exclusively at the tip of animal sperm. Losses of PKDREJ have occurred in ray-finned fishes and anurans. Multiple losses of PKDREJ in frogs are unique and suggest major shifts in fertilization

biochemistry. In squamates, duplication events due to unequal crossing over occurred in the common ancestor, providing a unique opportunity for phylogenetic analysis of squamates without using *Sphenodon* as an outgroup. Using the PKDREJ paralogs, phylogenetic analyses of both amino acids and DNA provides moderate support for *Dibamus* and *Gekkota* as the sister taxon to all other squamates. Cases of pseudogenes in both amphibians and squamates are rare but show some interesting patterns of duplications and deletions. In the allotetraploid *Xenopus laevis*, PKDREJ has many tandemly duplicated pseudogenes on one chromosome, but no copies are present on the corresponding duplicate chromosome. This research provides insights into the evolution of PKDREJ in amphibians and squamates and its potential use as a phylogenetic marker.

## Density and Abundance of Juvenile White Shark (*Carcharodon carcharias*) Prey Species along Southern California Beaches

Elizabeth Jahn, Christopher G. Lowe

The Southern California Bight (SCB) coastline is considered nursery habitat for juvenile white sharks (JWS) in the Northeast Pacific, where JWS form loose aggregations at specific “hotspot” beaches, or nursery aggregation sites. Prey resources are likely a driver of fidelity to aggregation sites, as these beaches require adequate prey availability for JWS foraging and growth. However, it is unknown to what extent potential JWS prey abundances may vary across Southern California beaches. This study describes the prey community composition and prey species abundance at aggregation sites and non-aggregation sites in Southern California using baited remote underwater videos (BRUVs) and beach seines. Six BRUVs were deployed monthly at four sites (two aggregation sites, one former aggregation site, and one non-aggregation site). Two seine nets (23m and 30m long) were towed along sandy, nearshore beaches monthly across three sites (one aggregation site, one non-aggregation site, and one former aggregation site). Over 300 hrs of BRUV footage was collected, and >30 unique potential prey species were sampled from >200 beach seine tows. Preliminary beach seine data suggest differences in the prey community assemblage of the aggregation site compared to both the former aggregation site and non-aggregation site (PERMANOVA,  $p = 0.006$ ). JWS prey community composition and relative abundances at JWS aggregation sites and non-aggregation sites may help to predict future aggregation “hotspot” sites.

## Molecular Evolution of Visual Opsin Genes in Side-neck and Hidden-neck Turtles

Golnar Jalilvand, Rayna Bell, Belinda SW Chang, Wali Mir, Anjela Ajei, Ryan Schott

Visual adaptation to different light environments tends to be reflected in the opsin genes that encode the protein component of the light-sensitive visual pigments. In vertebrates, there are five classes of visual pigments found in the retinal photoreceptors, each with a distinct opsin gene that is maximally sensitive to a different portion of the light spectrum. Four of these are expressed in the bright-light sensitive photoreceptors providing vertebrates that express them all the basis for tetrachromatic color vision, such as birds and turtles. However, our understanding of visual opsin diversity in turtles is based solely on hidden-neck turtles (Cryptodira) and we lack information from side-necked turtles (Pleurodira). Here we begin to fill this ~200 Ma evolutionary gap by investigating the molecular evolution of visual opsins from both groups using an eye transcriptome from *Pelomedusa subrufa* as well as four pleurodiran and 24 cryptodiran whole genomes. We found that *P. subrufa* expressed all five visual opsin genes, like Cryptodira species investigated to date, and that these genes were also present in all four pleurodiran genomes. To investigate how vision may have diverged between these species with different visual ecologies, we analyzed differences in spectral tuning and estimated selective pressures using models of codon evolution. Overall, we found that turtle visual opsins were remarkably conserved despite ~200 My of divergence. We also found evidence for positive selection in a subset of the opsin genes and variation in selective constraint between discrete ecological and life history categories reflecting potential functional adaptation.

## Phylogenetic relationships of the North American catfishes (Ictaluridae, Siluriformes): investigating the origins and parallel evolution of the troglobitic species

Francesco Janzen, Rodolfo Pérez-Rodríguez, Omar Domínguez-Domínguez, Dean Hendrickson, Mark Sabaj, Gabriel Blouin-Demers

Insular habitats have played an important role in developing evolutionary theory, including natural selection and island biogeography. Caves are insular habitats that place extreme selective pressures on organisms due to the absence of light and food scarcity. Therefore, cave organisms present an excellent opportunity for studying colonization and speciation in response to the unique abiotic conditions that require extreme adaptations. One vertebrate family, the North American catfishes (Ictaluridae), includes four troglobitic species that inhabit the karst region bordering the western Gulf of Mexico. The phylogenetic relationships of these species have been contentious, and conflicting hypotheses have been proposed to explain their origins. The purpose of our study was to construct a time-calibrated phylogeny of Ictaluridae using first-occurrence fossil data and the largest molecular dataset on the group to date. We test the hypothesis that troglobitic ictalurids have evolved in parallel, thus resulting from repeated cave colonization events. We found that *Prietella lundbergi* is sister to surface-dwelling *Ictalurus* and that *Prietella phreatophila* + *Trogloglanis pattersoni* are sister to surface-dwelling *Ameiurus*, suggesting that ictalurids colonized subterranean habitats at least twice in evolutionary history. The sister relationship between *Prietella phreatophila* and *Trogloglanis pattersoni* may indicate that these two species diverged from a common ancestor following a subterranean dispersal event between Texas and Coahuila aquifers. We recovered *Prietella* as a polyphyletic genus and recommend *P. lundbergi* be removed from this genus. With respect to *Ameiurus*, we found evidence for a potentially undescribed species sister to *A. platycephalus*, which warrants further investigation of Atlantic and Gulf slope *Ameiurus* species. In *Ictalurus*, we identified shallow divergence between *I. dugesii* and *I. ochoteranai*, *I. australis* and *I. mexicanus*, and *I. furcatus* and *I. meridionalis*, indicating a need to reexamine the validity of each species. Lastly, we propose minor revisions to the intrageneric classification of *Noturus* including the restriction of subgenus *Schilbeodes* to *N. gyrinus* (type species), *N. lachneri*, *N. leptacanthus*, and *N. nocturnus*.

## Urban Red-Backed Salamander Population Monitoring as a Vehicle for Public Science Outreach

Kortney Jaworski

Urbanization is increasingly becoming a global threat to biodiversity and population persistence, fundamentally changing the adaptive landscape that wildlife experience compared to natural areas. In addition, humans living in urban areas may never even experience native wildlife, even in their own backyard. For this project, I had two goals: to initiate a long-term monitoring project aimed at assessing salamander biodiversity within an urban landscape, and to create an opportunity for local volunteers to observe wildlife and participate in science, firsthand. In September 2022, I developed a training course and certified a group of 12 dedicated volunteers that assisted in setting up survey plots, surveying, collecting, and entering data. We established four replicate arrays of 50 artificial cover objects (ACO) that have since produced 73 red-backed salamanders (*Plethodon cinereus*) in just 10 surveys this first season (October 2022 to April 2023). For each animal, we identified its age, sex, and color morph, and measured its body size. We also recorded local microhabitat and environmental data, and noted if other organisms were sharing the ACO. I then uniquely marked each salamander via visual implant elastomer tags. For this poster, I provide an overview of the project and summary of our findings to-date. By continuing and expanding upon volunteer participation in these surveys over the next few years, I hope to ultimately build a long-term life history and social network dataset of *P. cinereus* in this urban environment accessible to the general public and fellow scientists alike.

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## Killin' Fish and Saving Lives: Examining Gene Expression Profiles of Killifishes with Different Life Histories. (Cyprinodontiformes: Nothobranchiidae)

Chi Jing Leow, Kyle Piller

The Turquoise Killifish (*Nothobranchius furzeri*; Nothobranchiidae) has recently become a model organism used in the study of human aging and age-related diseases. The Turquoise Killifish possess a lifespan between 4-8 months in the wild with the GRZ strain being the shortest-lived vertebrate in captivity. Its annual life cycle is due to its occupancy of the seasonal/ephemeral habitats in east Africa. Within the Nothobranchiidae, there are annuals, completing their life cycle in less than a year, as well as non-annual and semi-annual life histories. In terms of longevity, DNA repair plays a crucial role in maintaining genomic integrity, and previous studies have shown that long-lived species possess higher expression levels for DNA repair genes compared to short-lived species. In this study, we investigated the overall gene expression profiles of these killifishes with different life histories. In addition, we took a specific approach and focused on DNA repair genes. Our results show that, overall, non-annual and semi-annual species have more similar gene expression profiles compared to the annuals. The highest number of differentially expressed genes were found between annuals and non-annuals. Annuals exhibit higher levels of expression in some DNA repair genes compared to non-annual and semi-annual species. These upregulated DNA repair genes include X-ray repair cross complementing 3, DNA polymerase, tumor protein tp53, etc. Gene ontology enrichment analysis shows that annuals are most enriched in the ribosome, structural constituent of ribosome, and translation compared to non-annuals and semi-annuals. KEGG pathway analysis shows that annuals are most upregulated in metabolic pathways.

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## Putative Batesian Mimicry of Gelata by Larvae of Marine Fishes Based on in-situ Images from Blackwater Photographers

G. David Johnson, Ed Brothers, Richard Collins

The epipelagic zone of the open ocean is a high energy, nutrient rich arena that supports a myriad of planktonic life forms and serves as a nursery ground for many. Among the latter are the early life history stages of marine fishes that, as adults, occupy diverse habitats, most of them fundamentally different from the epipelagic zone; this is reflected in the diverse morphologies of the adults. The larvae of these fishes differ strikingly from the adults, having evolved to be successful in the relatively uniform, ubiquitous planktonic arena. Despite this environmental uniformity, these larval forms exhibit a remarkable array of morphological diversity that has captured the fancy and moved the pen of ichthyologists and evolutionary biologists since their earliest descriptions. Perhaps the most obvious and extraordinary of these are elaborate, often highly ornamented extensions of the fin rays that have repeatedly evolved independently in numerous unrelated taxa, most prominently within acanthomorphs. Much has been written about the possible functions of these structures, with the recognition that they must come at a selective price in terms of active predator avoidance, i.e., they create varying levels of drag and thus inhibit rapid swimming and maneuvering. Among the most commonly suggested functions is Batesian mimicry of toxic or "low carbon" gelata such as jellyfish and siphonophores. The recent popularity of blackwater photography of live, in situ individuals provides a new, enlightening visual perspective on this hypothesis. We highlight and discuss this fresh perspective with comparative images of various fish and invertebrate taxa.

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## Maternal Provisioning Interacts with Incubation Temperature to Affect Mercury Exposure in Hatchling American Alligators (*Alligator mississippiensis*)

Josiah Johnson, Christopher Smaga, Samantha Bock, Benjamin Parrott

The thermal environment experienced by developing embryos can influence the utilization of maternally-provisioned resources. Despite these processes being particularly consequential for oviparous ectotherms, many of which are threatened by human driven global change, these dynamics are largely unexplored within ecotoxicological frameworks. Here, we test if incubation temperature interacts with maternally-transferred mercury to affect

subsequent body burdens and tissue distributions of mercury in hatchling American alligators (*Alligator mississippiensis*). Nine clutches of alligator eggs were collected from a mercury-contaminated reservoir and incubated at either female- or male-promoting temperatures. Total mercury (THg) concentration was measured in egg yolk collected during incubation and in a suite of tissues collected from hatchlings. THg concentrations in residual yolk and blood were higher in hatchlings incubated at cooler, female-promoting temperatures compared to the warmer, male-promoting temperatures. THg concentrations were highest in tail muscle ( $0.300 \pm 0.022$  ppm ww) and kidney ( $0.257 \pm 0.018$  ppm ww), and lowest in fat body ( $0.036 \pm 0.004$  ppm ww) and liver ( $0.035 \pm 0.004$  ppm ww). THg concentrations in most tissues were positively correlated with THg concentrations in blood, and egg yolk THg concentration was the best predictor of THg concentration in many resultant tissues. Our results highlight a hereto unknown role of the developmental environment in mediating tissue specific uptake of contaminants in an oviparous reptile.

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## Habitat Utility by Herpetofauna at the Northern Terminus of Longleaf Pine Ecosystem with Comparison to Adjacent Habitats

Julianne Jones, Erik Yando

Amidst drastic habitat loss worldwide, the southeast US's longleaf pine ecosystems provide a critical conservation opportunity, supporting unique assemblages of flora and fauna. Longleaf pine ecosystems support the highest amphibian and reptile biodiversity of any temperate zone terrestrial ecosystem, and, although numerous species of herpetofauna have adapted to ecological pressures in these fire-maintained habitats (ie. Frosted Flatwoods Salamander (*Ambystoma cingulatum*), Gopher Tortoise (*Gopherus polyphemus*), etc.), Virginia is far out of the range for such longleaf specialists. This presents an opportunity to study how widespread "generalist" species interact with the landscape when a longleaf pine ecosystem is present among wetland habitat patches more typical of southeast Virginia. Blackwater Ecological Preserve (BEP) in Isle of Wight, Virginia, supports the region's northernmost communities of intact longleaf pine savanna and lacks thorough herpetological records. Within BEP, I have identified four distinct, adjacent habitat units to be surveyed: a mature longleaf pine savanna, pocosin wetland, maple-gum swamp, and a longleaf restoration site. Data collection is on-going and highlights relevant ecological factors, such as vegetation communities, hydrology and soil characteristics, etc., in addition to herpetofauna presence and biometrics. Preliminary data suggests 1) Longleaf pine ecosystems are underutilized at the northern extent of their range 2) BEP's pocosin wetlands may not serve as suitable refuge for amphibians and 3) Maple-gum swamps yield higher capture rates than adjacent habitats.

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## Tracking individual toads: Linking inter-individual variation to population-level partial movement

Nathalie Jreidini, David M Green

Animal movement ecology can be studied at various temporal scales, resulting in a wide range of data resolutions. High resolution movement data obtained from observations at a fine temporal scale can play a crucial role in teasing apart personality from stochastic individual patterns. These short-scale displacements accumulate to form large-scale movements, otherwise measured at relatively lower resolution, and can therefore shed light on the underlying mechanisms of various movement behaviours. Individuals within a population can either display different movement strategies, often leading to "partial movement", or even switch between strategies during a movement phase. We radio tracked 13 individual nocturnal Fowler's Toads (*Anaxyrus fowleri*) in Long Point, Ontario, Canada, on the northern shore of Lake Erie following their breeding season to understand their movement behaviour during the night. We obtained individual coordinates every 30 minutes from the moment they emerged until they burrowed, and used a hidden Markov model with measures of step lengths and turning angles to assess behavioural states and state-switching. We found that individuals switched between a 'dispersive' state and a 'searching' state as a function of both time and distance from the lake (i.e., microhabitat), whereby toads were more likely to perform long directional movements at the start of the night followed by shorter exploratory movements towards the end of the night. Our results also show that individual



stochastic variation in movement patterns, which conform to a random walk, lead to population-level trends that appear to be deterministic and suggestive of partial movement. Population-level movement patterns, or data measured at low resolution, may therefore be misleading when assessing behavioural movement strategies.

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### Successful Development and Reproduction of *Anaxyrus houstonensis* F1 and F2 Offspring Produced from Cryopreserved Sperm

Allison Julien, Carrie Kouba, Andrew Kouba, Isabella Burger, Diane Barber

In the last decade, cryopreservation of amphibian sperm has been successful in over 20 species worldwide; however, successful fertilization using frozen-thawed sperm and subsequent offspring development has been reported in far fewer species. While demonstrating successful reactivation of frozen sperm following cryopreservation is an important step for genetic management utilizing biobanked samples, ensuring healthy offspring production from cryopreserved sperm is crucial for conservation application. The aims of this study were to compare: 1) clutch sizes and fertility rates between cryo-produced (CP) females and non-cryo-produced (NCP) females; and 2) development and reproductive output of the F2 generation compared to unrelated cohorts. In 2021, we produced two female *Anaxyrus houstonensis* from frozen sperm at the Fort Worth Zoo (FWZ) and bred them in 2022. Average weights were 71 g and 46.7 g from CP and NCP females, respectively, with CP females laying an average of 5830 eggs and NCP females laying 4292. Fertility rates from CP females were 80%, and twenty individuals from both females were retained within the breeding program. Of these, 6 females and 12 males metamorphosed and survived to sexual maturity. Importantly, growth rates did not differ ( $p = 0.31$ ;  $t = 0.5$ ) between CP and NCP F2 offspring, and clutch sizes were comparable with CP F2s producing 4563 eggs on average, and NCP F2s producing 4143 eggs. These results demonstrate that reproductively healthy F1 and F2 offspring can be produced from cryopreserved sperm, and that individuals develop and reproduce comparably to offspring produced from natural breeding.

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### Female and Male Genital Shape of Invasive Burmese Pythons in the Florida Everglades

Rachel Keefte, Ian Bartoszek, Ian Easterling, Brandon Hedrick, Patricia Brennan

The extensive morphological diversity of snake genitalia has historically been explained by the “lock and key” hypothesis, wherein differing genital morphologies between species precludes interspecies mating events. However, this early hypothesis was generated primarily only through examinations of male anatomy. Recent work on watersnakes examined female and male genital morphology together and found evidence that sexual conflict between males and females may be a significant driver in the evolution of genital traits. In our study, we examine the genital morphology of a different family of snakes to compare evolutionary drivers of genital morphology more broadly. Our sample includes 20 males and 25 females of *Python bivittatus*, the Burmese python, from their invasive range in Florida. We use 3D geometric morphometrics to quantitatively compare the complex shapes of vaginal pouches and hemipenes in *P. bivittatus*, as well as comparing the degree of asymmetry between the right and left hemipenes of male *P. bivittatus*. Unlike watersnakes, python hemipenes do not bear any spines, yet are still bilobed, and have pointed ends that would appear to fit into the medial oviduct opening of females. Female genitalia are also bilobed, and shape coevolution seems likely. Genital shape correspondence and the lack of spines may suggest that the drivers of genital evolution in pythons are different than in watersnakes. This work will test if assumptions of the lock and key hypothesis or sexual conflict can explain genital diversity in this system.

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### International fisheries - how science informs conservation and management measures

Bryan Keller

Regional fisheries management organizations, or RFMOs, are intergovernmental fora charged with the conservation and management of certain transboundary fish stocks. Each organization defines an area of competence, which is the geographic boundary of the area subject to management. These fora create binding and non-binding conservation and management measures (CMMs) that are implemented by member nations for target stocks or species. Other species caught in association with those fisheries, such as sharks or sea turtles, may also be covered by these measures. The subject fisheries are often diverse, not only in gear type, but also in the spatiotemporal distribution of fishing effort. Collectively, these factors demand a complex process for identifying the best available science to inform CMMs. Here we describe that process through which scientific findings are incorporated into international fisheries management actions. Some believe that RFMOs can rely on any available peer-reviewed science. In fact, the designated scientific bodies for each RFMO, with participation of national scientists, typically play a pivotal role in analyzing and discussing data and recommending action to be considered in RFMO decision making. Without specific recommendations from an RFMO's designated scientific body, it can be challenging to gain the necessary support for the adoption of new CMMs. This unique process highlights the importance of scientists ensuring that relevant work is presented in this context, as it may otherwise not be considered by RFMOs. We hope this presentation will help non-government scientists to understand better the RFMO process, facilitating an increased participation in international fisheries management.

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### Population Survey of *Necturus punctatus* in a Perennial Stream within the Duke Forest

Sarah Kelso

The Dwarf Waterdog, *Necturus punctatus*, has unknown population trends with the potential to be declining in much of its range. To protect aquatic caudata, we need current data regarding threats and population changes. *N. punctatus* has not been studied in New Hope Creek since 1969. Urban land cover around New Hope Creek has increased since 1969 and has changed the chemical composition and physical structure of the creek. It is likely that this has contributed to changes in the *N. punctatus* population. This project aimed to establish a baseline population estimate for future studies to determine the population trend and to assess whether the New Hope Creek dam changed water characteristics such that it generated different density of animals, particularly *N. punctatus* above and below the dam. To study the population, we used Visual Implant Elastomers to mark and identify new/recaptured individuals caught in baited minnow traps from November 2022-March 2023. We also collected data on stream turbidity, pH, dissolved oxygen, temperature, specific conductance, and bycatch. Five *N. punctatus*, 40 crayfish, and 22 fish were captured over five months. Due to the low salamander capture numbers, with zero recaptures, a mark-recapture analysis was not completed. Crayfish, fish, and *N. punctatus* capture numbers were combined to look at spatiotemporal changes in animal capture rates. Water characteristics were also analyzed for these changes. The data show differences in water characteristics above and below the dam, as well as seasonal patterns, with only the spatial patterns consistent with animal capture rates.

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### Association of Anti-Mullerian Hormone Concentrations in Female Sharks in Relation to Sexual Maturity and Reproduction

Ashlynn Kemp, Jim Gelsleichter

Anti-Mullerian Hormone (AMH) is a protein hormone that has been shown to play an important role in early development of ovarian follicles in female mammals. Because of this, AMH levels in circulation have been shown to be a valuable indicator of reproductive condition in human females, particularly for ovarian reserve (i.e., the number of viable eggs capable of fertilization). The presence of AMH has recently been confirmed in chondrichthyans using molecular analysis. However, no studies examining the role of AMH in the reproductive biology of sharks and rays have been published. Therefore, the

purpose of this study was to determine if AMH is an indicator of reproductive biology in sharks and rays. Immunohistochemistry was used to examine the presence of AMH-like proteins in the ovary of female sharks. In addition, attempts were made to validate assays for measuring circulating AMH levels in female bonnethead sharks (*Sphyrna tiburo*) collected from multiple stages of sexual maturity and reproduction. AMH-like proteins were detected in vitellogenic ovarian follicles, suggesting that AMH may play a role in yolk production. Initial attempts to validate commercially available AMH assays for use with bonnethead plasma were unsuccessful; however, additional efforts are currently underway. Studies on AMH in chondrichthyans may improve our understanding of the evolution of this protein. Furthermore, if successful, efforts to develop assays for measuring AMH levels in shark plasma may provide us with new methods for characterizing shark reproduction using nonlethal approaches.

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## Modular organization of the Weberian apparatus in Cypriniform fishes

*Claudia Kern*

Adult phenotypes result from dynamic interactions among developmental stability mechanisms and developmental instability. Among the stability mechanisms, modularity has been targeted as a primary evolutionary developmental component facilitating the evolvability of complex systems. Among the various definitions of modularity (genetic, developmental, morphological, ecological), a module is a network of interacting elements behaving as quasi-independent units, or as semi-autonomous sets of highly correlated traits within larger units. Here we explore the modular organization of the Weberian apparatus among key cypriniform taxa with respect to their gonorynchiform relatives. This complex system consists of a series of anterior centra, neural arches, supra-neurals, and pleural ribs that form a mechanical linkage transmitting motion of the swim bladder to the inner ear. We employ micro-CT technology and 3-D geometric morphometrics to test modularity of the anterior vertebrae with respect to more posterior vertebral regions. We also investigate covariation among elements within the Weberian apparatus. Preliminary results suggest that the Weberian apparatus evolved from a non-modified vertebral column such as that in the outgroup Chanos chanos. This occurred within a modular framework, under which heterochronic events occurred in various otophyans, contributing to the disparity seen among Cypriniformes.

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## Exhumation of carbonate rock facilitates dispersal-mediated allopatric speciation in freshwater fishes

*Daemin Kim, Maya Stokes, Sandy Ebersole, Thomas Near*

A fundamental goal of evolutionary biology is to understand the mechanisms that generate and maintain biodiversity. Discovery and delimitation of species represent essential prerequisites for such investigations. We investigate a freshwater fish species complex comprising *Etheostoma bellator* and the federally endangered *E. chermocki* which is endemic to the Black Warrior River system in Alabama, USA, a global hotspot of temperate freshwater biodiversity. Phylogenomic analyses delimit five geographically disjunct species masquerading as *E. bellator*. Three of these new species exhibit microendemic distributions comparable to that of *E. chermocki* raising the possibility that they also require protection. The six species in the complex are restricted to stretches of rivers flowing over carbonate rock and they are separated from each other by waterways flowing over siliciclastic rock, a geographic pattern dictated by the underlying structural geology. Over time, rivers have incised downward through layers of clastic rocks in the basin, gradually exposing underlying carbonate rock, the substrate of suitable habitat today. Our results suggest that episodic dispersal events to patches of suitable habitat set the stage for allopatric speciation in the species complex. Our study suggests that the presence of heterogeneous rock can facilitate dispersal-mediated allopatric speciation in freshwater organisms even in the absence of external tectonic or climatic perturbations.

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## The physiological and ecological drivers of diversity in tuna life histories

*Holly Kindsvater, Maria José Juan-Jordá, Cat Horswill, Jason Matthiopoulos, Marc Mangel*

Understanding how fish growth and reproduction evolve in response to changing environmental conditions is a fundamental question in evolutionary ecology. Prior studies focused on the physiological effects of climate change upon life histories and population demography often ignore size-dependent foraging and risk of predation. We embedded a state-dependent energetic model in an ecosystem size spectrum to model prey availability (foraging) and risk of predation (fear) experienced by individual fish as they grow. We examined how spectrum richness and temperature interact to shape growth, reproduction, and survival; we found that richer spectra led to larger body sizes, but effects of temperature on body size were relatively small. We applied our model to scenarios corresponding to species representing the three ecological lifestyles (ecotypes) of tunas, in some cases including seasonal variation in conditions. We predicted realistic patterns of growth of tunas and found that seasonality in resource accumulation and compression of reproduction into short time periods favors growth to larger body sizes. Our framework for predicting emergent life histories combines direct and indirect effects of productivity (foodscapes), individual risk (fearscapes), and metabolic processes and offers a promising approach to understand fish life history responses to changing ocean conditions.

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## Tasting the Rainbow: The Palatability of Poison Frogs Along an Aposematic to Cryptic Gradient and its Relation with Chemical Defenses

*Jeremy Klank, Francesca Protti-Sánchez, Hannah M. Rowland, Federico Bolaños, Jennifer L. Stynoski*

Poison frogs (Dendrobatidae) are a well-known model for understanding the relationship between aposematism, toxicity, and predation. In this study, we investigate the palatability and composition of dermal secretions along a cryptic-to-aposematic gradient of five species of poison frogs (*Dendrobates auratus*, *Oophaga granulifera*, *Phyllobates vittatus*, *Silverstoneia flotator*, and *Allobates talamancae*). We used non-lethal methods to obtain and prepare extracts of the frogs' skin secretions with the objective to evaluate their palatability to ecologically relevant reptile and arthropod predators. We will also quantify the diversity and abundance of chemical compounds in their skin secretions, and analyze the relationship between palatability, chemical composition, and coloration. This cryptic-to-aposematic gradient is rarely studied in vertebrates. However, by studying it in poison frogs, it will allow for a better understanding of ecological interactions and ultimately the evolution of aposematism in these species. In addition, by testing the hypotheses that chemical compound diversity and abundance are related to palatability, this study will clarify the mechanisms of anti-predator defense in poison frogs and their impact on the evolution of alternative anti-predator strategies.

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## The Relationship Between Relatedness, Distance, and Local Density in Red-backed Salamanders (*Plethodon cinereus*)

*Marissa Knight, Stephanie Coster, Kristine Grayson*

The red-backed salamander (*Plethodon cinereus*) is a terrestrial salamander that lives in forests throughout the Northeastern United States. Many studies of red-backed salamanders have found territorial behavior and competition for cover objects, but these behaviors may change in high density populations. Our study site in suburban Richmond, Virginia was established as part of the Salamander Population & Adaptation Research Collaboration Network (SPARCnet). The local density of our plots was estimated using spatial capture-recapture methods and ranged from 2.86 – 6.26 individuals per m<sup>2</sup>. Multiple individuals are often found under the same 1 m<sup>2</sup> coverboard (as many as 10 – 12 during peak levels of activity). In high competition environments, related individuals may be more tolerant of each other as a form of altruism. This research explored whether proximate individuals are more likely to be related, which could suggest tolerance or altruism as an explanation for cover object sharing. We sampled salamanders from coverboard plots with higher and lower local densities. We genotyped 163 individuals at 14 microsatellite loci

to determine pairwise relatedness and compared the average relatedness of individuals at 0 distance to a null distribution. Our results indicated that individuals found in proximity were not more related than expected, suggesting there is no relationship between relatedness and distance at higher or lower density sites for this population. Our study provides additional insights on the spatial population structure of terrestrial salamanders, where familiarity may play a larger role than relatedness in intraspecific interactions.

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### **I like to move it, move it: advances in the application of acceleration data loggers in fisheries research**

*Ryan Knotek, Jeff Kneebone, Connor White, Tobey Curtis, Caroline Collatos, Nick Whitney*

Electronic tagging, such as satellite and acoustic telemetry, is a ubiquitous approach for monitoring fish movement and fate in post-release mortality (PRM) studies. However, acceleration data loggers (ADLs) also provide large amounts of fine-scale information on animal swimming behavior during and after the fishing event, yielding insights into animal fate and post-release recovery time. Here we present various ADL applications in shark PRM studies and discuss the benefits, limitations, and best practices of using this technology. Because ADL tag packages require physical recovery, they are best suited for nearshore environments where multiple animals can be tagged around the same time to increase the efficiency of tag package recovery. ADLs can also be a cost-effective strategy for gathering relatively large sample sizes because of their low cost and capacity to be re-used on multiple animals. In addition, tag package dimensions can be customized to expand ADL use to varying-sized sharks. Monitoring fine-scale body acceleration can also help identify more nuanced events that can be difficult to distinguish with other tagging technologies, including predation on tagged animals, scavenging dead (tagged) animals, and attempts to dislodge the tag by jumping or rubbing on the seafloor. Finally, ADLs can also be integrated with fishing gear in order to characterize the capture behaviors of hooked fish. The collective insight provided by ADLs may also help explain the variability reported in physiological stress and post-release outcomes between species and individuals.

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### **Long Dead Lizard Shows Critically Endangered Species Engages in Cannibalism**

*Krista Koeller*

*Lerista allanae*, a nearly limbless skink, was thought to be extinct for decades. Though rediscovered, its range is estimated to be under 1,000 km and it has been ranked the 7th most endangered squamate in Australia. Almost nothing is known about their natural history, but as every individual is precious, alternative methods to sacrificing specimens are preferred for such investigations. Here, I am reporting on the first known diet information for this species, obtained using computed tomography of a museum specimen collected in 1929. This specimen was found to have another lizard in its stomach. Anatomical comparisons demonstrate that the consumed lizard was also *Lerista allanae*, making this the first reported instance of cannibalism in this genus. The tendency towards intraspecific predation, when combined with the restricted range size, may be an additional factor threatening the survival of the species.

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### **Out of breath? Modularity & diversification in the air-breathing fishes and their allies**

*Matthew Kolmann, Matt Friedman*

Changes in the complexity of vertebrate body plans are linked to the degree to which modules within these bodies are covariable - how much does changing one structure influence adjacent structures? The adaptive significance of certain structures, key innovations or novelties, may exert considerable influence on the phenotypic evolution of body plans across entire clades. We explored whether a key innovation, air-breathing organs, influence body shape modularity and evolution in the labyrinth fishes (Anabantaria). The anabantarian fishes exhibit a suite of different air-breathing structures, from vascularized pharyngeal sacs to complex air-breathing organs called labyrinth organs. We radiographed 155 species and 402 individuals from all

major clades and then used 2D geometric morphometrics to characterize changes in body shape across air-breathing and non air-breathing lineages. We tested whether air-breathing lineages exhibited greater degree of phenotypic integration than non air-breathers at both intraspecific and interspecific (macroevolutionary) scales. We also tested whether morphospace occupation was inherently different among air-breathing and non air-breathing lineages. Anabantarians show some indications of an early burst in body shape disparity and we found differences in morphospace occupation among air-breathing and non air-breathing lineages. However, we found near identical magnitudes of integration among air-breathers and non air-breathers, both phenotypically and evolutionarily. This suggests two seemingly disjunct conclusions: (1) that phenotypic patterns of modularity can be mirrored across biological scales and that (2) these modes of phenotypic change are not enough, in isolation, to explain macroevolutionary patterns.

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### **One Man's Crime is Another's Study: Using confiscated snakes to study extreme starvation**

*Brandon Kong, Robin Bedard, Haley Moniz, Savannah Weaver, Amanda Avrit, Ryan Singer, Emily Taylor*

The ability of some snake species to withstand periods of a year or more without feeding have made them particularly interesting subjects in the study of fasting and starvation. However, due to ethical constraints on experimentation, the upper bounds of their capacity for starvation are not well explored. In this study, we utilized 53 Pacific Rattlesnakes (*Crotalus oreganus*) confiscated by the California Department of Fish and Wildlife to produce a novel dataset that allowed us to investigate the effects of extreme starvation. The snakes were confiscated from a collector who had hoarded them in poor conditions for up to a year or more. The specimens ranged from normal body condition to severely starved. We dissected the snakes to harvest organs (e.g., heart, liver, gallbladder, kidneys) and bones (ribs and vertebrae). We measured the wet mass and dry mass of the organs to assess starvation effects on organ size and tissue water content. A Rockwell testing machine was used to determine how starvation affects bone hardness and a tensile testing machine was used to discern effects on bone strength. We found that the wet mass of most organs decreases with starvation. Additionally, we show that the tissue water content of organs decreases with degree of starvation. Bone integrity also diminishes as starvation progresses. Although these results were expected, our findings shed light on specific effects of extreme starvation on the skeletal system and multiple organ systems of a highly fasting-adapted species. Our findings provide insight into snake starvation at their limits.

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### **Arctos: Bridging the gap between museums and zoos with the multidimensional extended specimen**

*Michelle S. Koo, Mariel Campbell, Emily Braker, Elizabeth Wommack, Andrew Doll, Lindsey Frederick, Teresa Mayfield-Meyer, Carla Cicero*

Natural history museums and zoos share important missions in herpetological research, education, and conservation. However, much of the historic and current gulf between the two institution types can be characterized as either cultural and social or logistical and technical. We will focus here on how the online platform Arctos, by leveraging a multidimensional extended specimen concept, can address these challenges and thus bridge some of the gaps between these biodiversity institutions. For example, Arctos can manage living entities and track related samples with links to external databases over time and space. Its connections to global online public aggregators and use as a discovery platform ensures that researchers have access to the invaluable data and resources for biodiversity and conservation studies. With its fine-scale permissioning and thematic hubs of dynamic data, Arctos provides access to related, allowable information and a web of knowledge focused on entities or specimens to many different kinds of users, from students to agencies. The Arctos consortium is a community of professionals with a track record of incorporating new collection types and functional needs in a supportive, community-based decision-making process. In other words, we have experience in bridging gaps and making connections. While some aspects are still nascent, the Arctos infrastructure is built over two decades of robust development, and we anticipate connections between zoos and Arctos consortium

museums will continue to grow with the increasing need to make these critical partnerships between institutions that have more in common than its current divisions.

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### **AmphibiaWeb: Connecting Amphibian Lovers to Data**

*Michelle Koo, Carol Spencer, Vance Vredenburg, Rebecca Tarvin, Ann Chang, Joyce Gross, Rayna Bell, Molly Womack, Emma Steigerwald, David Blackburn, David Cannatella, Alessandro Catenazzi, Kyle Summers, Jodi Rowley, Jimmy McGuire*

Amphibians are the most threatened vertebrate group on the planet and have been seen as harbingers of the Sixth Mass Extinction. It is with this alarm call that AmphibiaWeb was founded in 2000 and has been operating uninterrupted since then. AmphibiaWeb ([amphibiaweb.org](http://amphibiaweb.org)) is a non-profit initiative whose mission is to connect people around the world by synthesizing and sharing information about amphibians to enable research, education, and conservation. We do this by providing up-to-date scientific information on amphibian natural history, conservation, taxonomy, and global declines. The website synthesizes information from scientists and experts with the latest in biodiversity informatics. Features include: up-to-date tracking of newly described amphibian species, expert-based species accounts for almost 35% of the world's amphibians, some in multiple languages; dynamic species range and occurrence mapping; detailed pages on amphibian declines and its causes with ongoing literature updates; community-contributed multimedia files (sound, video and photos); and a community of scientists responsive to the latest taxonomic and research developments in amphibian biology and conservation. Here we will provide a guide to effectively use this amphibian resource. The current list of AmphibiaWeb photographers, species account and content writers is growing and we welcome new contributors. We will outline how herpetologists like you can contribute and share your knowledge and photos!

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### **Genomic and Morphometric Analyses of Diversification Dynamics in the *Pristurus rupestris* Species Complex**

*Thore Koppetsch, Bernat Burriel-Carranza, Benjamin Wipfler, Elizabeth Glynne, Xiao-Zhu Luo, Dean Adams, Michael Matschner, Salvador Carranza*

Numerous species of the ecologically diverse gecko genus *Pristurus* can be found across Afro-Arabia, offering great opportunities to study diversification dynamics. Previous studies indicate that particularly the *Pristurus rupestris* complex includes high levels of undescribed diversity. Here, we performed state-of-the-art population genomic and phylogenomic analyses based on datasets including ddRADSeq data and whole-genome sequencing (including a reference genome for *P. rupestris*) to investigate if and how diversification dynamics are associated with the presence of cryptic phylogeographic lineages in this complex across Arabian mountain ranges. Zones of gene flow within the *P. rupestris* complex have been identified, and species delimitation analyses revealed the presence of five distinct taxa. Molecular species delimitation results have been corroborated with morphological data in order to clearly define the tempo and mode of cryptic diversification. To understand phenotypic adaptations and their underlying constraints, 86 individuals representing 16 genetically identified lineages have been studied with  $\mu$ -computed tomography. Based on these scans, 3-dimensional models of skulls and limbs were created and subsequently used for geometric morphometrics. Newly identified species are described based on the analysis of phenotypic and molecular data. In addition, the retrieved morphological results of the landmark-based 3D shape analyses will be linked with a broad set of ecological parameters, and are aligned with the retrieved phylogenomic tree from the molecular analyses. This sophisticated methodological approach of studying associations or interactions of morphological traits with dynamics of diversification allows to trace the different adaptation patterns and evolutionary trends within a single species-rich and phenotypically diverse clade.

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### **Impacts of Microplastics Exposure on Tadpole Digestive Tracts**

*Scott Kornfeind, Sara McClelland*

Microplastics (MPs) are defined as plastic fragments between 0.1  $\mu$ m - 5 mm in dimension. MPs are a common pollutant found in multiple environments throughout the world. MPs are increasingly found in the aquatic habitats of toads, frogs, and tadpoles leading to direct MPs exposures. Previous work in marine fish has shown that MPs cause damage to the gills, buccal cavities, and guts. The research on this is limited in amphibians. To begin filling this knowledge gap we exposed Northern Leopard Frog tadpoles (*Lithobates [Rana] pipiens*) to an environmentally realistic concentration of MPs (0.5 ppb, 34-50  $\mu$ m). We then analyzed the impacts that the MPs had on the gut tract. In an additional experiment, tadpoles were exposed to different concentrations of fluorescent MPs (38-45  $\mu$ m) to visualize the location of MPs throughout the gut. Results from this study provide new insights into how MPs may be affecting amphibian development in contaminated habitats.

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### **An Integrative Data Approach to Modeling the Migratory Patterns of the Shortfin Mako in the Western North Atlantic**

*Colby Kresge, Jeremy Vaudo, Cami McCandless, Enric Cortes, Mahmood Shivji, Bradley Wetherbee*

The shortfin mako shark (*Isurus oxyrinchus*) is a widely distributed oceanic species frequently caught as bycatch in commercial fisheries and heavily targeted by recreational fishers. Because of declines in mako populations, there is a great deal of interest in reducing fishing mortality to rebuild stocks. Migratory patterns of mako sharks are poorly understood, which detracts from informed management to assist recovery of their populations. Assessment of stock status and migratory pathways of marine fishes relies heavily on fishery-dependent data; however, such information is often biased towards locations where fishing is concentrated. We investigated the migratory patterns of mako sharks in the Western North Atlantic Ocean using data derived from both fishery-dependent and -independent sources, including the Pelagic Longline Observer Program, Cooperative Shark Tagging Program, and satellite transmitters. Each data source has shortcomings for modeling migratory pathways of mako sharks associated with methodology of data collection. An integrated approach to model migratory routes of mako sharks based on data combined from all sources provided a more robust model of migration patterns than any single source of data. Mako sharks exhibited seasonal shifts in distribution as well as discrete core areas of habitat occupied throughout the year. Examination of the major data sources that have been historically used to document the spatiotemporal distribution of mako sharks in the Western North Atlantic reveals a number of gaps that contribute to an incomplete understanding of their migratory patterns.

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### **On the Origin of Patterns of Temperature-Dependent Sex Determination**

*Caleb Krueger, Fredric Janzen*

Species with temperature-dependent sex determination (TSD) exhibit significant variation in the relationship between incubation temperatures and the sex ratios they produce, making this an ideal system for comparing processes producing variation above and below the species level. Furthermore, a deeper mechanistic understanding may help reveal the currently unknown adaptive significance of this variation or of TSD as a whole. Here we probe these topics by examining the macro- and microevolutionary dynamics of this sex-determining mechanism in turtles. Our ancestral state reconstructions of discrete patterns of TSD suggest that producing females at cool incubation temperatures is derived and adaptive. However, the ecological irrelevance of these cool temperatures and a strong genetic correlation across the sex-ratio reaction norm in *Chelydra serpentina* both contradict this interpretation. We further find the phenotypic consequence of this genetic correlation in *C. serpentina* reflected across all turtle species, suggesting that a single genetic architecture underlies both intra- and interspecific variation in TSD in this clade. This correlated architecture can explain the macroevolutionary origin



of discrete TSD patterns without assigning cool-temperature female production an adaptive value. However, this architecture may also constrain adaptive microevolutionary responses to ongoing climate change.

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## ROSIE, a Database of Reptilian Offspring Sex Ratios and Sex-Determining Mechanisms

*Caleb Krueger, Fredric Janzen*

In contrast to genotypic sex determination (GSD), temperature-dependent sex determination (TSD) in amniotic vertebrates eludes intuitive connections to Fisherian sex-ratio theory. Attempts to draw such connections have driven over 50 years of research on the evolution of sex-determining mechanisms (SDM), perhaps most prominently among species in the order Testudines. Despite regular advancements in our understanding of this topic, no efforts have been published compiling the entirety of data on the relationships between incubation temperature and offspring sex in any taxonomic group. Here, we present the Reptilian Offspring Sex and Incubation Environment (ROSIE) database, a comprehensive set of over 7,000 individual measurements of offspring sex ratios in the order Testudines as well as SDM classifications for 149 species. As the name suggests, we plan to expand the taxonomic coverage of ROSIE to include all non-avian reptiles and will regularly release updates to maintain its comprehensive nature. This resource will enable crucial future research probing the ecology and evolution of SDM, including the presumed sensitivity of TSD to rapid environmental change.

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## The Scientific and Conservation Value of Collaborating with Shark-based Ecotourism Operators in Florida, USA.

*Gretchen Kruizenga, Deborah Menna, Cassandra Scott, Hannah Medd*

Shark-based ecotourism has the potential to expand our understanding of shark biology, ecology, and conservation. However, it may also negatively impact sharks at individual and population levels based on the physiological and behavioral responses of the specific species involved in the interactions at the activity site. There is a high concentration of shark-based ecotourism operators that use an area of approximately 135 km<sup>2</sup> in Federal jurisdiction along the Atlantic coast of southeast Florida, USA. A subset of the current seven operators has contributed to a long-term data collection program recording environmental variables and observations of shark species diversity between 2016 – 2021. They recorded fourteen elasmobranch species, including the smalltooth sawfish (*Pristis pectinata*) which is listed on the Endangered Species Act. Of the species observed on more than 5 dives over multiple years, 93% are within the Threatened Categories of the IUCN Red List. A collaboration with this industry represents an opportunity for species-specific and interspecific research with high conservation value. We also conduct detailed surveys using non-invasive techniques including paired laser photogrammetry and in-water sample collections, including a citizen science project for underrepresented students. This collaboration led to the development of a Code of Conduct for the operators to minimize their impacts on the sharks, one of the first steps of self-regulation. We are also co-producing studies to determine the long-term effects of these activities in a limited geographic area. These are the crucial first steps to mitigating the consequences of the effects of ecotourism.

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## Which Species of Pseudolaguvia (Sisoridae: Siluriformes) are Present in Nepal?

*Kole Kubicek, Heok Hee Ng, Kevin Conway*

Members of the genus *Pseudolaguvia* are small bodied crypto-benthic sisorid catfishes found in hill streams and large rivers of the Himalayan, Shan Hills and Western Ghats mountain ranges. The genus currently comprises 24 valid species, most of which have been described only within the past 10 years based on morphometric characters, coloration and external morphology (e.g., shape of the thoracic adhesive apparatus). Despite these diagnosable characters, the identification of species of *Pseudolaguvia* can be complicated by their small body size (~ 30 mm SL) and overall similarities in appearance as well as the relatively few records of occurrence available for each species. The recent examination of *Pseudolaguvia* material collected from the Ganges

River drainage of Nepal has led to the discovery of specimens resembling *P. shawi*, a species which has not previously been reported to occur in Nepal and is found primarily in the Brahmaputra River drainage. To date, only 4 nominal species (*P. assula*, *P. kapuri*, *P. nepalensis* and *P. ribeiroi*) have been recorded from the Ganges river drainage in Nepal. Based on available data and original species descriptions we suspect that *P. nepalensis* is a junior synonym of *P. assula*. To clarify which species of *Pseudolaguvia* are present in Nepal, we provide a detailed osteological comparison of the members of this genus known to occur in Nepal. Our analysis of micro-CT data has revealed several distinct and previously undocumented osteological characters that can be employed in the identification and delimitation of *Pseudolaguvia* spp.

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## Phylogeographic variation in the *Plethodon wehrlei* complex

*Shawn Kuchta*

Species delimitation is critical for biodiversity studies, yet species complexes characterized by high levels of population structure and subtle phenotypic differentiation can be challenging to delimit. Here, I report on a preliminary molecular systematic investigation of woodland salamanders in the *Plethodon wehrlei* species complex. In an earlier study using mitochondrial DNA and five nuclear loci, I found substantial phylogeographic diversity in this complex, including multiple geographically cohesive clades. In this study, I revisit patterns of genetic variation in the *P. wehrlei* complex using 350 orthologous genes sequenced using anchored hybrid enrichment. Using species tree approaches, analyses of population clustering, and other methods, I evaluate patterns of phylogeographic variation in the *P. wehrlei* complex. Species formation via fragmentation will be briefly discussed.

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## Historical climate change structures contemporary patterns of diversity in widespread insular snakes

*Arianna Kuhn, Sara Ruane, Isaac Overcast, Frank Burbrink*

Oceanic islands are considered natural laboratories for studying evolution. The entire biota of these islands is derived from a few initial colonization events followed by local diversification, which generates high levels of endemism and ecomorphological differentiation. Thus, the biota of oceanic islands can be interpreted as the result of successful independent evolutionary experiments starting with a single or multiple colonization events from the continent. Using sub-genomic data from population level sampling across the island's snakes within a comparative statistical phylogeographic framework, we investigated patterns of population structure and pulses of simultaneous demographic shifts for Madagascar's pseudoxyrhophiine snakes to determine if demographic histories been individualistic or synchronous relative to Pleistocene climate change. We identified 21 populations with a high probability of recent expansion and 12 with a history of population bottleneck. We conducted a hierarchical Random Forest analysis to estimate the proportion of lineages experiencing synchronous demographic dynamics and the timing of these events. For expanding populations, we recovered a strong signal of synchronous expansion in the Late Pleistocene after the Last Glacial Maximum. Contracting lineages showed evidence of temporally concordant bottlenecks before the onset of widespread anthropogenic Holocene fire disruption, indicating historical climate was more important in structuring contemporary patterns of diversity. Lack of geographic, ecological and taxonomic signal in sensitivity to climate change suggests that areas of past demographic stability and potential resilience to future climate change for broadly distributed taxa may be more influenced by localized habitat features and species interactions than the biogeographic histories and ecoregion generalizations.

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## The Population Level Response of a River Breeding Frog to Dam Removal

*Sarah Kupferberg*

Dam removal provides opportunities for river restoration, but also presents challenges to keep sensitive fauna out of harm's way during deconstruction and monitor populations as instream physical habitat changes post-demolition. Such was the case for Benbow Dam (South Fork Eel River, Humboldt Co., CA). Constructed in 1931 to generate hydropower and acquired in 1958 by California State Parks, it was operated seasonally until 2008 to create a

lake for recreation. Between 2008 and 2016 when dismantling began, Foothill Yellow-legged Frogs (*Rana boylii*) colonized the river flowing through the former lakebed. To avoid harming frogs during demolition (13,000 yd<sup>3</sup> of concrete removed), egg masses were relocated in spring at weekly intervals via kayak to safe sites, and during summer construction season, temporary block nets were used while clearing areas of tadpoles and frogs. Before-After-Control-Impact comparisons of reproductive output (number of clutches laid per river km) indicate that these strategies were successful. The population in Benbow State Park has grown relative to an upstream control reach on the Angelo Coast Range Reserve. Breeding aggregations shifted spatially when sediment movement after dam removal made some areas too swift and shallow for egg deposition, but reproductive output over a 3.4 km reach remained robust despite a 30% decrease in the first year post-removal. At the State Park, pre-removal mean ( $\pm$ SE) output increased from 105 $\pm$ 44 clutches per km (2012-2016), to 199 $\pm$ 6 post-removal (2017-2022). Control site densities did not change significantly; 90 $\pm$ 9 pre- and 105 $\pm$ 10 per km post-removal.

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## Antimicrobial Host-defense Peptides in Appalachian salamanders

*Steve Kutos, Randall Jiménez, Owen Osborne, Amy Ellison, Brian Gratwicke, Timothy Cleland, Carly Muletz-Wolz*

Animal immune systems interact and respond to many external factors including microorganisms. Immune related host-defense peptides can act as an innate barrier and are useful for animals to attack pathogenic microbes occurring on the epithelial surfaces, while allowing beneficial or commensal microbes to remain. Host-defense peptides from many amphibians are still unclassified, particularly in salamanders. Therefore, we explored host-defense peptide diversity of red-backed salamanders (*Plethodon cinereus*, n=31), two-lined salamanders (*Eurycea bislineata*, n=25), and eastern newts (*Notophthalmus viridescens*, n=35) sampled in Maryland and Virginia, USA at 12 sites. We quantified overall peptide yields from skin soaks of acetylcholine-stimulated and control salamanders, and then used mass spectrometry, transcriptomics (n=13 salamanders) and antimicrobial peptide database querying to identify candidate host-defense peptides. We found that acetylcholine led to increased peptide yields and greater identification of peptides in mass spectrometry compared to our control group. In transcriptomic data, each salamander species had 10-20 transcriptionally active candidate host-defense genes (including ubiquitin and cathelicidins). When we used mass spectrometry to detect host-defense peptides, most individuals did not have any identified (68/93 salamanders). Overall, the host-defense peptides were generally unique in each salamander species; red-backs produced Plastacin-like peptide, eastern newts Lividin- and Maximin- like peptides, and two-lined salamanders produced a wide variety of peptides. Data suggests that three Appalachian salamander species produce host-defensive peptides, especially two-lined salamanders, which can interact with the microbes colonizing the skin. Further understanding of host-defense peptide diversity released by amphibians could be critical to understanding skin disease outcomes.

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## Mosaic Evolution in Gymnotiform Brains due to Natural Selection and Phylogenetic Constraints.

*Shannon Kuznar, James Albert*

The Amazon and adjacent river basins are hotspots of diversity and species richness, home to the most diverse fauna of continental freshwater fishes on Earth. Unique systems of survival are given the opportunity to develop and expand in these evolutionarily nurturing environments, exemplified by Gymnotiformes, an electric fish clade found throughout tropical Central and South America. These fishes evolved a novel behavior: active electroreception, in which they generate and detect weak electric fields for use in navigation, foraging, and communication. Brain morphology within this clade is likewise modified to accommodate these behaviors, such as presence of the electrosensory lateral line lobe and expanded cerebellum. Additionally, this group occupies wide habitat and trophic ranges, from floodplain lakes to deep river channels and piscivorous to planktivorous. Here we examine patterns of brain evolution using a 3D landmark-based topology alongside volumetric allocation. We use contrast-stained computer tomography (CT) scans representing 23 gymnotiform species and 17 genera to visualize brain

regions defined by functional, structural, and developmental criteria. We use generalized Procrustes analysis and principal components analysis (GPA/PCA) to generate a phylomorphospace of the clade and multidimensional scaling to compare volumetric allocations of brain regions to habitat and trophic traits. We expect natural selection to be the greater influence of brain morphology if species occupying similar habitat types and trophic levels cluster in the morphospace, regardless of phylogeny. Clustering between closely related species is expected if a hypothesis of phylogenetic constraint, perhaps due to the specialized neurobiology of Gymnotiformes, is a better model.

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## Assessing populations of spotted seatrout in the Texas bay systems using genotyping-in thousands by sequencing.

*Michaela Labare, Alison Monroe, Katherine Lanoue, Christopher Hollenbeck, David Portnoy*

Spotted seatrout (*Cynoscion nebulosus*) is an important recreational estuarine fish that inhabits the U.S. South Atlantic and Gulf of Mexico. Overfishing concerns led Texas Parks and Wildlife Department (TPWD) to begin a seatrout stocking program in 1993 that releases captive-bred fingerlings into the major Texas bays. Aquaculture-based enhancement can be effective in maintaining populations. However, these efforts need accurate, cost-effective, and time-efficient means of monitoring their success. Genetic monitoring that involves genotyping of hatchery brood stock and wild-caught individuals can be used to identify familial relationships, assess genetic variation, and determine population demographics. We designed a Genotyping-in Thousands by sequencing (GT-seq) panel, which allows thousands of individuals to be genotyped at hundreds of SNP-containing loci for a modest cost. We performed parentage and kinship analyses to determine hatchery contribution to wild populations as well as brooder contribution to an array of fingerlings. We also assessed patterns of variation within and among Texas Bays and characterized standing genetic diversity. This work contributes to a larger project to assess fine and large-scale population structure of spotted seatrout across the Gulf of Mexico and identify localized adaptation, to inform management and stocking strategies.

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## Monitoring the Population Status of *Crotalus adamanteus* in North Carolina Using the AHDriFT Camera Trap System

*Emily Langer, Matthew Gacheny, Jayme Waldron, Shane Welch, Emily Gaydos*

Eastern diamondback rattlesnakes (EDB; *Crotalus adamanteus*) are endemic to southeastern pine savannas and woodlands, a fire-climax system that includes longleaf pine (*Pinus palustris*) wiregrass sandhills, clayhills, and flatwoods. The northern extent of the EDBs range is in Onslow County, NC at the Marine Corps Base Camp Lejeune, where individual-based monitoring efforts have used visual searches to locate and capture individuals for mark-recapture and radio telemetry surveys. The EDB is cryptic and difficult to detect, however, making visual searches impractical for surveying large study landscapes like the MCBCL. To complement current EDB monitoring efforts at the MCBCL, we built 20 Adapted-Hunt Drift Fence Technique (AHDriFT) arrays that spanned 17 training compartments. We affixed a Reconyx Hyperfire 2, PIR camera at each end of the 50-foot arrays to capture images of individuals moving through the trap. Images were stored and analyzed using the camtrapR package in RStudio. We will use camera trap data to estimate site occupancy relative to habitat suitability and prey abundance. The MCBCL EDB population is the northern-most limital EDB population, and occupancy models from this population will help identify covariates that limit the species' distribution and help land managers to prioritize conservation efforts for EDBs.

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## Does arboreality influence the evolution of hydrothermal physiology in neotropical frogs?

*Julia Laterza Barbosa*

The relationship between organisms and their climate is a complex one, and it is influenced by hydrothermal physiology, behavior and evolutionary history. Microclimatic variation at a local scale provides a variety of conditions that animals can select, and in that way influence selective pressures that act

on physiology, particularly for small ectotherms such as anurans. One of the major sources of microclimatic variation in forested environments is provided by trees, since temperature and humidity vary vastly along the ground-to-canopy microhabitats. Therefore, arboreal habits and microhabitat selection will influence the evolution of physiological traits. I have collected detailed microhabitat use of frogs from ground to canopy in two localities in the Atlantic forest of Brazil, in addition to characterizing the vertical temperature and humidity gradient using data loggers. Then I obtained critical thermal limits, dehydration and rehydration rates for 30 species of frogs and used phylogenetic comparative methods to test the hypothesis that frog perch height influences evolution of physiological traits.

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## Where Temperature and UV Collide: Photoregulation Trade-Offs in Divergent Microhabitats

*Matthew Lattanzio*

Over a century of studies on lizard physiological ecology have perpetuated the assumption that so-called behavioral 'thermoregulation' between sunlit and shaded microhabitats is driven by temperature needs alone. However, those basking and shuttling behaviors also modulate access to ultraviolet (UV) wavelengths of light, which are essential for proper bone development, organ function, survival, and reproductive success. Recently, my lab has shown that lizards can actively regulate their UV exposure and may also prioritize UV needs overall, even when facing thermal costs. However, the interaction between competing UV and thermal demands, particularly in extreme environments, remains unclear. My prior work on high-elevation (>2,000 m) mountain spiny lizards (*Sceloporus jarrovi*) during 2012-2017 revealed a clear microhabitat use preference for cooler, shadier edge areas over hotter, sun-exposed boulders. For example, lizards using edge areas tend to exhibit better performance capacity (bite force and stamina), lower body temperatures, and greater social network connectivity than lizards in the open areas. However, because UV exposure requires direct sunlight, exploitation of shadier edge areas should limit access to UV light. Here I expand on prior work to assess trade-offs in UV and temperature regulation efficiency by *S. jarrovi* across these divergent microhabitats. My findings should reveal whether lizards exploiting preferred edge microhabitats sacrifice UV over temperature regulation efficiency and/or whether boulder microhabitat lizards prioritize their UV regulation despite potential thermal costs. Ultimately, my findings should provide novel insight into how a species balances potentially competing UV and thermal demands across divergent microhabitats.

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## DIY Herpetology: Using Hobby Electronics to Enhance Innovation and Accessibility in Scientific Research

*Matthew Lattanzio*

One of the biggest constraints on field and lab research endeavors is the cost associated with purchasing expensive data loggers and/or sensor devices. In some cases, the ability to ask integrative, novel research questions is limited by that constraint, or not possible if, for instance, the needed equipment does not exist (yet). Appreciation for the utility of hobby electronics (e.g., Arduino, Raspberry Pi) for scientific research has grown in recent years due to their affordability, versatility, and comparable accuracy to often expensive commercial devices. These electronics can also be easily assembled and programmed to perform various functions by novices and experts alike. Here, I discuss my recent dive into hobby electronics for lab and field use, focusing mainly on two systems that have 1) consolidated data collection for a field study on salamander ecology and 2) enabled us to directly assess behavioral regulation of UV light exposure by lizards in the lab and field. I also provide insight into the process itself, including programming, assembly, and validation of homemade devices, highlighting the supportive user community as well as the challenges I encountered along the way. Overall, I argue that hobby electronics offer an exciting avenue for scientists to address novel and often underappreciated aspects of organismal biology and physiology, in a manner that maximizes information gain while minimizing costs to the researcher. More broadly, I argue that the normalization of using low-cost hobby electronics in scientific research will help make doing science more accessible to a broader audience.

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## Comparative Osteology of the Kukri Snake genus *Oligodon* Fitzinger, 1826 (Squamata: Colubridae)

*Justin Lee, Aaron Bauer*

Kukri snakes (*Oligodon* Fitzinger, 1826) represent the most diverse radiation of snakes on the Asian continent, and the second largest snake genus in the world. Besides its remarkable species richness, members of *Oligodon* possess intriguing ecological attributes, including unique feeding strategies, seasonal territoriality, and Batesian mimicry with venomous snake species. In an attempt to understand the morphological underpinnings of these behaviors, we used high resolution computed tomography scanning to describe and digitally reconstruct the skulls of over 30 kukri snake species, encompassing all major clades recognized by past authors. Enlarged 'blade-like' maxillary teeth are found across most species. In contrast, the number of teeth on the pterygoid and palatine is considerably variable and in some species these bones are completely edentulous. Intrageneric variation was also noted in other elements of the skull, such as the shape of the premaxilla, relative size of the neurocranium, and robustness of the suspensorium and mandible. Taken together, we provide insights on how osteological features found in *Oligodon* might correlate with aspects of feeding and habitat ecology. We also summarize several characters that may be useful for kukri snake systematics.

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## Quantitatively Exploring Morphological Patterns of the Cephalic Lateral Line among Major Lineages of Osteichthyes: A Preliminary Finding

*Derrick Leong, Zehua Zhou, Juan Liu*

The cephalic lateral line system includes enclosed canals and superficial neuromasts allowing fish to detect local motion in water. While the diversification of cephalic sensory canals (SC) has been reported, their morphological complexity has largely been unexplored with a quantitative framework. To explore how skull roof bones covary with SC, we use the supraorbital sensory canals (SOSC) and associated bone as a model system. We scanned alcohol-preserved and skeletal specimens from 11 extant actinopterygian taxa and two fossil specimens using X-ray Computed Tomography (GE Phoenix Nanotom). The skull roof bones and SOSC were segmented using Avizo, and analyzed using 3-D geometric morphometrics methods with two landmark schemes: 12 landmarks to represent the SOSC shape and their distance to bone junctions along the midline, and 18 landmarks which additionally captured associated bone morphology. First, principal component analyses distributed taxa in a morphospace created by PC1 and PC2 (PC1 36.9%, PC2 22.1%; PC1 30.1%, PC2 22.7%, respectively), in which sarcopterygians and actinopterygians consistently occupied different regions. However, from subsequent morphological disparity analyses, only the dataset with landmarks including bone morphology showed significant differences when comparing sarcopterygians with neopterygians, teleosts and euteleosts (p-value 0.03, 0.034, 0.049, respectively). Our results suggest that the morphology of SOSC is relatively conserved, and their diversification may have co-evolved with the bones. Although this empirical study requires an expanded dataset to observe ecological or phylogenetic trends, it inspires further investigation of cephalic sensory canals variations and their implications of vertebrate evolution.

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## Estimating Whole-Body Metabolic Rate from a Point Measurement at the Gills in Sandbar Sharks

*Andrea Leontiou, Jonathan Cohen, Aaron Carlisle*

As water breathing animals, shark's gills are the primary site of oxygen uptake for aerobic metabolism. Studies on shark oxygen consumption rates provide insight into many physiological processes; swimming efficiency, bioenergetics, and overall fitness. The ability to determine whole body metabolic rate from a single point measurement taken at the gill will make it possible to better capture accurate field based metabolic rates. These field-based rates will be more representative of an animal's actual metabolic demands through time in a dynamic environment. Currently, metabolic rates determined from laboratory-based experiments in conventional respirometers are limited by size constraints, and biased by the stress of handling and the lab environment. In this study, we compare estimates of metabolic rate obtained from a point measurement of oxygen consumption at the gill to whole animal standard

metabolic rates (SMR) estimated using standard static respirometry in Sandbar Sharks (*Carcharhinus plumbeus*). We use the point measurement of oxygen consumption, area of gill openings, estimates of the size of gill surface area, and flow rate to calculate a metabolic rate taken via oxygen consumption at a single gill. Multiple methods of calculation were then tried to determine if this rate could be comparable to the whole-body metabolic rate acquired from the tank itself. Our results suggest that it is possible to calculate an animal's whole body metabolic rate from a single gill measurement. These data will set the ground work for better methods of collecting metabolic rates from gill breathing animals in the wild.

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## Understanding Species Composition of Fish Maw in China— for Future Trade Monitoring, Fishery Improvement & ETP Species Conservation

*Baian Lin, Min Liu*

Fish maw is made of the swimming bladder and has long been consumed as a luxury and nourishing food for its supposed nutritional and medicinal benefits within Chinese communities. Historically, the maw has been exclusively made from the swimming bladder of a few croaker species (Acanthuriformes: Sciaenidae). However, increased demand for fish maw has led to large-scale global international trade with 100 countries currently exporting fish maw to China. In order to understand the species composition of fish maw for future trade monitoring, fishery sustainability, and effective management and conservation, we collected 907 maw samples in 2016-2022 from various trade sources in China and applied molecular techniques for species identification. A total of 73 species from 17 families and 8 orders were recognized, including 34 croaker species and 15 catfish species. More than 50% of the species were estuary-associated and more than 60% of species were large-sized (>0.8m SL). Among the 73 species, only three species have stable population status, according to IUCN red list, and 21 species show a decreasing population size. Currently, only one species has been sustainably managed in the fishery in its distribution range. Eleven endangered species (IUCN: CR, EN, VU) and four protected species (by international or national regulations) were found in the fish maw trade, suggesting the need for further management from fisheries to wildlife trade. Based on the natural distribution of traded species we identified here, Southeast Asia and Northeast South America are the hotspots for fish maw fisheries activity. Our study serves as a baseline to understand the species composition in fish maw trade and calls for attention to the fishery management of endangered, threaten and protected (ETP) species to ensure resource and biodiversity sustainability.

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## Endemic and Endangered: Conservation of Fishes of the Galápagos

*Christi Linardich, Kent E. Carpenter*

Marine fish endemism in the eastern tropical Pacific is highest in the isolated environments of the Galápagos Archipelago (Ecuador), which is also a global-level biodiversity hotspot. Some additional species occur elsewhere only in the adjacent oceanic islands of Malpelo (Colombia) and Cocos (Costa Rica). To promote sustainable management in this area, a decree that expanded the Galápagos Marine Reserve and a multi-national agreement to preserve the marine corridor connecting these island groups were announced in 2022. To inform the planning process for these conservation efforts, the IUCN Marine Biodiversity Unit led an initiative to identify priorities by producing IUCN Red List extinction risk assessments of all endemic marine fishes. A Red List assessment workshop was held in the Galápagos in September 2022 with 20 marine fish scientists. The 84 marine endemics, which is represented by 36 families, primarily inhabit rocky reef and nearly half occur at deep depths >50 m. Preliminary results indicate 8%, or 7 species, are threatened, and 21%, or 18 species, are Data Deficient. Specific highlights include overfishing of the restricted range Sailfin Grouper (*Mycteroperca olfax*) and Camotillo Sand Bass (*Paralabrax albomaculatus*), the possibly extinct Galápagos Damselfish (*Azurina eupalama*), the status of recently discovered deep fishes and the need to survey diminutive inshore fishes, especially the Intermediary Blenny (*Cottoclinus canops*) of Isla Española. Actions that prevent biodiversity loss

in the Galápagos should include improving fishery management, sustaining/increasing marine monitoring work and supporting international policies that increase resilience in the face of climate change.

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## Seasonal Physiological Responses to Experimental Immune Challenge in a Snake Population Afflicted with Ophidiomycosis.

*Craig Lind, Joseph Agugliaro, Jason Oretaga, Jenna Palmisano, Terence Farrell*

In wildlife, successfully coping with infection requires the coordination of a suite of physiological processes. Seasonal patterns in temperature, reproduction, and disease may constrain physiological coping responses and impact disease outcomes. We currently know little about physiological responses to infection in reptilian wildlife, and we know even less about how coping processes vary with respect to seasonal biotic and abiotic conditions. To examine how seasonal factors impact the immune response, we used a sterile antigen, bacterial lipopolysaccharide (LPS), to induce a physiological immune response in pygmy rattlesnakes, *Sistrurus miliarius*, housed in outdoor mesocosms in winter and summer. We measured components of the stress response (corticosterone and glucose) and acellular immune performance (bacterial killing assay) in plasma taken from 104 individuals exposed to either LPS or a saline control. In a subset of snakes, we implanted temperature dataloggers to monitor thermal responses to LPS. The sampled population included snakes afflicted with an emerging mycosis, ophidiomycosis, in both seasons and pregnant females in the summer. We hypothesized that the demands of ophidiomycosis and pregnancy would impair responses to LPS. Counter to our hypothesis, LPS induced an increase in corticosterone in snakes of all disease and reproductive statuses. Immune challenge did not result in a significant glycemic or acellular immune response in any group. Immune challenge elicited a significant febrile response in both summer and winter, but thermal responses were constrained by differences in seasonal operative environments. Our results highlight thermal and glucocorticoid responses as potentially important disease coping mechanisms across seasons.

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## Cycling Temperature Treatments Affect Estimates of Digestive Performance in Prairie Lizards (*Sceloporus consobrinus*)

*Allison Litmer, Steven Beaupre*

In nature, organisms experience a range of body temperatures over the course of a day or season. Ectotherms typically experience warmer daytime and colder nighttime temperatures, due to increased difficulty thermoregulating at night. Many physiological studies measure performance at stable temperature treatments and extrapolate to predict function in a cycling environment. Additional factors, including circadian rhythm and temperature pattern, may influence rates of physiological processes. The current study quantified food consumption, digestive passage time, and metabolizable energy intake (MEI) across three stable temperature treatments (30, 33, 36°C) and two temperature cycles. Cycles rotated through 30, 33, 36°C daily, with equal durations of time at each temperature. Cycle one consisted of warm days and cool nights and cycle two was the reverse of cycle one. For analyses, results at stable temperatures were compiled into a single dataset and compared to cycles. If stable temperatures accurately represent cycling regimes, then performance averages from stable treatments should compare favorably to performance under cycling treatments. Physiological parameters responded differently to temperature treatments. Passage rate was quickest in cycling regimes, while MEI and food consumption varied. Consumption and MEI were similar between stable trials and cycle one (warm days), but comparably lower in cycle two (cool days). Circadian rhythm did not appear to be influential. Metabolizable energy intake is driven primarily by consumption, and whereas lizards passed food quicker with warm days, they did not increase food intake. Physiological data collection requires careful consideration of potential effects of cycling versus stable temperature treatments.



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## A Revision of Paleocene Catfish from Sanshui Basin, South China

Juan Liu, Jiangyong Zhang

Paleocene-Eocene catfish from East Asia were previously assigned to the extant family Bagridae (Siluriformes), leading to a long-standing ghost lineage. Furthermore, two nominal species, *Mystus dalangshanensis* and *Mystus spinipectoralis* (Wang et al 1981), from the Paleocene Buxin Formation in Sanshui Basin, south China represent a 30 Ma gap from the estimated mitogenomic divergence time for *Mystus*. These discrepancies suggest either problematic taxonomic assignment, and/or a 'morphology vs. molecules' conflict exacerbated by an incomplete fossil record. Based on our analyses of new materials from recent fieldwork, we propose a substantial revision of Paleocene Sanshui catfish by removing them from the suborder Siluroidei, and by extension, Bagridae, based on key new observations. First, the principal caudal fin rays of Sanshui specimens are 18 (i,8,8,i), which is a basal character absent in extant catfish (i,7,8,i) except Diplomystidae. The Paleogene <sup>†</sup>Hypsodoridae and <sup>†</sup>Astephidae also have 17. Second, the coronoid process of the dentary is elevated and prominent. This condition is seen only in <sup>†</sup>Hypsodoridae. The moderately developed coronoid process is present in diplomystids and some bagrids, but not in the rest of the siluriforms. Third, interdigitation is absent at the suture of ceratohyal and epihyal, a known synapomorphy of Siluroidei. All these characteristics indicate Sanshui catfish belong to a basal lineage of catfish and suggest early morphological diversification hidden by previous taxonomic assignments. These new findings offer clarification critical to untangling puzzling aspects of the evolutionary history of catfish and highlight the importance of systematic review and revision as evidence of divergence times.

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## Bonnethead shark (*Sphyrna tiburo*) morphology and trophic ecology: A comparison across geographies, sex, and maturity

Kathy Liu, Jasmin Graham, Jayne Gardiner, Sora Kim, Hyejoo Ro, Tonya Wiley, Catherine Macdonald

Bonnethead sharks (*Sphyrna tiburo*), a small hammerhead species recognizable by their rounded spade-shaped head, are the first documented shark species to exhibit both sexual dimorphism in cephalofoil morphology and omnivorous feeding preferences. Males have been found to have a more discernably pointed snout than females. Studies have shown seagrass to be a large component of their diet though this varies across life stage, region, and sex. Bonnetheads are present in shallow tropical and subtropical waters along both coasts of the Americas with sizes being well defined for only a few populations. The bonnetheads in Biscayne Bay, Florida have not been studied in detail and this project is the first in-depth assessment of their characteristics utilizing bonnetheads from Tampa Bay, Florida as a direct comparison to identify differences in cephalofoil shape, gape size, and trophic position. Body measurements and biological samples were collected in the field and both sides of the cephalofoil were photographed to plot specific landmarks in ImageJ. Stable isotope analysis was conducted on muscle biopsies. Preliminary analyses suggest that cephalofoil curvature is significantly different between sexes at both locations. Multiple factors including sex, maturity, eye-to-eye width, girth, and gape size correlate with total length and cephalofoil curvature slope of individuals. This project provides more comprehensive information about bonnethead shark traits and diet across populations and is an initial step in longer-term research on bonnetheads in Biscayne Bay.

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## Swimming beneath the Sahara: the thermal biology of *Scincus scincus*, the sand-swimming skink.

Ethan Livingston, Anya Shevchik, Alyssa Head, Brooke Bodensteiner, Eric Gangloff

Little is known about the natural history of the sandfish skink (*Scincus scincus*), despite its range spanning two continents and being common in the pet trade. In arid and hot environments across North Africa and the Arabian Peninsula, these lizards spend up to 90% of their day beneath sand and are uniquely adapted to this environment. However, much about the basic biology of this species remains unknown, especially in regard to its adaptations to its thermal

environment. With this study, we compiled a robust suite of thermal biology parameters to assess within-individual patterns in sandfish skinks (N = 8). We first quantified thermal preferences and the use of sand horizons to thermoregulate. We monitored individual lizard body temperatures day and night for one week in an environment where they had the ability to select their ideal temperature during both active and inactive periods. Additionally, we quantified the thermal dependence of performance by recording sprinting speed and diving speed, important for prey capture and predator avoidance, across a range of temperatures. Finally we measured the critical thermal minimum (CTmin) and maximum (CTmax), allowing us to build a complete thermal performance curve. This then allowed us to test the hypothesis that thermal preferences and the optimal temperature for diving and sprinting performance are correlated, suggesting co-adaptation of behavior and physiology. Overall, these data provide understanding of how this unique ectotherm survives in the extreme thermal conditions of a scorching hot desert.

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## Stress Responses of *Sternotherus odoratus* to Urbanization in Southeastern Louisiana

Oliver Ljustina, Florence Wen, Brandon Hedrick

Like most vertebrate species, herpetofauna typically respond poorly to habitat modification associated with urbanization. Species that persist in urbanized environments are subjected to a variety of potential stressors, including environmental pollutants, exotic predators, road mortality, inadequate nutrition, among others. However, these negative impacts may be offset by factors such as resource subsidies and a potential decrease in native predators. While many turtle species occur in urbanized areas throughout the southeastern United States, negative impacts on individuals and populations may be obscured by their relatively long lifespans. We hypothesized that individuals from an urban population of Eastern Musk Turtle (*Sternotherus odoratus*) in Kenner, Louisiana would exhibit greater signs of stress and decreased body condition compared to those from a population found in a natural, non-urbanized area. To assess stress levels, we examined two body condition metrics and heterophil: lymphocyte ratios. Contrary to our hypothesis, we found that individuals from the urban population were significantly larger and had greater body condition relative to individuals from the natural population. Though body condition was better, turtles from our urban population had significantly higher heterophil: lymphocyte ratios, suggesting they were more chronically stressed. These results suggest that environmental factors other than lack of foraging opportunity act as stressors of turtles found in urban areas, and that even populations that appear to thrive in urban areas may be at risk of decline.

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## (Re)Imagining Snakes: Lessons from a meme

Stephen Lchetto

Snakes are at the top of American phobias. Where does this attitude originate? To what extent is it biological or cultural? Can snakes be painted in a more appealing light? In this presentation, I explicate the myriad of reasons that serve to promulgate and reinforce what I call a "phylogenetic hegemony," a dominant discourse that advances negative perceptions of reptiles in general and snakes in particular. Fortunately, such attitudes can be culturally manipulated. I present evidence culled from digital ethnography that disrupts this hegemony. Changing attitudes towards snakes and other reptiles can have conservation implications. This presentation highlights the importance of new kinds of evidence in herpetological studies and the potential for a biological humanities approach.

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## The California Shark Beach Safety Program – Using Research, Education and Outreach to Assess Shark-Human Injury Risk and Reduce Economic Impacts.

Christopher G. Lowe, James Anderson, Patrick Rex, Emily Spurgeon, Yamilla Samara-Chacon, Zachary Merson, Elizabeth Jahn, Corina Silva, Gabriella Hancock, Katie Dudley, Dede Long

Increase numbers of white sharks along California beaches over the last 10 years, driven by successful conservation, has raised public safety concerns for ocean recreationists. Juvenile white sharks form aggregations of up to

40 individuals ranging in size from 1.25-3 m TL, often at public beaches, and may remain resident in these aggregations for months. In 2018, the State of California funded the Shark Beach Safety Program which include a research, education, and outreach arm. Research focuses on understanding and predicting when and where aggregations form, what sharks are doing there, and how often they interact with water users. Using satellite and acoustic telemetry monitoring, with some novel real-time acoustic detection tools, shark behavior data are provided directly to water safety officials to inform beach safety management. Shark and human water user encounter rates are monitored across beaches using UAV surveys, aided by community partnerships. Socio-economic studies are underway to quantify public perceptions of sharks and beach risks, as well as quantify economic impacts associated with shark bites and beach closures. Education programs designed for lifeguards, provide current research to help reduce shark-human interactions and minimize beach closures. Biological and socio-economic research is used in public outreach program including mobile beach pop-ups, comic book series, and K-12 STEM curriculum to inform perceptions about marine wildlife. Despite annual white shark bites on humans and occasional fatalities, additional protections have been added for white sharks in California to better protect the species from fishery interactions and human harassment.

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### Deterrent Effects of the Electromagnetic SMARTTM Hooks and the Newer “SMARTER” Hooks on Sharks in Recreational Hook-and-Line and Longline Trials

*Ryan Lowndes, Kristina Flanigan, Craig O’Connell, Abigail Cavaris, Shae Schreckengost, Derek Crane, Samuel Gary, Daniel Abel*

As bycatch continues to impact global shark populations, there is a continuing need for effective bycatch reduction devices. Prior research has shown promise in exploiting sharks’ electrosensory ability to this end. We tested the deterrent efficacy of the Select Magnetic and Repellant Treated (SMARTTM) and the newly developed “SMARTER” hooks in experimental longline and hook-and-line trials. Both are magnetized and contain an electropositive metal component made of magnesium (SMART) and a magnesium alloy designed to extend longevity (SMARTER). We deployed 127 longlines with SMART hooks, SMARTER hooks, controls, and procedural controls from 2021-2022 in Winyah Bay, South Carolina, and caught 134 sharks composed of 7 species (*Carcharhinus isodon*, *Carcharhinus leucas*, *Carcharhinus limbatus*, *Carcharhinus plumbeus*, *Negaprion brevirostris*, *Rhizoprionodon terraenovae*, *Sphyrna tiburo*). Additionally, hook-and-line trials testing the SMART hook alongside controls were conducted over 73 days from 2021-2022 at Myrtle Beach (South Carolina) State Park and caught 117 sharks composed of 5 species (*Carcharhinus acronotus*, *C. plumbeus*, *Mustelus canis*, *R. terraenovae*, *Sphyrna lewini*). Catch-per-unit-effort did not significantly differ among SMART, SMARTER, and control hooks in longline or hook-and-line trials. Further testing of SMART and SMARTER hooks with other species and increased strength of the hooks’ electromagnetic fields is needed to determine if electropositive hooks are a viable option for reducing shark bycatch.

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### Resolving the Evolutionary Relationships of the Order Centrarchiformes using Genomic Data and Dense Taxonomic Sampling

*William Ludt, Peter Unmack, Michael Sandel, Jean-Paul Hobbs, Giacomo Bernardi, Thomas Near, Prosanta Chakrabarty*

The order Centrarchiformes contains approximately 300 species classified into 19 families that are distributed worldwide in both freshwater and marine systems. These families contain many commercially important species, as well as species popular in the aquarium trade. While a variety of centrarchiform fishes are economically important, many also provide essential ecosystem functions. Despite their economic and ecological importance, little is known about the evolutionary history of these fishes, as this order wasn’t recognized until the inclusion of molecular data in large-scale fish phylogenetic studies. However, there are considerable differences in the hypothesized phylogenetic relationships of this group among the molecular studies that have included multiple families from this order. Insufficient genomic and taxonomic sampling likely cause these disparities, as most previous studies fail to include representatives from all centrarchiform families, have limited taxonomic

sampling within these families, or only sample a handful of loci. Here we take a phylogenomic approach to examine the evolutionary history of this order using approximately 500 ultraconserved elements with dense taxonomic sampling that includes 85% of the accepted centrarchiform genera and 66% of the recognized species diversity. We discuss how our findings differ from previous studies and the implications that these findings have for the biogeography of this order. Lastly, we highlight future directions that will further our understanding of this large order of fishes.

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### Preliminary Characterization of Crude Venom from Spines of the Atlantic Stingray, *Hypanus sabinus*

*Carl Luer, Cathy Walsh, Craig Doupnik*

Stingray venom compounds and cellular mechanisms responsible for their excruciating pain and tissue necrosis are poorly understood. Crude stingray venom (SRV) was obtained by scraping tissue from grooves on the ventral surface of Atlantic stingray (*Hypanus sabinus*) spines. Harvested tissue was gently homogenized to release venom compounds, which were separated from cellular and tissue debris by centrifugation. Aliquots of the resulting supernatants were lyophilized and either analyzed by electrophoresis or stored at -80 C for bioactivity assays. SRV compounds demonstrated reproducible protein banding patterns on SDS-PAGE and venom preparations yielded comparable total protein. SDS-PAGE patterns appear unchanged following crude venom collection from spines wrapped in foil and frozen in liquid nitrogen for two or four weeks. Cytotoxic effects of SRV on normal human dermal fibroblasts (NHDF) and human neuroblastoma cells (SH-SY5Y) were monitored in real-time by an impedance-based biosensor system (xCELLigence Real-Time Cell Analyzer) and found to be both time- and concentration-dependent. At 100 µg/mL, SRV caused a rapid (<15 min) decrease in cell impedance of cultured NHDF, consistent with acute cytotoxicity with an EC50 of ~10 µg/mL. At lower concentrations (3 µg/mL), SRV slowed the 24-48 hour cell growth curve, consistent with an inhibitory action on cell proliferation. These responses were absent in heat-treated SRV (95-100 deg C for 10 min). Unlike box jellyfish venom, whose cytotoxic effects can be inhibited by cell membrane depletion of cholesterol, pretreatment of SH-SY5Y cells with a cholesterol-depleting reagent had no effect on either acute or chronic effects of SRV.

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### Costs of Parthenogenesis in the Zebra Shark

*Kady Lyons, Lance Adams, Jennifer Wyffels*

The zebra shark (*Stegostoma tigrinum*) is known to readily reproduce both sexually and through facultative parthenogenesis while in human care. Artificial insemination trials that took place between 2011 and 2013 resulted in the hatching of two sexually-produced (herein “heterozygotes”) and ten parthenogenic sharks that allowed for a retrospective comparison of growth, feeding and longevity between offspring. Parthenogenetic offspring were generally smaller at hatch than their heterozygous counterparts and after the first several months post-hatch failed to increase in mass and length at the same rate as heterozygotes. Parthenogenic offspring exhibited non-normal swimming behaviors and a reduced ability to properly suction feed. Median lifespan for parthenotes was 1.05 years while one heterozygote lived to 2.37 years of age and the other is alive at present. By contrast, the two longest surviving parthenotes perished at ~5.5 and ~6.5 years of age, respectively. This study demonstrates substantial negative costs to fitness in parthenogenetic offspring compared with their heterozygous siblings.

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### Values, Attitudes, and Media Exposure: US Public Perception of Sharks and Shark Conservation

*Catherine Macdonald, Kela McEntee, Julia Wester*

Public values and attitudes are increasingly relevant to wildlife conservation and management. For species historically perceived negatively, including sharks and other predators, conservation action may be shaped by public perceptions. We present the results of the first large-scale, in-depth survey of public attitudes toward sharks and their conservation in the United States (n = 640). The survey covered a wide range of attitudes, beliefs, and practices, including where participants encounter information about sharks, their perceptions of current conservation threats to sharks and “attack” risk, and their

interest in and self-reported knowledge about sharks. It also explores what value they perceive sharks to have, what management policy approaches they most support, and their beliefs regarding their personal responsibility for shark conservation. Results deepen our understanding of cultural trends around wildlife conservation by demonstrating the influence of large-scale value shifts on other, more specific attitudes. Findings also highlight a need for a stronger conservation focus in media and messaging, as public perceptions, including potential biases or misperceptions, track closely with aggregate media coverage.

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### **Combining Long-Term Datasets to Answer Landscape Level Questions: Opportunities with the North American Amphibian Monitoring Program Data-Set.**

*Zira MacFarlane, Shannon McCauley*

The North American Amphibian Monitoring Program (NAAMP) produced one of the richest amphibian biodiversity datasets globally, with 15 years (2001-2016) of standardized surveys across 26 states in the USA. This presents an excellent opportunity to study the ways in which trends of amphibian community and diversity are shaped by landscape level drivers including pollution, agricultural development, and urbanization. This poster firstly presents a review of the present suite of research that has been carried out using the data from the NAAMP with a focus on that which integrates other long term landscape level datasets such as the National Landcover Database; and, secondly, identifies new areas to delve into with a focus on aquatic water quality and pollution levels in conjunction with development.

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### **Plasticity of *Ambystoma texanum* life history traits in response to environmental conditions**

*Mariah Mack*

The length of time a water body remains inundated is an important environmental factor for developmental stages of aquatic-breeding amphibians. In addition, predation risk has direct and indirect effects on amphibians at various stages of their life cycle. Some amphibians exhibit plasticity during development to mitigate these environmental stressors and may incur costs and tradeoffs in life history traits. I conducted a mesocosm experiment with three hydroperiod treatments (short, medium, constant) and two predator treatments (presence or absence of *Lepomis macrochirus* cues) and measured life history traits at metamorphosis to determine the degree of developmental plasticity exhibited by *Ambystoma texanum* (smallmouth salamander). Overall survival to metamorphosis averaged 56.7% and was consistent across all treatments. Larval period, mass, and body size increased with longer hydroperiods but were not significantly affected by predator cue or the interaction of hydroperiod and predator cue, suggesting a lack of synergistic effects between these stressors for the levels we used. Larval periods ranged from 50 to 100 days, with an overall average of 65.35 days. Compared to the control, salamanders in the short hydroperiod metamorphosed 14.5% earlier (mean = 60.55 days), 49% lighter (mass; mean = 0.82 g), and 14.8% shorter (SVL; mean = 32.25 mm) on average. Salamanders from the medium hydroperiod generally metamorphosed at an intermediate time and size between the short and constant hydroperiod treatments. We hypothesize that *A. texanum* has a plastic development rate that is sensitive to some degree to their larval environment and specifically to water levels. Although they were able to metamorphose earlier to escape drying conditions in the short hydroperiod, the observed cost of this accelerated development was a significant reduction in mass and size at metamorphosis. Smaller mass at metamorphosis is associated with lower adult fitness in other *Ambystoma* species, suggesting that the cost of plasticity may eventually have population level effects for *A. texanum*. Understanding the effects of environmental stressors on *A. texanum* life history traits is important for predicting their adaptability to climate change-induced shifts in water availability and introduced predators.

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### **DEI: Building Safe and Inclusive Spaces in Field Settings: You have a Right to Know!**

*Maisie G. MacKnight, Allyson B. Muth*

Open communication, setting culture of the work environment and expectations from the beginning, has been shown to empower individuals by providing information to help them identify and reduce risk and fosters discussion between field members. Appropriately assessing risk requires supervisors to acknowledge differences in individual identity and experience level. However, many people, especially from minority groups or those with less institutional power (e.g., students, untenured faculty etc.) have reported feeling unsafe while doing fieldwork at some point in their careers. Additionally, there is still uncertainty in how to apply outlined best-practices to specific settings. Therefore, we present a collection of field safety resources that follow the best practice of increasing communication between field members. The goals of these resources are two-fold. The first is to inform technicians of expectations regarding field work risks and codes of conduct so that technicians can provide informed consent prior to starting work. The second goal of this document is to provide guidance as supervisors discuss issues beyond physical safety with their technicians while also considering the impact of race, gender, and background on experiences. Implementation of these resources in individual lab groups has been shown to improve individual sense of belonging and empowerment of students in the field.

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### **Tag, you're it: Chasing down considerations for using tag and survey methodologies in wildlife systems**

*Maisie MacKnight, Samantha Bryn, David Miller*

Ecologists are developing novel ways to track individuals across time and space. Technologies such as external marks or implanted tags and their associated survey methodologies can inform survival and growth rates and directly track movement of individuals. However, there lacks a consistent standard for considerations when quantifying the cost and benefits of novel methodologies and their applications. We argue that the costs and benefits of any marking method should be defined by the research objectives and can be evaluated based on four main criteria: information quantity, information quality, biases, and costs. We discuss the processes in which we hope will motivate a greater standardization of assessment strategies. We then present a case study from our own work assessing the use of PIT tags in our study species, the eastern red-backed salamander (*Plethodon cinereus*). We tested for differences in annual survival, detection probabilities, and body condition between a tagged population of 164 individuals across 5 plots and compared this population to our untagged populations at those same sites. We found that the use of PIT tags did not affect detection probability using pre-existing surveys, and we failed to detect any negative effects to long- or short-term survival or body condition between populations. We also found improvements in detection between the two survey methodologies, meaning that PIT tags are a viable option to on their own or in conjunction with long-term research systems.

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### **Descriptive Myology of Stargazers, Genus *Uranoscopus* (Percomorphacea; Uranoscopidae)**

*Leo MacLeod, Murilo Pastana*

The family Uranoscopidae, commonly known as stargazers, includes > 50 species in seven valid genera and are found worldwide in shallow waters of the tropical to temperate oceans. More than half of the species in the family belong to the genus *Uranoscopus*, found from the Mediterranean to the Indo-Pacific. In this study we investigated the myology of several species of *Uranoscopus*, describing and illustrating its main muscular complexes. We provide special attention to the cranial myology, addressing homology hypotheses for each of its muscle components. Our analysis identifies major evolutionary changes related to the mandibular, opercular, branchial, and paired-fin myology, which are translated into several phylogenetic characters. This represents the second study to investigate the myology of the genus *Uranoscopus*, and provides a detailed look at characteristics that have been under-explored. This increased understanding of the anatomy may help to understand the evolutionary relationships of the genus and provide new characters with which to compare to the rest of the family.

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## Visual Opsin Evolution and Diversity in South American Electric Fishes (Gymnotiformes) is Associated with Photic Environment

*Esme Macpherson, Frances Hauser, Ahmed Elbassiouny, William Crampton, Belinda Chang, Nathan Lovejoy*

Vision allows for the extremely rapid relay of information between organisms and their environment. Core molecular components of the visual system are visual opsins: light-sensitive proteins that initiate the phototransduction cascade. The structure, sequence, and evolution of opsins are often tightly correlated with aspects of visual ecology such as foraging, mating, and habitat. In addition to vision, South American electric fishes (Gymnotiformes) have evolved a novel, sophisticated electrosensory system that has allowed them to navigate and communicate. Since Gymnotiformes are thought to have poor vision given their evolution in frequently dim-light habitats, we investigated whether the known complement of gymnotiform visual opsins (RH1, the dim-light sensitive opsin, and LWS, the long wavelength-sensitive opsin) exhibit evidence of relaxed selection. We used targeted sequence capture to sequence the visual opsins from 90 gymnotiform species, and used codon-based likelihood models to explore molecular evolution in LWS and RH1. We also investigated whether RH2 was present in the genome of these species, and if this could be inferred via the sequence capture method. Contrary to our expectations, we did not find evidence for relaxed selection in gymnotiform opsins. Rather, we found evidence for positive selection in both LWS and RH1, and that the evolution of both these genes was strongly associated with light environment. Finally, we also investigated whether eye size was associated with shifts in selection in the opsins. Together, our results highlight that despite their reduced opsin repertoire, opsin genes are under strong selection in Gymnotiformes to adapt to diverse photic environments.

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## Site fidelity of Pacific nurse sharks (*Ginglymostoma unami*) to a Marine Management Area in the Costa Rican North Pacific.

*Sergio Madrigal-Mora, Christopher Lowe, Mario Espinoza*

Endangered Pacific nurse sharks (*Ginglymostoma unami*) are often observed in Santa Elena Bay, a newly established Marine Management Area (MMA) in the north Pacific coast of Costa Rica. Although sightings of this species seem to be relatively common within the Bay, little is known about their spatial ecology. In this study, we used passive acoustic telemetry to quantify the site fidelity and movement patterns of *G. unami* in the Santa Elena Bay MMA and adjacent unprotected waters in the Gulf of Santa Elena. Thirteen sharks ranging from 85-256 cm total length (TL) were acoustically tagged and monitored between September 2018 and December 2022. Pacific nurse sharks had low residency index (days detected/days monitored; mean $\pm$ SD: 0.22 $\pm$ 0.19) in Santa Elena Bay, and were detected at adjacent Matapalito Bay and Gulf of Santa Elena with similar frequency, suggesting home ranges that exceed the MMA. Additionally, a male shark was detected by a receiver array located ~200 km south of Santa Elena Bay, which represents the longest movement so far reported for this species. Using Generalized Additive Mixed Models, residency in Santa Elena Bay was found to have fluctuating seasonal patterns. Pacific nurse sharks show peaks in residency index inside the Santa Elena Bay during dry season, correlated with changing water temperature due to seasonal upwelling events that occur during this time of the year. Understanding Pacific nurse shark seasonal patterns and movement ecology is essential to inform management and protection of the species throughout its distribution.

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## Climate may drive salamander variation in size at maturity with life history and demographic constraints on responses to climate change

*John Maerz, Cynthia Carter*

Behavioral, physiological, or morphological adaptations determine whether plethodontid salamander populations persist under a given climate. Identifying and estimating constraints on such adaptations is key to predicting population responses to climate change. Larger body size reduces evaporative water loss, therefore, we hypothesized that plethodontids evolve delayed maturation to achieve a larger body size in drier climates. To test this hypothesis, we used

a generalized linear model of minimum sizes of mature large *Plethodon* spp. from more 24,000 individuals observed among 56 sites across north Georgia, western NC, and eastern Tennessee including 26 sites within the Coweeta Basin. Minimum mature male and female size were strongly, negatively correlated with mean daily vapor pressure deficit (VPD) during the active season (2010-2021). This pattern was stronger when constrained to sites within the Coweeta Basin. Minimum size at maturation differences between wetter and drier sites likely represents 2-4 years difference in age at maturity among sites. Abundance ranged from 0 to 4.5 *Plethodon* per m<sup>2</sup> squared, and was strongly, negatively correlated with mean daily VPD. We propose that delaying maturity to achieve a larger body size is an important way that *Plethodon* have evolved to persist at sites with drier climates, but this life history change is constrained demographically by reduced survival to adulthood. As a result, *Plethodon* are already excluded from some drier climates, and if future climates are drier at occupied sites, *Plethodon* abundance is expected to decline with constraints on the potential for adaptation to offset those effects.

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## Estimating the Effects of Hemlock Imidacloprid Treatment on Terrestrial Salamander Abundance

*John Maerz, Jade Samples, Danielle Bradke, Shelby Bauer, James Martin, Vanessa Terrell, Cassandra Waldrop, Elizabeth McCarty*

When considering the use of pesticides, managers need to know whether pesticides will negatively affect non-target organisms to judge the trade-off between management goals and non-target consequences. Hemlock woolly adelgid (HWA) has decimated hemlock populations in the southern Appalachians. Imidacloprid, a neonicotinoid insecticide, is currently used to suppress HWA populations and conserve hemlock trees and the habitats they create. Recent concerns have emerged about possible non-target impacts of hemlock imidacloprid treatments on amphibians including salamander populations. Robust estimates are needed on the magnitude of any potential impacts of imidacloprid application on salamander populations to inform decisions on hemlock conservation in sensitive areas. We conducted repeated depletion samples among 15 replicate plots at each of 14 treated and 11 reference sites across north Georgia, western NC, and eastern Tennessee, and then used a Bayesian, hierarchical, N-mixture model to account for differential salamander availability and detection as well as climatic effects on abundance to estimate the effects of imidacloprid application on terrestrial salamander (*Plethodon* spp.) abundance. We found no measurable correlation and a weak positive correlation between *Plethodon* abundance and imidacloprid dose at a 15 m and 3 m buffer scale, respectively. Variation in *Plethodon* abundance among treated and untreated hemlock sites was related to climate (mean daily vapor pressure deficit). Our results suggest that imidacloprid application to conserve Eastern hemlock does not negatively impact *Plethodon* spp. abundance.

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## Diversification and convergence following the transition from saltwater to freshwater in stingrays

*Autumn Magnuson, João Pedro Fontenelle, Matthew Kolmann*

One of the most fundamental questions in biology is why some groups of organisms are more diverse than others. Classic hypotheses for explaining differences in diversity consider distinctions in time, place, resources, and competitors as the staging grounds for differential diversification. Freshwater and saltwater environments have similar levels of diversity despite significant differences in size, so studying transitions between the two systems can provide insights into evolutionary processes. Despite the challenges associated with this transition, stingrays have invaded freshwater habitats multiple times across different continents, making them useful for better understanding these systems. In this study, we evaluated the frequency of saltwater-freshwater invasions in stingrays, examined three types of diversification among freshwater and saltwater stingrays, and assessed the degree of convergence among freshwater stingrays. We found that, like nearly all other aquatic taxa, stingrays overwhelmingly only transition from saltwater to freshwater. After independent freshwater invasions, river rays did not demonstrate a pattern of increasing morphological or lineage diversification. However, the phenotypic disparity of saltwater stingrays did not follow the Brownian prediction and appeared to spike around two global climatic events. Despite not being morphologically distinct from saltwater stingrays, freshwater stingrays do



push the boundaries of morphological diversity. Diet guilds did demonstrate morphological differences, with piscivores and molluscivores being distinct from other diet guilds. Freshwater stingrays did not appear to converge morphologically, which may be because there has not been enough time for this to occur among more ancient and more recent freshwater lineages.

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## DNA sequencing of marine fishes preserved in ethanol for 115 years

*Sharon Magnuson, Chris Bird*

There is a wealth of genomic information preserved in museum specimens that can be used to address current questions. Though the literature reports some successes in accessing this information, it is mostly in desiccated specimens. Even relatively well-preserved specimens present challenges caused by DNA degradation, low-copy numbers, and carry-over of enzymatic inhibitors during extraction. We compared the performance whole genome shotgun libraries to those with an additional capture probe step in Philippines fishes collected both contemporarily collected from 1907-1909 by the scientific research vessel, the U.S.S Albatross. The historical specimens were stored in ethanol and transferred to the Smithsonian Museum of Natural History. Whole genome shotgun libraries were prepared using the KAPA HyperPlus kit. Some libraries were further enriched using targeted capture probes that had been designed for that species and then both sets of libraries were sequenced on Illumina sequencers. The success of the libraries was determined by comparing standard mapping statistics with sequencing effort (quantity of raw reads). As expected, contemporary specimens required lower sequencing effort to achieve the desired depth of coverage, but we were successful in sequencing many of the historical specimens at scale for population genomic analysis. The performance of the targeted capture probes varied widely across species for both historical and contemporary specimens, while the whole genome sequencing was more reliable and produced more data per unit sequencing effort. We concluded that while targeted capture could be more cost effective if optimized, the whole genome sequencing did not require the same level of optimization.

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## Rethinking the Sea Turtle “Lost Years”

*Kate Mansfield*

Sea turtles are late-maturing marine animals that spend most of their time far from their terrestrial nesting habitats. Very little is known about young sea turtles from the time hatchlings depart their natal beaches and enter offshore, oceanic waters, until they return to coastal waters years later as large juvenile turtles, remaining in neritic waters until maturity. The early oceanic period is otherwise known as the sea turtle “lost years” due to the historic lack of empirical data on the young turtles’ distribution and behavior. Understanding sea turtle behavior during all life stages is critical for ensuring the conservation and survival of these protected species; however, we often rely on “best available data” for management decisions. Historically, sampling logistics and lack of appropriate tracking technology limited our knowledge of the sea turtle lost years to opportunistic sightings, near shore manual tracking, and laboratory studies. These “best available data” resulted in three long-held assumptions/hypotheses about this early sea turtle life stage: 1) the turtles are oceanic; 2) they remain at the sea surface; and 3) passively drift in available ocean currents for years before returning to coastal habitats as larger, neritic juveniles. Recent work using novel technologies (with a little help from a manicurist) combined with ocean modeling, genetic and diet analyses has provided new insight to the sea turtle “lost years”. This work challenges long-held assumptions about early turtle behavior and is changing how we perceive sea turtle biology, ontogeny, and conservation on a global scale.

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## Relative Energetic Importance of Different Regions of the U.S. East Coast for the Migratory Sand Tiger Shark

*Benjamin Marsaly, Noah Motz, Dewayne Fox, Danielle Haulsee, Matthew Oliver, Bradley Wetherbee, Aaron Carlisle*

Recent decades have been characterized by rapid technological advances, providing researchers access to new tools to investigate various aspects of the ecology of marine species. However, aspects of the biology of many large

elasmobranchs remain poorly understood, especially their migratory behavior and the relative importance of different regions to the energy budgets. The sand tiger shark (*Carcharias taurus*) seasonally migrates along the east coast of the United States and has experienced historical population declines to the point where the species is now listed as ‘Critically Endangered’ on the IUCN Red List. Improved understanding of the ecological role of sand tigers, and how this may vary across their seasonal migrations, will also inform management of their populations. We used a combination of acoustic telemetry and stable isotope analysis (SIA) to investigate relationships between movements and feeding ecology of sand tigers by tagging sharks with acoustic transmitters and collecting muscle tissue biopsies for SIA to estimate carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) ratios. We used a modified Bayesian isotope mixing model integrating acoustic telemetry and SIA data as well as information on tissue turnover rates and stable isotope discrimination factors to estimate the relative importance of different regions used by sand tiger sharks during their migration towards their overall diet. This information will enhance our knowledge of the relationship between migratory behavior and foraging in sand tigers and advance our ability to directly integrate distributional and chemical tracer data to better characterize the migratory behavior of large marine predators.

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## Changes in reptile and amphibian communities across urbanization gradients in northeastern North America

*David Marsh, Ian Kinney, Thomas Finnegan, Cecily Stern, Sellers Smith*

Urbanization is expected to alter animal communities, but few studies have generalized beyond patterns from individual cities. We used citizen-science data on reptiles and amphibians from 17 cities in North America to determine: 1) how species richness and diversity differs between cities and suburban areas, 2) whether the degree of landcover change in cities vs. suburbs is related to the magnitude of change in species diversity, 3) whether urban communities are more similar to each other or to their suburbs, and 4) what characteristics of reptile and amphibian species predict how sensitive they are to urbanization. We found that species richness and diversity tended to be somewhat lower (10-20%) in cities as compared to surrounding buffers. For reptiles, the magnitude of decrease in richness and diversity appeared to be related to the degree of change in impervious surface cover, whereas for amphibians, these changes tracked differences in forest cover. There was little evidence of urban homogenization—city communities tended to cluster with their surrounding buffers rather than with other cities. For individual species, those that were less common in cities tended to be more dependent on forest cover and specialized breeding habitats such as streams or vernal pools. Our results suggest that cities are capable of supporting a relatively high diversity of reptiles and amphibians. However, maintenance of this diversity may require attention to habitat protection and restoration, particularly for less common aquatic habitats.

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## Body Size Responses to the Combined Effects of Climate and Land Use Changes within an Urban Framework

*Amanda Martin, Jennifer Sheridan*

Alterations in body size, an important fundamental trait, can strongly impact an organism’s life history and ecology with long-lasting effects that span multiple biological scales. Climate and land use change, two of the largest current threats to biodiversity, can influence animal body size. Over time, climate warming has led to smaller body sizes of many species due to impacts on growth (i.e., Bergmann’s rule and temperature-size rule). Altered precipitation patterns related to climate change have received less attention, but organisms may experience size increases due to increased resource availability in wetter areas and for those particularly sensitive to desiccation in drier areas. Conversely, urbanization, an example of land use change, has largely been demonstrated to cause size increases due to selection for enhanced dispersal ability and increased resource availability. However, few studies have examined the combined influences of climate and land use on organism size. Therefore, we present background theory, summarize existing evidence of impacts of climate and land use changes on size, make several recommendations to guide future research, and provide preliminary data evaluating amphibian size responses to the interactive effect of climate and land use

change over time using museum collections. Given the rapid pace of climate change and urbanization, understanding the combined effects of climate and land use changes on body size is imperative for biodiversity preservation.

### **Social Security: Can rattlesnakes reduce acute stress through social buffering?**

*Chelsea Martin, Gerad Fox, Breanna Putman, William Hayes*

Social buffering comprises the reduction of stress an organism experiences when in the presence of a companion and has been well documented in highly social animals, such as birds and mammals. To date, it has not been reported in reptiles. Rattlesnakes are cryptically social, exhibiting kin recognition and forming subtle social networks in certain contexts. We tested for the presence of social buffering against an acute stressor in 25 wild-caught adult southern Pacific rattlesnakes (*Crotalus helleri*) by measuring heart rate increase during a standardized disturbance when alone, in the presence of a rope (inanimate object control), and in the presence of a same-sex companion. We further tested for a greater proclivity of social buffering in montane snakes, which overwinter communally, than in lowland snakes, which mostly overwinter alone; and for a greater proclivity in females, which often aggregate during gestation and maternally attend their offspring. Results indicated that the presence of a companion significantly reduced emotional tachycardia (change in heart rate) after disturbance compared to the other treatments. We detected no difference in social buffering proclivity between montane and lowland populations, but lowland snakes exhibited a subdued acute stress response across treatments and had a longer heart rate recovery time. No difference in emotional tachycardia existed between the sexes. This novel study can benefit our understanding of social buffering, including its expression, adaptive roles, and practical applications for captive animals.

### **Evolutionary Mechanisms Shaping Major Histocompatibility Complex Immune Gene Variation and Trans-Species Polymorphism in Four Species of Sea Turtle**

*Katherine Martin, Kate Mansfield, Anna Savage*

Major histocompatibility complex (MHC) immune genes comprise one of the most diverse gene families in vertebrates. Several non-mutually exclusive evolutionary mechanisms, including pathogen-mediated balancing selection, favor high allele number and sequence diversity within individuals and populations. Similar and even identical MHC alleles are found in species millions of years diverged, in a phenomenon known as trans-species polymorphism (TSP). Two primary scenarios can produce TSP: 1) maintenance of ancestral polymorphism in descendant species via balancing selection, and 2) convergent evolution of similar or identical alleles that confer the same pathogen resistance in different species. Immune genes like MHC are ideal candidates for understanding selective processes that shape genetic variation across millions of years of diversification, especially in disease-threatened taxa. Sea turtles are also ideal species for studying immune system evolution given their long lifespans, worldwide conservation threats, and species-level susceptibility differences to the viral-associated tumor disease fibropapillomatosis. However, sea turtle immunogenetic variation is minimally explored. We sequenced the  $\alpha 1$  peptide-binding region of MHC class I genes from 88 loggerhead (*Caretta caretta*), 268 green (*Chelonia mydas*), 65 leatherback (*Dermochelys coriacea*), and 39 Kemp's ridley (*Lepidochelys kempii*) sea turtles. We reconstructed phylogenetic relationships among all sequenced MHC alleles and recovered extensive allelic variation, evidence of TSP, and significant evidence of positive selection shaping allele evolution. We present simulation analyses assessing whether identical alleles arose from common ancestry or convergent evolution. Our study is the first comparative MHC analysis across turtle families, providing insight into the evolutionary forces shaping immunity in reptiles more broadly.

### **Investigation of the Anatomy and Morphology of Bioluminescent Light Organs**

*Rene Martin*

Fishes have evolved the ability to produce bioluminescence at least 27 independent times. This light is produced either endogenously via light-producing cells or symbiotically via a relationship with bioluminescent bacteria housed and maintained in specialized light organs. The majority of bioluminescent lineages of fishes are found in the deep sea (areas below 200 m depth). The adaptive significance of bioluminescence in these deep-sea fishes includes camouflage, predation, predator avoidance, and communication. Across the tree of fishes, bioluminescent organs vary greatly in their size and shape, in their light intensity and color, and in their location and number. The variation in the anatomy and morphology of light producing organs across deep-sea fishes continues to be an understudied avenue of research in our pursuit of knowledge regarding fish evolution and adaptation in the deep sea. Using a combination of methods and techniques, including dissecting, magnetic resonance imaging, and histology, we describe the anatomy and morphology of light organs in previously uninvestigated species of deep-sea fishes.

### **Using a citizen-science approach to characterize shark depredation in the recreational fisheries of the southeast United States**

*Michael McCallister, Luran Brewster, J Marcus Drymon, Cliff Hutt, Matthew Ajemian*

Shark depredation (i.e., partial removal of target catch prior to retrieval) is an emerging issue that is purportedly affecting several fisheries in the southeastern US. While NOAA Fisheries has begun assessment of shark depredation in commercial fisheries, there have been few attempts to quantify these interactions in the recreational sector. These data are needed to characterize the scale of the issue and determine the need for mitigation measures. To address this knowledge gap, we initiated a citizen-science-based project with goals to: 1) acquire depredation data in the recreational sector, and 2) evaluate fishermen perceptions of the issue. We are achieving these goals via multiple approaches, including data acquisition from an online survey of randomly selected Florida saltwater anglers, social media groups, and genetic sampling of depredated fish carcasses landed by participating charters. From July 2021 – July 2022, online angler surveys generated 2206 responses with 53% of respondents having experienced depredation. Experiencing depredation had little effect on how often anglers went fishing, however 43% said it does affect where they choose to fish, and most anglers responded to depredation by changing locations or ending fishing for the day. 87% of respondents feel that depredation rates have stayed the same, slightly increased, or greatly increased over the last 5 years. Results from this study will be imperative towards building a comprehensive understanding of this emerging human-wildlife conflict.

### **Exploring Index Standardization Using Habitat Suitability and Spatiotemporal Modeling to Improve Model Fit and Biological Plausibility of Scalloped Hammerhead Estimates**

*Cami McCandless, Dan Crear, Tobey Curtis, John Carlson, Lisa Natanson*

Federally managed Atlantic shark species are assessed through the Southeast Data Assessment and Review (SEDAR) process. Indices of abundance used in SEDAR shark assessments are typically standardized using a generalized linear or generalized linear mixed model in a two-step delta-lognormal approach that models the proportion of positive catch with a binomial error distribution separately from the positive catch, which is modeled using a lognormal distribution. The time series of scalloped hammerhead catch-per-unit-effort data from a fishery-independent survey conducted off the United States Atlantic Coast was standardized using this method and the resulting trend in relative abundance was biologically unsupported. Additional analyses were undertaken that modified the spatial coverage of the survey (excluding non-repeated stations and excluding areas), model development (excluding year until all explanatory variables were incorporated), and model distribution (Tweedie). Although some improvements were seen in model fit the resulting trend was still not biologically supported, indicating that some explanatory

variables may be missing. Habitat suitability modeling was conducted based on observer data from the shark bottom longline fishery that overlapped with the timing of the fishery-independent survey. The results from the habitat suitability modeling helped to identify some of the issues in the standardization process and led to the use of spatiotemporal modeling to account for latent variables not accessible in the original standardization process.

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## **Atrazine Causes Changes in the Brains of Larval Amphibians**

*Sara McClelland, Janniry Cabrera Belen*

Environmental pollutants are a major contributor to the global decline of amphibians. Atrazine is a triazine herbicide that inhibits photosynthesis and is used to help control weed growth across multiple sectors. Atrazine is a known endocrine disrupter in non-target organisms where it interferes with the hormone production, physiological function, and behavior. Multiple studies have analyzed the impacts of atrazine on reproductive structures and behavior in amphibians, but none have analyzed its impact on the amphibian brain. We hypothesized that atrazine exposure during development would result in morphological changes to the brain. To test this hypothesis, *Xenopus laevis* tadpoles were exposed for three weeks to either a vehicle control or 3 µg/L atrazine, a concentration that is considered safe by the Environmental Protection Agency (EPA). Following exposure, behavior and brain morphology were measured. Preliminary analysis of the data found that when tadpoles were exposed to 3 µg/L atrazine there were changes in the shape of the brain. Further, tadpoles exposed to atrazine also behaved differently in open arena trials. The results from this study add a new layer to what we know about atrazine's effects and offers new insights into how freshwater vertebrates are being impacted by atrazine exposures.

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## **Using Genetics to Further the Understanding of Istiophorid Billfishes: How Far Have We Come?**

*Jan R. McDowell, Nadya R. Mamoozadeh, Jackson L. Martinez, John E. Graves*

Over the past 30 years, a variety of molecular markers have been used to survey stock structure and evaluate the taxonomic relationships of istiophorid billfishes (marlins, spearfishes, and sailfish), including allozymes, restriction fragment length polymorphism analysis of mitochondrial DNA (mtDNA), sequencing of mtDNA, sequencing of nuclear gene regions, nuclear microsatellite loci, and genotyping-by-sequencing of single nucleotide polymorphisms (SNPs). These analyses have contributed to the understanding of genetic population structure in sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*) in the Atlantic and Pacific oceans, striped marlin (*Kajikia audax*) in the Pacific and Indian oceans, white marlin (*K. albidus*) in the Atlantic Ocean, and to clarify range uncertainties among the spearfishes (*Tetrapturus*). More recently, genetics have been used to resolve long-standing uncertainty about the relationship of white marlin and striped marlin and to understand the stock composition of striped marlin in the central North Pacific Ocean. This talk will review progress to date and remaining knowledge gaps.

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## **Seasonal Movements of Bowfin (*Amia calva*) and Northern Snakehead (*Channa argus*) in Virginia: Comparison of Native and Invasive Species**

*Patrick McGrath, Eric Hilton*

Bowfin, *Amia calva*, are a native, carnivorous fish inhabiting freshwater systems throughout eastern North America. Although Bowfin are one of the top predators in these systems, little research has been conducted on its seasonal movements. Northern Snakehead, *Channa argus*, are native to eastern Asia but have been introduced throughout the world, and was first documented in the Piankatank River, Virginia, in 2013. The Northern Snakehead is also a top predator, overlapping the ecological niche of Bowfin. In this study, 14 Bowfin and 8 Northern Snakeheads were acoustically tagged to compare their spatial overlap and seasonal movements. In the winter, both species inhabited the same deep (3-4 m) portion of the river. In March, almost every Bowfin moved upstream 9.5-25.8 km, presumably to spawn. The movement upstream correlated with a rain event that significantly increased water levels allowing access

above beaver dams and other obstructions. Northern Snakeheads during this time did not have a directed upstream movement, instead moved rapidly upstream and downstream 0-6.5 km. During April and May, 7 tagged Bowfin returned to the original tagging area. The other half remained upstream during the summer, likely confined in sections of the river due to low water levels restricting movements. This past fall an additional 4 Northern Snakeheads and 1 Bowfin were tagged, as well as 5 more receivers put in service to increase coverage of upstream habitat. The results of this study will contribute to the understanding of how native and invasive species that occupy similar niches interact spatially.

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## **Watery Having for Dinner? Variation in Blunt-nosed Leopard Lizard Dietary Water**

*Tess McIntyre, Savannah Weaver, Michael Westphal, Emily Taylor*

Insectivorous desert lizards are assumed to meet their water needs through their diet, but prey water content is seldom measured. Understanding how water content varies among arthropod species and over time can provide insight into the dietary hydration strategies for desert reptiles that are facing water scarcity due to drought and climate change. Blunt-nosed Leopard Lizards (*Gambelia sila*) are an endangered species endemic to California's Central Valley, whose diet primarily consists of arthropods. We collected arthropods biweekly in known *G. sila* habitat during their active season, measured water content as percent wet mass of each specimen, then compared arthropod water content across taxa and over time. We hypothesized that climate variation (e.g., increasing temperatures and decreasing water availability) over the active season would affect the water content of *G. sila* arthropod prey. We found little variation in prey water content across the active season, but there was a significant difference between Hymenoptera species (lowest percent water) and Coleoptera species (highest percent water). As the effects of climate change intensify, megadroughts will increase hydric stress on *G. sila* and all desert species, so it is important to identify how these endangered lizards stay hydrated. For example, it would be interesting to observe whether lizards preferentially feed on arthropods with higher water content during drought years.

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## **Multi-parasite Surveys in North-Central Florida Reveal Variable Infection Patterns Among Resident Snakes**

*Cory McKinstry, Arik Hartmann, Faith Dunlap, Tristan Vratil, Robert J. Ossiboff, David Rodriguez, Ana V. Longo*

Free-ranging herpetofauna is increasingly threatened by emerging pathogens. One such pathogen, *Ophidiomyces ophiodiicola* (Oo), has potentially contributed to the decline and local extirpation of species across the United States from snake fungal disease (SFD). Other infective agents, such as Ranaviruses and *Cryptosporidium*, may also contribute to additional snake declines as pathogen pressure increases within the landscape. However, in snakes, little is known about the compounding effects of multiple pathogens on host susceptibility. The presence of one infective agent may facilitate secondary infections, or alternatively, generate cross-reactive immune responses increasing host resistance to subsequent infections. To begin understanding how Oois interacting with endemic parasite communities and if these interactions are modulating host infection dynamics, we monitored the presence of three pathogens (i.e., Oo, Ranaviruses, and *Cryptosporidium*) using quantitative PCR on samples from a wild snake community in north-central Florida. Our preliminary data revealed Ooinfections in 5 of the 14 species sampled. We detected correlations with specific species and habitats, indicating that some snake communities may be disproportionately impacted by emergent diseases. Ooinfections were not always coupled with visible signs of disease, but high pathogen loads of Oo were detected in rare and declining species with moderate-severe signs of SFD. Ranavirus infections were less prevalent and observed in only 2 individuals across 2 species. Overall, we show that several emerging pathogens of snakes infect a variety of species across taxonomic and ecological groups in north Florida, which may be used to guide future disease mitigation and conservation strategies.

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## Systematics of cichlids in the genus *Chuco* (Teleostei: Cichlidae) from Northern Middle America

Caleb McMahan, Dylan Maddox, Sophie Picq, Josué García-Pérez, Diego Elías

Middle American cichlids are a group of fishes with a long history of systematic work, and the relationships of many species have long been enigmatic. In recent years our understanding of the evolutionary and biogeographic history of Middle American cichlids has greatly advanced. This study focuses on the genus *Chuco*, which contains three species (*C. intermedium*, *C. godmanni* and *C. microphthalmus*) distributed allopatrically in Atlantic drainages from southern Mexico, along Belize, northern Guatemala, and Honduras. In addition, two species of the genus, *C. godmanni* and *C. microphthalmus*, are considered diagnostic of areas of endemism in northern Middle America. Although all three species have been included in molecular phylogenetic analyses of Heroine cichlids, these studies have included only a few individuals of each species. In this study we investigate the systematics and biogeography of the genus and evaluate phylogeographic patterns of the three species of *Chuco* throughout their distribution using mitochondrial markers, as well as genomic-scale data (genotyping by sequencing, ddRAD-seq). Our results support the general hypothesis of phylogenetic relationships of species within the genus. Based on our biogeographic analysis, we propose that the Polochic and Motagua faults have played an important role in the diversification of the genus at different temporal and geographic scales. Finally, preliminary results suggest discrepancies in the biogeographic boundaries of these three species, where individuals recovered within recognized clades are distributed in aquatic systems outside the known allopatric range of each species, as currently understood.

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## Creating exciting exhibits for Conservation.

Dennis McNamara

The herpetology team at the Virginia Zoo strives to create naturalistic exhibits that mimic the regions where animals in the collection are endemic to. We add additional animals when possible to add variety to the exhibits to help accentuate the animal with higher conservation value. Our goal is to have visitors spend more time at each exhibit and appreciate the animals in a naturalistic set up. The longer we can have a visitor in front of an exhibit, the more we can have them appreciate the animal and hopefully educate them about their place in the world.

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## Mercury concentrations in sharks from southeast U.S. estuaries

Elliot McPhatter, Bryan Frazier, Jim Gelsleichter

Mercury is a nonessential toxic metal that often accumulates in sharks, posing health threats to these animals as well as human consumers of shark meat. Because of this, numerous studies have been conducted on mercury levels in sharks from coastal habitats throughout the U.S. east coast; however, few studies have focused on estuarine and nearshore sites on the South Atlantic Bight. The goal of this study was to examine mercury accumulation in sharks from South Carolina, southeast Georgia, and northeast Florida estuaries, focusing on two abundant species: the bonnethead *Sphyrna tiburo* and the sandbar shark *Carcharhinus plumbeus*. In addition to measuring and comparing muscle total mercury (THg) levels in individuals of these species from multiple sites along the southeast U.S. coast, we also examined site-associated differences in red blood cell THg concentrations in *C. plumbeus* to determine if this tissue could be used as a more specific indicator of recent mercury exposure. Muscle THg concentrations in *S. tiburo* varied little by site of collection and were often above thresholds for fish consumption and toxicity; however, they were comparable with levels previously reported for this species, which has been well studied with regards to mercury exposure. Muscle THg levels in juvenile *C. plumbeus* also varied little by site of collection and were largely below thresholds for human consumption and toxicity, a novel finding given the limited research conducted on Hg accumulation in this species. THg concentrations in juvenile *C. plumbeus* red blood cells also varied little by site.

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## Combining multiple isotope approaches and acoustic telemetry to determine trophic ecology and habitat use of the Atlantic Stingray (*Hypanus sabinus*)

Emily N. Meese, Jason Williams, Gregory W. Stunz, Jay R. Rooker, R.J. David Wells

This study combines dietary tracer (bulk stable isotope analysis, SIAbulk, and compound specific isotope analysis of amino acids, CSIAAA, of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) and acoustic telemetry methods to investigate the trophic ecology and habitat use of Atlantic Stingrays (*Hypanus sabinus*) in a northwestern Gulf of Mexico subtropical estuary. Atlantic Stingrays are demersal batoids commonly distributed in shallow waters from the Chesapeake Bay, USA to Campeche, Mexico; however, despite their wide distribution, abundance, and trophic significance, little work has been done in situ to quantify Atlantic Stingray trophic ecology and habitat use. Atlantic Stingrays were collected throughout Matagorda Bay, Texas, USA from June 2020 – September 2022. Stingrays ( $n = 50$ ) were externally fitted with acoustic transmitters and biopsied for isotope processing. Bulk  $\delta^{13}\text{C}$  values exhibited a strong spatial gradient depending on their capture location within the estuary along the marine-freshwater salinity gradient. Bulk  $\delta^{15}\text{N}$  values were high in areas of increased freshwater inflow to the estuary. Stingrays exhibited relatively high site fidelity near the locations they were originally tagged. CSIAAA values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  should infer more detailed information on stingray estuarine habitat use. Linking trophic ecology with habitat use information, and potential environmental drivers of individual behaviors, provides context and may infer more complete functional roles of specific habitats to mesopredator elasmobranchs. By establishing data on the movement patterns and habitat use of a common batoid species within a large estuarine system, this study may support conservation strategies for similar vulnerable elasmobranchs and effective management of estuarine habitat.

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## Phylogeny and species delimitation in the African genus *Bryconaethiops* (Characiformes: Alestidae): evidence from mitogenomes

Bruno Melo, Melanie Stiassny

Five species of the African alestid genus *Bryconaethiops* are found in freshwater environments ranging from the coastal rivers of southern Nigeria to the Chambezi river in northeast Zambia, with a majority in the Congo basin. No study has investigated interspecific phylogenetic relationships or the molecular diversity of species across their distribution range. We used entire mitochondrial genomes containing 13 protein-coding genes and 16,764 base pairs from 24 specimens of four species of *Bryconaethiops* and other alestid taxa as outgroups. According to the resulting phylogeny, *B. macrops* and *B. yseuxi* are the sister species of *B. boulengeri* and *B. microstoma*. Species delimitation analyses reveal the presence of a genetically separate lineage of *Bryconaethiops* in the Ogooue-Ivindo basin of Gabon. This genetic lineage with four specimens most likely represents an undescribed species that is sister to the clade that contains *B. boulengeri*, *B. microstoma*, *B. macrops*, and *B. yseuxi*. Mitogenome polymorphisms indicate that *B. macrops* is subdivided into three subgroups: (i) Lulua/Congo, (ii) Dja/Cameroon, and (iii) Malagarasi/Tanzania. Mitogenomes of the widespread species *B. boulengeri* and *B. microstoma* have reduced genetic diversity, with relatively long branches in *B. macrops* from Lulua and *B. boulengeri* from Mai-Ndombe region. Findings provide evidence for undescribed species diversity in *Bryconaethiops* and provide new avenues for studying the biodiversity of alestid fishes.

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## The Discovery of Some Rare and New Species of Brazilian Deep-Sea Fishes

Marcelo Melo, Amanda Gomes, Barbara da Silva, Cesar Romero, Flávia Masumoto, Guilherme Faria, Heloisa Caixeta, Julia Ibanhez, Marcos Reis, Pollyana Roque, Rayane França, Rodrigo Caires, Claudio Oliveira

The deep ocean is the largest environment available on Earth and the home to some of the most fascinating living creatures. The first collections of deep-sea fishes in the Western South Atlantic were by the HMS Challenger Expedition in the 1800's, followed by a few oceanographic cruises between the 1980's and early 2000's. Indeed, the knowledge concerning the diversity of deep-sea fishes in the Brazilian waters is still relatively scarce in comparison



to other ecosystems. The Project DEEP-OCEAN has the goals to increase the taxonomic sampling in the Brazilian EEZ and to foment integrative taxonomic studies using both morphology (e.g., microCT) and molecular (e.g., DNA barcoding) analyses. Two cruises were made onboard the Brazilian R/V Alpha Crucis focusing the continental slope off São Paulo and Santa Catarina States, in depths between 250 and 1,500 meters. A total of 50 hauls were made using a semi-balloon otter trawl and traps to collect fish and other invertebrates, and CTD for the oceanographic data. The collections resulted on the capture of a species of Myxini, 22 species of Chondrichthyes, and about 120 species of Actinopterygii, including some rare species, and putative eight new species which are under investigation, including two chimaereas (*Chimaera*), a lantern shark (*Etmopterus*), a scorpaenid genus (*Phenacoscorpius*), a beardfish (*Polymixia*), and a gernardier (*Ventrifossa*), or were recently described, such as the myrocongrid eel *Myroconger pietschi*, and the blind clusk eel *Sciaenodon alphacrucis*. Funding: FAPESP 2017/12909-4.

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### eDNA Metabarcoding Reveals Differences in Potential Prey Community Between Juvenile White Shark (*Carcharodon carcharias*) Aggregation and Non-Aggregation Sites

*Zachariah Merson, Matthew Barnes, Christopher Lowe*

Juvenile white sharks (JWS) aggregate at coastal beaches along the Southern California Bight, which serves as a nursery habitat, providing protection from larger predators, warmer environmental conditions, and sufficient food resources. While water temperature is thought to be important for aggregation site selection, the influence of prey abundance and community in aggregation site residency is unknown. Northeastern Pacific JWS are thought to feed on a variety of coastal teleosts and elasmobranchs. I use metabarcoding to compare the community composition and relative abundance of potential JWS prey at aggregation and non-aggregation sites. Water samples were collected at four beaches, two current and two former aggregation sites, and filtered for eDNA. The MiFish-U/E primers were used to amplify samples, sequencing was performed with an Illumina MiSeq, and taxonomic assignment was done in the DADA2 bioinformatics package using a California current reference library. At all sites, the dominant species were forage fish (e.g., *Engraulidae*, *Atherinopsidae*, *Clupeidae*). The community composition differed significantly between sites, though the aggregation sites were more similar to each other than to the non-aggregation sites. Benthic elasmobranchs (*Myliobatiformes*) and croakers (*Sciaenidae*) were the potential prey species that contributed most to the difference between sites. The results indicate that aggregation sites correlate with certain prey species, which could be a contributing factor to aggregation site selection. Understanding which species are associated with JWS aggregations may help predict future aggregations, discern the top-down trophic pressure JWS may exert, and determine the overlap with recreational and commercial fisheries.

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### Twelve Years of Field Surveys

*Michael Meyer*

Field surveys are an excellent way to understand and appreciate local herpetofauna. For the last twelve years during the spring semester at Christopher Newport University, Herpetology Lab [BIOL 440L] students have conducted surveys of the reptiles and amphibians throughout Hampton Roads, Virginia. To date, 48 species have been digitally documented from five local natural areas ranging from city parks to a National Wildlife Refuge.

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### An assessment of osmoregulatory gene regulation in two populations of the Atlantic stingray, *Hypanus sabinus*

*Joseph Miguez, Gavin Naylor*

*Hypanus sabinus* is a common species of euryhaline stingray belonging to the family *Dasyatidae* and subfamily *Dasyatinae*. *H. sabinus* is unique in its genus, as it is the only species that has a resident freshwater population. *H. sabinus* regulates its internal ion concentration as it moves between habitats of different salinities through control systems housed in the kidneys, gills, and rectal gland. The change in the deployment of genes that allow *H. sabinus* to tolerate both marine and freshwater environments has not yet

been comprehensively examined. My current research will allow for a better understanding of the physiological changes that are involved in the transition between marine and freshwater environments and the role that different tissues play in the change. Tissues of both freshwater and marine specimens of *H. sabinus* were collected to generate individual sequences using RNAseq and align with a reference genome. Results will be contrasted with similar findings in scientific literature that are mainly based on experimental physiology. While we expect that there will be an overlap with the results of this study and the previous findings based on gene expression and experimental physiology, we anticipate a discovery of new genes associated with the physiological response to a shift in salinity that have not yet been characterized. The genes identified will be mapped to candidate biochemical pathways to identify the potential mechanisms involved. I anticipate that the results from this work will serve as a baseline for comparative studies with other species of euryhaline and stenohaline elasmobranchs.

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### Opening Pandora's box: Phylogenomics reveals continuous innovation in a deep-sea radiation (Lophiiformes)

*Elizabeth Miller, Rose Faucher, Pamela Hart, Melissa Rincon-Sandoval, Aintzane Santaquiteria, Ricardo Betancur-R, Luke Tornabene, Kory Evans, Dahiana Arcila*

The deep sea is thought to be a harsh environment that imposes many evolutionary constraints. Alternatively, the deep sea could also provide a release from usual constraints in the shallow realm, such as competition or visually-driven predation. It is unclear how the push-and-pull of these opposing factors influences macroevolution. Here we examine rates of evolution in cranial shape using the anglerfishes (Lophiiformes), an order containing lineages that inhabit continental shelf, slope, and bathypelagic habitats. In particular, the ceratioid anglerfishes are an iconic radiation containing half the family and species diversity of the order, all of which evolved in the extreme environment of the bathypelagic zone. We estimated a novel phylogenomic hypothesis for Lophiiformes based on 1,000+ exons. Our taxonomic sampling expands on previous studies and includes 10 of 11 families and 40% of all species, the largest tree yet. Phylogenies were time-calibrated using 18 well-justified fossil calibrations. To infer evolutionary rates in skulls, we generated micro-CT scans of museum specimens representing two-thirds of the species in the tree. In this presentation we will discuss the relationships among the five suborders of Lophiiformes and their constituent families (frogfishes, batfishes, monkfishes, coffinfishes, and deep-sea anglerfishes) and the timescale of evolution. We will also present results of geometric morphometric analyses of skull shape and compare evolutionary rates among groups.

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### A novel phylogenomic hypothesis of Eupercaria, the new bush at the top

*Elizabeth Miller, JoJo West, Rose Faucher, Sandra Alvarez-Carretero, Giorgio Carnevale, Aintzane Santaquiteria, Emily Troyer, Carole Baldwin, Mark Westneat, Guillermo Ortí, Lily Hughes, Kory Evans, Ricardo Betancur-R, Dahiana Arcila*

Here, we present novel phylogenomic hypotheses for Eupercaria, a clade within the spiny-rayed fishes (Acanthomorphata) with similar diversity to passerine birds or placental mammals. The systematics of this group have long been plagued with uncertainty, largely due to short internodes associated with early rapid radiations (hence the 'new bush at the top'). Using >1000 exons obtained through target capture approaches, we built concatenation- and species-coalescent trees comprising 1,051 species. Our taxonomic sampling covers 80% of families, 40% of genera and 20% of species in Eupercaria (of 171 families and ~5,000 species total). We accommodated uncertainty in relationships and divergence times by generating additional trees from independent gene subsets. We time-calibrated the resulting trees based on 46 well-justified fossil and 15 geologic calibrations. In this poster, we present preliminary inferences of relationships among major lineages, which include reef fishes such as groupers, wrasses, butterflyfishes, surgeonfishes and pufferfishes; deep-sea fishes such as anglerfishes; coldwater groups such as icefishes, eelpouts, and sculpins; and freshwater groups such as darters and basses. We will discuss which clades are stable and which relationships remain uncertain even with the use of genome-wide approaches. These trees will form

the backbone of a re-evaluation of the classification of orders and families in Eupercaria, the timescale of evolution of major lineages, and a comparison of evolutionary rates among groups.

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### **Assessment of Density, Spatial Distribution and Movement Patterns of *Plethodon cinereus* (Eastern Red-backed Salamander) Using Spatial Capture-Recapture**

*Ryan Miller, Jennifer Deitloff, Jillian Fleming*

Spatial capture-recapture methods are an accurate method to measure population, and these methods have been used in many studies on population dynamics. These studies are important because they improve understanding of how species act differently in different environments. The Eastern Red-backed Salamander, *Plethodon cinereus*, is a terrestrial, lungless salamander that is common in Eastern North America from Quebec to North Carolina. *Plethodon cinereus* is small in size and can be easily found and studied in forest ecosystems. This species will defend their territory through aggressive behavior and are known to be territorial. However, the extent of territoriality of males and females, and how salamanders spatially disperse has not been thoroughly examined in Pennsylvania. In this study, we looked at the density, spatial distribution, and movement patterns of both sexes of *P. cinereus* using spatial capture-recapture methods. We used plots of coverboards in the salamander's natural habitat for this study. The following hypotheses were tested: (1) males have larger foraging territories than females and (2) both sexes display site fidelity by staying within the same plot and area within plot. Individuals were marked with a unique four-color code through injection of Visible Implant Elastomer tags. The goals of this study are to estimate the population density and understand the population dynamics of *P. cinereus* in central Pennsylvania. We found that males and females use approximately the same amount of area, in contrast to our first hypothesis. We found support for our second hypothesis that both males and females exhibit site fidelity.

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### **Rare MHC Alleles Are Associated with Lower Burdens of a Specialist Gill Parasite in Darters**

*Kara Million, Curt Lively*

The mechanisms that maintain high diversity at major histocompatibility complex (MHC) genes in vertebrates is an ongoing conversation in biology. Two plausible hypotheses are: (1) heterozygote advantage and (2) negative frequency-dependent selection (rare advantage), both of which focus on the selection pressure imposed on vertebrates by pathogens and parasites. The first hypothesis is that individuals with more alleles in MHC genotypes are more resistant to disease. The second hypothesis is that individuals possessing a rare genotype are more resistant to disease than individuals with common genotypes. In this study we tested these two hypotheses in three species of darter using two of their natural parasites: monogenean gill parasites (which are highly specialized on their hosts) and acanthocephalans (which are generalists). We genotyped host individuals at the MHC class IIb gene, and we measured prevalence and abundance of each parasite on all individuals. We used generalized linear models to evaluate the relationships between MHC genotype (frequency and number of alleles) and prevalence and abundance of both parasites. We found a significant positive relationship between the frequency of the rarest allele in an individual's genotype and the number of gill parasites, but not acanthocephalans. No relationship was found between number of MHC alleles in a genotype and parasite burden for either parasite. These findings indicate that selection by specialist parasites favors rare MHC alleles in nature, and thus support the negative-frequency dependent selection hypothesis.

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### **Comparison of the juvenile morphology and ontogenetic changes in morphology of three species of seahorses *Hippocampus* spp. from south-eastern Australia**

*Tony Miskiewicz, Kerry Parkinson, Graham Short*

Seahorses are a charismatic component of the fish fauna in estuarine and coastal marine water. In addition to their unique morphology and reproductive strategy, they are threatened by habitat degradation. The taxonomy of

seahorses has been problematic with many species misidentified in museum collections. This poster will describe the morphology of newly hatched juveniles and ontogenetic changes with growth of three species of seahorses, *Hippocampus whitei*, *H. abdominalis* and *H. breviceps* from south-eastern Australia. We will describe and compare differences in morphological characters of juveniles, such as the number and location of spines on the body and the structure of the coronet on the head, which are important features for identification of adults of different species. The ontogenetic changes in morphology of the juveniles will also be compared with the adult morphology of the three species.

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### **Ocular lens morphology is influenced by ecology and metamorphosis in frogs and toads**

*Amartya Mitra, Molly Womack, David Gower, Jeffrey Streicher, Brett Clark, Rayna Bell, Ryan Schott, Matthew Fujita, Kate Thomas*

The shape and relative size of an ocular lens affect the focal length of the eye, with consequences for visual acuity and sensitivity. Lenses are typically spherical in aquatic animals with camera-type eyes and axially flattened in terrestrial species to facilitate vision in optical media with different refractive indices. This presents an optical challenge to organisms inhabiting both aquatic and terrestrial environments. In this study, we examined the lenses of frogs and toads (Amphibia: Anura), which are ecologically diverse, with many species shifting from aquatic to terrestrial ecologies during metamorphosis to investigate the influence of ecology on lens shape. We quantified lens shape and relative size using 179 micro X-ray computed tomography scans of 126 biphasic anuran species and tested for correlations with life stage, environmental transitions, adult habits and adult activity patterns. Across broad phylogenetic diversity, tadpole lenses are more spherical than those of adults. Biphasic species with aquatic larvae and terrestrial adults typically undergo ontogenetic changes in lens shape, whereas species that remain aquatic as adults tend to retain more spherical lenses after metamorphosis. Further, adult lens shape is influenced by adult habit; notably, fossorial adults tend to retain spherical lenses following metamorphosis. Finally, lens size relative to eye size is smaller in aquatic and semiaquatic species than other adult ecologies. Our study demonstrates how ecology shapes visual systems, and the power of non-invasive imaging of museum specimens for studying sensory evolution.

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### **When the data run dry: how Bayes' Theorem can crack the life-history code of data-poor populations**

*Brian Moe, Charles Cotton, Dean Grubbs, Joseph Travis*

A basic understanding of individual age and growth and the corresponding life history strategy of a given species is crucial in evaluating the health of fish populations and determining appropriate management strategies. However, the requisite data can be extremely difficult to gather for rare or understudied species. Traditional approaches for estimating growth parameters often result in unusual or illogical parameter estimates when applied to data poor species. Bayes' Theorem, however, provides the ability to reasonably predict age/growth parameters with very little data, provided we have a rudimentary idea of their birth size, size at maturity, maximum size, and some notion of how individuals grow through time. Using a Bayesian-informed Markov Chain Monte Carlo simulation cross-validated with a two-sex age- and size-based integral projection model, we provide updated growth parameters which are more biologically realistic than previous estimates for several species with varying degrees of data deficiency and provide new insights into the populations' growth rates and their sex-specific age and size structures. This is a new innovated approach to model-fitting using directly observed information about the population and has the potential to revolutionize growth modelling and management of data-poor species.

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### **Physiological changes in the skin of Pacific newts (*Taricha*) along a terrestrial-to-aquatic life history shift**

*Haley Moniz, Evan Odberg, Emily Taylor*

Pacific newts (*Taricha*) are found in a variety of ecosystems throughout California, using terrestrial habitats during the non-breeding season and migrating to

occupy breeding pools each year. This biphasic life history requires that newts adapt to the demands of two environments with dramatically different hygric qualities. In response, many newt species undergo anatomical changes such as decreasing skin rugosity which are likely accompanied by physiological shifts that decrease desiccation tolerance and facilitate movement of oxygen across the skin while in breeding pools. Adopting these changes may also impact organs that maintain osmotic concentrations which are essential to homeostasis and could have long-lasting effects if they become disrupted. If transitioning from a terrestrial to an aquatic form alters how the skin interacts with water, newts may be particularly sensitive to drought conditions, especially if aquatic habitats are ephemeral and dry more rapidly than newts can resume a desiccation-tolerant terrestrial form. This project will use a combination of cutaneous evaporative water loss (CEWL) and plasma solute analysis to identify changes in water loss across the skin surface associated with each life history phase, and how that impacts internal physiology (e.g., hydration) by way of dissolved solutes in the blood. Results of this work will provide a novel insight into the role that water loss may play in constraining newt activity by providing accurate measures of evaporative water loss across the skin, which can lead to better estimations of critical hygric limits imposed by projected climate change.

### **Morphology, ontogeny, and replacement of teeth on the bills and lower jaws of billfishes (Istiophoriformes)**

*Miguel Montalvo, Laura Habegger, Kaitlyn Cisz, Katherine E. Bemis, Eric J. Hilton*

Billfishes (Istiophoriformes), comprising the swordfish (Xiphiidae) and the marlins, spearfishes, and sailfish (Istiophoridae), are a remarkable and charismatic group of teleostean fishes; their fast-swimming abilities, predatory lifestyles, specialized muscles within their large eyes for endothermic heating, and other features makes them unique among fishes. Their elongated rostra (the "bills" of billfishes) are possibly the most striking features of their anatomy. Formed predominantly by a pair of elongated premaxillae, as well as other supporting bones at its base, the bill and the lower jaw in Xiphiidae bear exceptionally small teeth as juveniles but are edentate as adults. In contrast, the oral jaws of the Istiophoridae have small but well-developed teeth present throughout their lives. The ontogenetic transformation of teeth in either group and the mode of tooth replacement in adult marlins have not been fully studied. We present new observations on the development of the oral jaw teeth in Xiphiidae and Istiophoridae based on an ontogenetic series comprising larval to adult specimens, which we studied using whole specimens, cleared and stained specimens, computed-tomographic data, and LM histology. We also present a preliminary assessment on the process of tooth replacement in the oral jaws of Istiophoridae.

### **Factors Affecting Digestive Efficiency in Three North American Snakes**

*Chad Montgomery*

Optimal foraging theory predicts that a snake will prey upon food items that maximize net energy gain because net energy intake directly affects an organism's fitness, i.e. lifetime reproductive success. Digestive efficiency (DE), or the proportion of energy ingested that is absorbed across the gut relative to ingested energy, is important for determining the energy budget of an organism because not all energy ingested is available to the organism. We determined the effect of prey type, meal size and predator body mass on the DE of Western Ratsnake (*Pantherophis obsoletus*) and the Common Watersnake (*Nerodia sipedon*). We also determined the effect of meal size and snake body mass on the DE of the Common Garter Snake (*Thamnophis sirtalis*). We used prey types that are representative of those typically encountered by each species, including quail eggs, quail chicks and mice for *P. obsoletus*, tadpoles, fish and frogs for *N. sipedon*, and fish for *T. sirtalis*. *Pantherophis obsoletus* digested eggs more efficiently than mice, and in turn digested mice more efficiently than chicks, possibly a result of difference in prey composition. *Nerodia sipedon* digested all prey types with equal efficiency. In addition, there was no significant difference in digestive efficiency between *Nerodia sipedon* and *Thamnophis sirtalis* when feeding on fish, likely due to ecological and physiological similarities between the two species.

### **What species are in the feces? DNA metabarcoding reveals the diet of threatened eastern massasauga rattlesnakes**

*Jennifer Moore, Alyssa Swinehart*

Characterizing the diet of imperiled species using minimally invasive methods is crucial to understanding their ecology and conservation requirements. We applied a DNA metabarcoding approach to study the diet of the eastern massasauga rattlesnake (*Sistrurus catenatus*), a Federally Threatened snake found throughout the Great Lakes region. Eighty-three fecal samples collected across 10 different massasauga populations located in Michigan, USA, were sequenced, with 70 samples containing prey DNA. We used universal metazoan primers and developed a host-specific oligonucleotide blocker to characterize their diet. We identified at least 12 different prey species, with eastern massasaugas exhibiting opportunistic feeding and a strong preference towards small mammals. Meadow voles (*Microtus pennsylvanicus*) were the most common prey item (70% of diet) followed by the northern short-tailed shrew (*Blarina brevicauda*) and masked shrew (*Sorex cinereus*; 15.7% of diet each), along with occasional bird and snake prey. Adult individuals exhibited a more generalized diet, consuming a larger number of prey taxa on average. Younger snakes consumed a smaller variety of prey items and tended to consume smaller-sized mammals such as masked shrews (*Sorex cinereus*) and northern short-tailed shrews (*Blarina brevicauda*). We conclude that small mammals are a crucial part of eastern massasauga rattlesnake diet and recommend this be taken into consideration when conservation strategies are developed. The methods developed in this study can be applied to other reptile species, providing an accurate, minimally invasive, and thorough diet assessment for at-risk species.

### **The Effect of Sexual Dimorphism in Mosquitofish on Predation of Amphibian Larvae**

*John Moore*

Female mosquitofish are significantly larger than their male counterparts. Female mosquitofish display higher feeding rates per unit body size and show a preference for larger prey compared to males. While the sex ratio of immature mosquitofish is 1:1, biological factors may alter this ratio in the mature population. Differential predation by birds on female mosquitofish may lead to extremely high proportions of males in natural populations. However, it is more common to find female dominated populations due to their longer lifespans. This sexual dimorphism has been shown to lead to differences in diet in regards zooplankton, but little has been done to show if it also leads to differences in predation on amphibian larvae. As the introduction of mosquitofish is often implicated in the decline of amphibians, this is an important metric to quantify. To examine this, I will conduct a 2 x 2 factorial indoor microcosm experiment involving sex (male or female) and two species of amphibian larvae (boreal chorus frog or small-mouth salamander). Mosquitofish predation experiments will be conducted in 10-gal aquaria containing treated tap water and a layer of gravel 2 cm deep. There will be ten trials for each treatment. In each predation trial, I will add 10 amphibian larvae and 1 mosquitofish. I will then let the experiment run for 24 hours. After 24 hours, I will record the survivorship of prey and the number of survivors with visible limb or tail injuries. This experiment will be conducted during the first week of April 2023.

### **Comparing the Effects of 2 Deterrents (Magnetic and Electronic Pulse) to Reduce Depredation in the Commercial and Recreational Fisheries**

*John Morris, Demian Chapman, Valerie Hagan*

Conservation legislation along the eastern US has demonstrated marginal increases in species specific shark populations (e.g. blacktips, sandbars, hammerheads). However, these successes in population management have become problematic to commercial and recreational fishermen losing their targeted catch to depredation. To combat this problem 2 types of deterrents have been attempted in field applications to deter catch loss to depredation. These in-field trials only documented the prey acquisition and did not document individual behavior towards the devices. To determine the effectiveness of each device we exposed active and control devices with a desired prey item to (3) sandbar sharks (*Charcharhinus plumbeus*) in our large animal research

facility. A total of 22 exposure trials using a magnetic device, a non-magnetic device, an electronic pulse device and a non-electronic pulse device were conducted over a 30 day period. In all trials the sharks acquired the prey item (bonito filet). However, the time to acquire the prey varied between deterrent devices. The average time to acquire the prey item between magnetic (active) and control devices was 16.7 and 14.8 seconds, and 21.0 and 12.9 seconds for the electronic pulse (active) and control devices. Comparing the active devices the initial time to acquire the prey was 25.0 seconds for the magnetic device and 40.0 seconds for the electronic pulse device. However, the use of these devices showed a diminishing negative response to the stimulus with the final prey acquisition times of 2.9 seconds for the Magnetic device and 14.0 seconds for the Electronic Pulse device.

## Disentangling decision rules underlying flexible sex-reversal of parental care in a poison frog

Jeanette Moss, Katherine Julkowski, Molly Podraza, Eva Fischer

Flexible parental care strategies are widespread in nature, and factor particularly prominently into conflict between the sexes and the realization of sex roles. While one sex is typically the primary caregiver, the other sex may retain caregiving capabilities but limit their involvement to a compensatory capacity. While adaptive explanations abound, the mechanisms that underlie flexible 'sex-reversal' of care are not always clear. Here, I enlist a biparental frog (*Ranitomeya imitator*) with flexible sex-reversal of tadpole transport to investigate how multiple sources of environmental information are integrated to inform takeover decisions. Using standard mate removal experiments in the laboratory, I show that members of the flexible sex (females) express greater individual variation in transport behavior than members of the primary transporting sex (males). To differentiate whether successful females are responding to cues of an absent partner or of offspring need, I next manipulate acoustic and visual stimuli of mates following their removal. Preliminary data suggest that a female's perception of her social environment can affect her involvement in care, potentially overriding signals of offspring need. Acoustic cues (playback of partner calls) appear to have stronger effects on female behavior in this species than visual cues (frog dummy). These behavioral data lay the groundwork for future investigations into the neurogenomic mechanisms of sex-reversed care.

## Using a synthetic GnRH agonist to modulate aggression in male cownose rays (*Rhinoptera bonasus*): Evaluating effectiveness with an enzyme-linked immunoassay

Kat Mowle, Linda Penfold, Michael Hyatt

Enzyme-linked immunoassays are helpful for evaluating the reproductive status of elasmobranchs but validation of the assay for each species, including physiological validation, is imperative to determine usefulness. In this study, aggression control in cownose rays (*Rhinoptera bonasus*) was investigated by treating them with Suprelorin® implants and monitoring plasma testosterone concentrations. Suprelorin® is a synthetic GnRH agonist that is commonly used as a contraceptive agent across a range of mammalian species and has been shown to decrease concentrations of plasma testosterone in multiple species. In this study, three male *R. bonasus* were implanted with Suprelorin®, while a fourth male was used as a control and not given the implant. Plasma samples were collected from all four males before the implant was placed for baseline concentrations and three weeks post-implant. After these initial samples, all males were sampled monthly for a year. A double diethyl ether extraction method was utilized to extract the steroid hormones from the plasma. Samples were analyzed using a competitive double antibody enzyme immunoassay using an antibody and corresponding horseradish peroxidase (HRP) conjugate against testosterone (testosterone-6-carboxymethyl oxime:BSA; R156/7). Serial dilutions of pooled extracted plasma yielded curves parallel to the standard curve. Results showed no change in plasma testosterone in the control male, but sustained, elevated concentrations in the treated males that have continued up to 12 months post-treatment. These findings are similar to those documented for Bovidae species, which also demonstrated elevated testosterone post-treatment with Suprelorin®. Results indicate species differences in response to GnRH analogs.

## Feeding kinematics and ethology of Blue Sharks, *Prionace glauca* (Carcharhiniformes: Carcharhinidae)

Joshua Moyer, Jon Dodd

Studying the kinematic and behavioral aspects of feeding performance in large, pelagic sharks has historically been hindered by logistical limitations, such as a lack of access to specimens and the availability of durable, field-portable video systems. We report findings from high-speed videography of wild Blue Sharks, *Prionace glauca*, to present the first kinematic analysis of feeding in the species using bite duration as the principal metric of performance. The mean duration of bites in which food items were consumed in an uninterrupted bite sequence was  $0.41 \pm 0.016$  s. Bite duration varied across individuals. Indices of stereotypy indicate that feeding performance varied at the individual level. We report correlations between feeding behaviors and kinematics of *P. glauca* and discuss these findings with respect to biotic and abiotic variables, including food size, depth, and the presence of conspecific and heterospecific sharks. Based on the variability and modulation of feeding performance in the Blue Shark, we propose the feeding modulation in pelagic generalists (FMPG) hypothesis, which states that the ability to modulate feeding performance in an environment with patchy nutrient availability confers an adaptive benefit to pelagic sharks with varied diets.

## Diversity of Ichthyoplankton Collected Inside and Outside of Eddies in the Sargasso Sea

Sarah C. Muffelman, Eric J. Hilton, Deborah K. Steinberg

Ichthyoplankton collected in the North Atlantic near Bermuda from June-August of 2004 and 2005 were sorted from zooplankton samples and the fish larvae identified. The samples are from a project in which plankton were collected inside, on the periphery, or outside of persistent mesoscale eddy features using a Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS); surface ring-net samples were also collected during the cruises. Preliminary analyses indicate that the most abundant families found in MOCNESS samples from both inside and outside of eddies are Gonostomatidae, Myctophidae, and Sternoptychidae. For ring-net samples, the most abundant families are Gonostomatidae, Myctophidae, and Scombridae. Other frequently occurring families in MOCNESS samples include Phosichthyidae, Paralepididae, and Melamphidae. Additionally, some samples contain several families of coral reef associated taxa such as Scaridae and Labridae. Relatively rare taxa in the family Stomiidae were found in good condition in both MOCNESS and ring-net samples. These data will form the basis of future analyses of the influence of eddies on larval distribution.

## Eco-evolutionary processes in community assembly of Appalachian salamander skin microbiomes from a pathogen-protective trait view

Carly Muletz-Wolz, Randall Jimenez, Owen Osborne, Allison Byrne, Brian Gratwicke, Amy Ellison

We determined the eco-evolutionary processes structuring skin microbiomes and their association with *Batrachochytrium dendrobatidis* (Bd) infection in 10 salamander species. We sampled species from six genera (*Ambystoma*, *Desmognathus*, *Eurycea*, *Gyrinophilus*, *Notophthalmus*, and *Plethodon*) at 12 sites in Central Appalachians, USA ( $n = 222$ ) and their environment (pond, stream or forest,  $n = 118$ ). We found Bd infection in six species (*E. bislineata*, *P. cinereus*, *P. glutinosus*, *D. fuscus*, *G. porphyriticus*, *N. viridescens*), with *N. viridescens* having the highest Bd prevalence and loads. High richness and relative abundance of putative Bd-inhibitory bacteria were correlated with decreased Bd load. Both host evolutionary history and environment impacted skin microbiomes; host evolutionary history had a stronger effect and we detected phylosymbiosis (correlated microbiome change with evolutionary divergence), even after correcting for environmental microbiomes. In three salamander species with robust sampling (*N. viridescens*, *E. bislineata* and *P. cinereus*), we incorporated null-based assembly models and co-occurrence networks to investigate environmental selection and bacterial associations effects on skin microbiomes. Bd-infected *N. viridescens* had a high proportion of negative associations in bacterial networks (40%), while uninfected *N. viridescens*, *P. cinereus* and *E. bislineata* had more positive associations



(70%, 70% and 87%, respectively). Putative Bd-inhibitory bacteria were shared between environment and salamander species, ranging from 27 to 29 bacterial taxa, and were detected as hub taxa (highly connected) in networks. Our findings suggest that environmental bacterial reservoirs are important to maintaining microbiomes within the constraints of host evolutionary history and that microbial-Bd interactions drive competition in the microbiome.

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## Estimating the Species Composition of Global Shark and Ray Fisheries

*Christopher Mull, Elizabeth Babcock, Ana Martins, Zoya Tyabji, Devanshi Kasana, Luke Warwick, Demian Chapman, M. Aaron Macneil*

Shark and ray fisheries landings have decreased over the past decade. While more than 300 species are caught in global fisheries, reliable species-specific landings information is scarce as statistics are often reported as aggregate categories (e.g. “sharks, skates, and rays, nei”). Aggregate data masks important species-specific trends, complicating stock assessment efforts and obscuring the ecological footprint of fishing. Generating reliable species-specific landings estimates across the globe is critical for effective fisheries management. Here, we present an estimate of species-specific landings across more than 100 countries from 2012 to 2019 based on the FAO Fisheries Capture Production Database. Aggregate landings were allocated to species based on taxonomy, FAO Major Fishing Area, and estimates of fishing pressure. Unsurprisingly, blue sharks dominate global landings though batoids represent a greater proportion than expected. Our results provide species-specific trends in fisheries landings and will assist with estimating biological reference points across all landed shark and ray species globally. These refined landings estimates will improve stock assessments and strengthen the implementation and enforcement of conservation initiatives such as CITES.

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## Taxonomic Elevation of Two Pirate Perch Subspecies and Three New Species

*Tyler Muller, Andrew Simons*

Pirate Perches, Aphredoderidae, are a widespread lowland family of freshwater fishes native from the Mississippi River basin east to the Atlantic slope. Pirate Perch as of recently were described as two subspecies *Aphredoderus sayanus sayanus* on the Atlantic slope and *A. s. gibbosus* in the Mississippi River Valley and Great Lakes with a broad intergrade zone in the Gulf of Mexico. Here we use molecular and morphological data as evidence support elevating both subspecies, and describe three new species of Pirate Perch.

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## Comparing protein evolution in sexual versus parthenogenic whiptail lizards (*Aspidoscelis*)

*Zoë Müller, Matthew Fujita*

Asexuality has evolved in scattered groups across the animal kingdom. Given that the majority of animals are sexually reproducing, there must be evolutionary consequences to reproducing asexually. For example, Muller's Ratchet predicts that deleterious mutations accumulate in asexual populations compared to sexual populations. Using whiptail lizard (*Aspidoscelis*) transcriptomes, I compared synonymous and nonsynonymous mutations in a parthenogenic lineage, *Aspidoscelis tessellatus*, with its sexual parents, *Aspidoscelis marmoratus* and *Aspidoscelis gularis*. I expect to see more deleterious mutations in the asexual *A. tessellatus*, supporting Muller's ratchet theory. I also predict that there will be less nucleotide diversity in the asexual lineage in comparison with its sexual parents, reinforcing that recombination and segregation enforce sequence diversity. These analyses allow us to better understand how mutations accumulate in asexual lineages, thus illuminating how asexual lineages continue to evolve in vertebrates. Because *A. tessellatus* is a young species, this analysis also helps us identify how quickly mutations accumulate in asexual vertebrates.

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## The Spatiotemporal Impacts of Habitat Change on an Estuarine Predator, the Juvenile Bull Shark (*Carcharhinus leucas*)

*Lindsay Mullins, Eric Sparks, Kristine Evans, John Cartwright, John Mareska, Marcus Drymon*

Nearshore fisheries are highly vulnerable to the impacts of climate change and other stressors, including urbanization and overfishing, given the increasing pressure placed on coastal aquatic systems. Studies have demonstrated that coastal fish species distributions have altered in response to changing water quality parameters linked to climate change, particularly increasing temperatures. Few studies, however, have evaluated these variables in conjunction with additional habitat alterations, such as land-use/land-cover (LU/LC) changes. We used a multi-decadal gill net survey of coastal Alabama to examine spatiotemporal impacts of habitat change on juvenile bull sharks (*Carcharhinus leucas*). Boosted regression trees were used to assess the impacts of water temperature, depth, salinity, dissolved oxygen, riverine discharge, Chl-a, and LU/LC changes on the likelihood of capture and identified water temperature as the primary marginal effect. These models were then used to create species distribution models for early (2003) and recent (2020) portions of the times series, which indicated increased habitat suitability for juvenile bull sharks over time. Regression models identified significant changes in temperature, LU/LC, and Chl-a on the study area since 2000. These results align with observed increases in relative abundance of bull sharks across the study period and demonstrate the impacts of changing environmental conditions on the abundance and distribution of juvenile bull sharks.

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## Extinction Risk Assessments for members of the Flatfish Suborder Pleuronectoidei (Pleuronectiformes): Global Perspective on Conservation Evaluations for a Diverse Taxon

*Thomas Munroe, Gina Ralph, Christi Linardich*

Flatfishes constitute a diverse group of interesting fishes characterized by bilateral asymmetry. They occur in shallow to moderately deep (ca. 2000 m) demersal habitats worldwide. Most species inhabit marine waters, several reside within estuaries, and a limited number occur strictly in freshwater. Flatfishes range from 2.5 to 365.0 cm. Larger species support major commercial and recreational fisheries, while smaller, primarily tropical, species are an important component of non-selective trawl fisheries. In collaboration with 20+ international experts, we applied IUCN Red List methodology to assess conservation status of 811 flatfish species. Nearly 4% of species for which data are available are considered threatened (range 2-43%). Most threatened species are large, heavily exploited members of the Pleuronectidae, many of which are also impacted by warming waters associated with climate change. However, this family also includes key examples of species with well-managed fisheries, particularly those occurring in the northeast Pacific Ocean. In contrast, speciose families, Soleidae, Bothidae, and Cynoglossidae, record high numbers of Data Deficient species. Many of these species experience high levels of anthropogenic threats, though population-level changes could not be quantified because of limited data. Taxonomic issues can impede accurate application of IUCN Red List criteria, while assessments themselves highlight data gaps and can inspire new avenues for research. For example, inconsistencies in taxonomy applied to several subspecies of *Platichthys flesus* have implications when assessing conservation status of these taxa. This first comprehensive extinction risk assessment of flatfishes forms the baseline for evaluating future changes in biodiversity of these fishes.

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## Competition intensification drives century long declines in a common lizard species, *Sceloporus consobrinus*.

*Alexander Murray, Edita Folfas, Morgan Page, Zach Lange, Joe Mruzek, Luke Frishkoff*

Habitat modification and climate change have been identified as primary mechanisms responsible for ongoing changes in populations. However, species interactions may be of a similar level of importance. Here we assess the role of competition in recent population trends and current distribution of two closely related lizard species, the prairie lizard (*Sceloporus consobrinus*)

and the Texas Spiny lizard (*S. olivaceus*). Occurrence data reveals divergent population trends, *S. consobrinus* decreasing and *S. olivaceus* increasing in relative frequency over the last century. We spatially aggregated records of lizards into randomly placed pseudo-sites to determine the role of climate suitability, landcover and species interactions in shaping the occurrence patterns of *S. consobrinus*. Presence of *S. olivaceus* greatly reduces occurrence of *S. consobrinus* and explains occurrence better than either climate suitability or landcover. Over the last century rates of exclusion by *S. olivaceus* are increasing as is urbanization, which combine to account for the decline of *S. consobrinus*. To test whether patterns of co-occurrence detected using occurrence data are indicative of competition we conducted transects surveys to assess local lizard communities and paired this with behavioral trials. Surveys revealed lower abundance of *S. consobrinus* on transects containing *S. olivaceus*, only co-occurring on 2 of 176 transects. Behavioral trials revealed the dominance of *S. olivaceus*, which controlled the basking location, and initiated more interactions that led to retreats than *S. consobrinus*. Overall, we present an example of the importance of species interactions in structuring communities and suggest that human influences are strengthening such interactions.

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### Impacts of a common organophosphorus insecticide on amphibian development

*Santoshi Mutyala, Delanie Crabtree, Sara McClelland*

Organophosphorus pesticides target the nervous system of insects. Unfortunately, these pesticides can also be found in nature including in the aquatic habitats of many amphibians. While we know a lot about acute exposures to organophosphates, exposures to low and more environmentally realistic concentrations are less well studied. In this study, we wanted to better understand the impacts of organophosphates after a prolonged chronic exposure to environmentally realistic concentrations of an organophosphate. To do this, we exposed Northern Leopard Frog (*Lithobates [Rana] pipiens*) tadpoles to either a vehicle control or to 1 µg/L of the organophosphate malathion for approximately 20 weeks in a blind, controlled laboratory study. We are analyzing changes to development including impacts on body and brain morphology. By better understanding how these common environmental pollutants are impacting the nervous system of tadpoles, we may be more likely to improve how we apply pesticides. This could help to conserve herpetofauna worldwide.

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### Genomic Data Provide Insight into Adaptation and Demographic History of South American Rattlesnakes

*Edward Myers, Kevin de Queiroz, Rayna Bell*

The savannas of South America cover over 250 million hectares with a fragmented distribution, divided by the Amazonian rainforest into northern and southern blocks. Although these savanna blocks are presently separated, numerous species are found in both, indicating that these regions were likely recently connected. The timing and potential routes of connectivity, however, are contentious. By leveraging recent advances in conceptual and computational methods in the field of phylogeography, we can gain a better understanding of the drivers of diversification in such historically dynamic ecosystems. *Crotalus durissus* (the South American rattlesnakes) is widespread across South America and extremely variable in color and pattern, with 11 described subspecies, making it an excellent candidate for use in biogeographic studies to understand drivers of diversification. Here we sample across the distribution of this taxon, generate whole genome sequence data, and integrate these data with geographically explicit spatial simulations with ecological niche models to test if competing models of historical shifts in the distribution of *C. durissus* can explain the current genetic structure. With the extensive genomic data collected here we will be able to address the impact of late Quaternary climate fluctuations in driving habitat changes and the accumulation of biodiversity in South America.

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### Humans Disappear, Predators Move In: Anthropause Outcomes from a Stable Aggregation of Spiny-tailed Iguanas, *Ctenosaura similis*

*Ann-Elizabeth Nash*

Anthropause offers an opportunity to understand how animals behave when experiencing a sudden reduction of human activities. In March 2020, the SARS-CoV-2 pandemic resulted in the closure of Costa Rican national parks. At Palo Verde National Park, all Organization for Tropical Studies (OTS) visits were cancelled, and staffing was greatly reduced. In 2022, a stable aggregation of Spiny-tailed Iguanas, *Ctenosaura similis*, living at the OTS station and studied >10 years, was assessed to understand the effects of the anthropause. With reduction of human workers and visitors, animals including Ocelot, Grey Fox, and White-tailed Deer began using station areas. When the park reopened to visitors, some marked lizards were absent during surveys and lizards were perceived to have endured increased bodily trauma. Data analysis determined the persistence of individuals from first capture to current capture period. While the number of first-time captures varied across years, the quantity of known animals captured from each previous period was significantly consistent. For Spiny-tailed Iguanas, in the absence of suspected predator release, and in the presence of predator congeners, the anthropause did not change persistence for this population.

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### The effects of incubation temperature on larval morphology of the locally endangered Streamside Salamander (*Ambystoma barbouri*)

*Tatyana Natal, Julia Thulander, Joshua Hall*

The Streamside Salamander (*Ambystoma barbouri*) is a diminishing species within Tennessee. While habitat fragmentation is the primary cause, climate change will further negatively affect the species. In particular, rising nest temperatures may influence important morphological traits like body and head size which may interfere with locomotion and feeding. To determine how incubation temperature influences development and resultant morphology, we collected eggs from populations across the Streamside Salamander range and incubated them at a range of temperatures (5-25 °C). We report results of incubation temperature on key morphological traits of larvae which affect important behaviors like escaping predators (e.g. tail length, body length) and feeding (e.g. head size). This study will demonstrate how a range of incubation temperatures affect the morphology and resultant performance of Streamside Salamanders and how such thermal affects vary across populations.

Our results will aid targeted conservation efforts by demonstrating which populations are most susceptible to rising temperatures due to climate warming.

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### Spawning Phenology of Larval Pacific Sand Lance (*Ammodytes personatus*) and Arctic Sand Lance (*A. hexapterus*)

*Laurel Nave-Powers, Luke Tornabene, Alison Deary*

The Pacific Sand Lance (*Ammodytes personatus*) and Arctic Sand Lance (*A. hexapterus*) are important forage fish in the Pacific Northwest of the United States. There is a large knowledge gap for their larval life stage, and most information on spawning phenology is based on Pacific Sand Lance. Spawning phenology effects when fishes are found as well as the survival of the larvae and could be shifting with climate change. This study assessed the spawning phenology of Pacific and Arctic Sand Lance by analyzing a 20+ year dataset of plankton surveys from 2000 to 2022. Specifically, we examined the distribution and standard lengths of larvae caught for both species from the months of May through September. Weighted mean lengths (WML) and length-frequency distributions were used to back-calculate peak time of spawning. We hypothesized that Pacific Sand Lance spawn earlier in the fall (larger increase in WML) and Arctic Sand Lance spawn later in late fall/early winter (smaller increase in WML). We also expected length-frequency plots to show more larval Pacific Sand Lance in late spring and early summer compared to Arctic Sand Lance, reflecting this difference in spawning time. Given the effect that climate change has on spawning phenology in other marine species, we also hypothesized a general shift in spawning to earlier

in the year for both species over time. Understanding spawning phenology of these species and how they are changing in response to a warming ocean is critical for the management of these ecologically important forage fishes.

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## **Aging tuatara (*Sphenodon*): complexities for population management**

*Nicola Nelson, Susan Keall, Linlin Liu*

Aging individuals in the wild is complex where no physical characters enable definite age steps to be determined, yet age structure is a crucial parameter in population viability analyses. Size is often used as a surrogate for age, but in reptiles, growth may be variable and indeterminate based on resource availability. We estimated age of wild tuatara (*Sphenodon*) in a population of concern on North Brother Island, New Zealand. We applied von Bertalanffy growth equations using snout-vent length to confirm that both sexes grow in length until they are ~30 years old, then growth asymptotes. Aging following the size asymptote can then only be estimated based on the length of the monitoring period. Physical observations suggested a cohort are in poor condition, appearing 'old', but using 30 years of capture data on individually identified tuatara, we were unable to conclude an aging population structure. Furthermore, recent analysis of our data set estimates mean tuatara longevity at 137 years. Implications of these data for population viability are numerous. For example, we lack information on reproductive senescence in the wild, and whether individuals continue to contribute significantly to recruitment in their later years is likely confounded with condition which appears poor in the observed 'old' individuals. Low reproductive output by old individuals essentially reduces the effective population size. Moreover, size-age patterns are proving to be misleading in populations translocated for conservation due to increased resource availability, meaning estimating age of tuatara will require a monitoring period more commensurate with their longevity.

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## **Ontogeny Recapitulates Phylogeny in the Development of the Parrotfish Skull (Labriformes: *Scarus iseri*)**

*Mayara Neves, April Hugli, Simon Brandl, Kory Evans*

During ontogeny, multicellular organisms often undergo dramatic shape changes associated with increases in size. Rarely, juveniles may pass through stages that superficially resemble the adult stages of ancestral lineages in a pattern known as recapitulation. Recapitulation may have distinct effects on juvenile ecology if the morphology of the juvenile differs substantially from the adults. Parrotfishes (Eupercaria: Labriformes) are highly specialized herbivores that exhibit robust beaks and skulls that allow them to feed directly on hard coral skeletons. Recent studies have shown that parrotfishes inherited their beaks from a wrasse ancestor during the Oligocene. However, it is not entirely clear how the parrotfish beak and skull develop and change during growth. Here, we use micro-CT scanning and three-dimensional geometric morphometrics to analyze the skull of a parrotfish, *Scarus iseri*, to understand the ontogenetic transformations therein. We compared the ontogenetic changes of *S. iseri* to the skull shapes of 150 adult parrotfishes and 157 adult non-parrotfish wrasses to test for ontogenetic recapitulation. We find that the individuals of *S. iseri* in the early stages of development and juveniles have skull shapes that more closely resemble their non-parrotfish wrasse relatives. Throughout ontogeny, skull shapes become more similar to the skull shape of adult parrotfish species. These changes are mainly related to the neurocranium, though high-resolution micro-CT scans also reveal that the juvenile parrotfishes lack fully coalesced beaks and instead exhibit individual teeth. Therefore, the evolution of the highly specialized parrotfish skull may have occurred through terminal modifications of an ancestral wrasse developmental program.

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## **Re-examination on two closely related *Etmopterus* species, *E. decacuspidatus* & *E. evansi*: Is the cusplet number stable to separate lanternshark species?**

*Shing-Lai Ng, Katherine Bemis, Kwang-Ming Liu, Shouu-Jeng Joung*

The lanternshark genus *Etmopterus* Rafinesque, 1810 is distinguished from other Squaliformes by having multicuspoid upper teeth, and blade-like, cutting lower teeth. Cusplet number in the upper teeth have often been used as a

diagnostic character to separate closely related species, yet the sexual and ontogenetic variations in cusplet numbers have been rarely evaluated. One example is the high similarity between *E. decacuspidatus* Chan, 1966 known solely from the holotype, and *E. evansi* Last, Burgess & Séret, 2002, which only differ in cusplet numbers. In the present study, we aim to test the validity of cusplet number to separate the two species. We examined 100 morphological characters, including the cusplets of 31 specimens of *E. decacuspidatus* collected from commercial trawlers in the South China Sea, and 12 *E. evansi* specimens (including type specimens) deposited in the museums. We find that all the immature specimens have one or two cusplets on each side of the cusp, while large female specimens have two cusplets in both species. In contrast, mature males mostly have three cusplets, rarely four in both species. Our results suggested that the cusplet number cannot be used as a diagnostic character to separate the two species. In addition, we find limited morphological difference between the two species, which suggested that *E. evansi* is possibly a junior synonym of *E. decacuspidatus*. Further comparisons on the genetic structure and osteological characters between the two species may better clarify their relationships.

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## **Integrated approaches show taxonomic uncertainties in two lanternshark species *Etmopterus burgessi* and *E. moller*, with implications for future taxonomic work**

*Shing-Lai Ng, Nicolas Straube, Kwang-Ming Liu, Shouu-Jeng Joung*

The lanternshark genus *Etmopterus* Rafinesque, 1810 is the most diverse group among all extant shark genera containing at least 42 species. Although new species are continuously described, ambiguous taxonomic relationships between several species remain unresolved, which impedes essential studies regarding conservation and management approaches of these poorly known deep-sea sharks. Our study aims to clarify the taxonomic interrelationships of two species occurring off Taiwan: *E. burgessi* Schaaf-Da Silva & Ebert, 2006 and *E. moller* (Whitley, 1939). We use an integrated taxonomic approach based on 100 morphological characters and molecular analyses of the mitochondrial NADH2 marker (1044 bp) for 105 specimens from the North- and Southwest Pacific representing the species' distribution. Our analyses also include specimens of *E. moller* and *E. lucifer* Jordan & Snyder, 1902 from Australian waters. Our results show that *E. burgessi* closely resembles *E. lucifer* described from Japan, but is distinct from *E. lucifer* of Australian waters. Similarly, *E. moller* of Taiwan differed from *E. moller* of Australian waters. Our results suggested that: 1) *E. burgessi* is possibly a junior synonym of *E. lucifer*; 2) *E. abernethyi* Garrick, 1957 should be resurrected for specimens hitherto assigned to *E. lucifer* around Australian waters; 3) *E. schmidt* Dolganov, 1986 should be resurrected for specimens assigned to *E. moller* in the northwestern Pacific. We also conclude that several morphological characters such as the lateral flank-marking shape and length, as well as the body coloration, are quite variable within species, and should be used cautiously for species identification.

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## **Scanning for Oral and Pharyngeal Jaw Decoupling in Neotropical Cichlids**

*Benjamin Nicholas, Hernán López-Fernández*

A central question of biology is what promotes diversification in some groups and not others. Fishes exhibit remarkable phylogenetic and ecological diversity, with most fishes feeding with two sets of jaws: the oral and the pharyngeal jaws. Several groups of fishes have evolved additional skeletal and muscular modifications to their pharyngeal elements, such as the fusion of the fifth gill arch. In cichlids, a specialized pharyngeal jaw is hypothesized to have allowed for the functional decoupling of the two jaws, with the oral jaws specializing in prey capture and the pharyngeal jaws in prey processing. The proposed decoupling allows the jaws to evolve independently and promote trophic diversification. Neotropical cichlids are an ideal system to test whether the two jaws are decoupled as they are speciose and contain many diverse feeding ecologies. Previous studies on jaw decoupling in cichlids have generally focused on variation in the lower pharyngeal jaws and not the entirety of the feeding system. Focusing on the whole feeding system will preserve the functional and anatomical context of the jaws and allow for better understanding of how the two jaws have evolved in relation to the rest of the feeding system. Utilizing museum specimens and  $\mu$ CT scanning, we quantify

the shape of the entire feeding system of 108 species of Neotropical cichlids using homologous and semi-sliding landmarks. Using 3D geometric morphometrics and phylogenetic comparative methods, we test to what degree the oral and pharyngeal jaws are decoupled and whether dietary ecology influences patterns of integration.

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### Short-term Tissue Regeneration Rates in Lesser Sirens (Caudata: Sirenidae)

*Mackenzie Nichols, Lorin Neuman-Lee, Stephen Mullin*

The ability to respond to the presence of a stressor is vital to an organism's survival. Amphibians are model organisms to examine the effects of stress because their exposed integument makes them sensitive bioindicators. More specifically, Lesser Sirens (*Siren intermedia*) are appropriate species for use in physical stress research because of their ability to regenerate tissue following injury. These fully aquatic salamanders can be abundant in wetlands, and conspecific interactions can be aggressive and result in skin lacerations. To date, only one study has addressed the rate of tissue regeneration in *S. intermedia*, but short-term rates of regeneration (i.e., immediate response to injury) have not been examined. After collecting specimens from two sites in north-east Arkansas, we used iris scissors to remove a portion of each subject's dorsal tail fin that was less than half the diameter of the broadest region of the tail. We recorded photos of the excised area daily for a seven-day period after the tail tissue was removed. We used ImageJ to quantify the area and length of the tail scoop in each photo, and scaled these values using the subject's body condition index. The rates of tissue regeneration appeared to be similar and there was little repair to the damaged portion of the tail within the week-long period. These results indicate that sirens undergo long-term tissue regeneration rather than short-term tissue regeneration following instances of physical stress, regardless of individual body condition.

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### Factors Affecting Home Range Size in the Blunt-nosed Leopard Lizard (*Gambelia sila*)

*Emmeleia Nix, Nicole Gaudenti, Michael Westphal, Emily Taylor, Brian Todd, Kathleen Ivey, Paul Maier, Nargol Ghazian, Malory Owen, Mario Zuliani*

Understanding space use of threatened species is vital in conservation planning because it can inform the minimum patch size needed to support a population and the importance of connectivity among patches. The Blunt-nosed Leopard Lizard (*Gambelia sila*) is one species in North America that is highly threatened due to human land-use and habitat conversion. Small populations persist in the southern Central Valley and adjacent foothills of California, USA in isolated populations separated by agriculture, residential areas, and industrial drilling sites. Here, we radio-tracked 106 Blunt-nosed Leopard Lizards (*Gambelia sila*) from May to mid-July from 2018–2020, during which precipitation, temperature, and NDVI varied among years. We calculated home range areas using both 95% Minimum Convex Polygon (MCP) and time-based Local Convex Hull (tLoCoH) methods. We evaluated whether home range area was correlated with sex, body temperature, body mass, precipitation, air temperature, and NDVI. Preliminary analyses show that males had larger home ranges than females, mass was not correlated with home range area, and NDVI and precipitation positively correlated with home range area.

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### Kako Morita: A Japanese Artist and his Struggle to Survive as a Scientific Illustrator in early 20th century America

*Ai Nonaka, Lisa Palmer, Laurence Dorr*

Kako Morita, a Japanese illustrator, worked for DS Jordan, Bashford Dean, Barton Evermann, and other prominent ichthyologists of the time in the early 1900s in the U.S. During his two years as Jordan's assistant at Stanford, he drew approximately 100 drawings of fishes, which are beautiful, precise and important contributions to ichthyology as of today. His illustrations were used often in Jordan's publications. However, we do not know where Morita met Jordan or how he came to work for him. We learned that during Morita's stay in the U.S., he worked as an illustrator, moving from place to place throughout

the U.S, partly depending on Jordan's recommendation to his colleagues. We discovered a number of Morita's letter to Jordan, in which Morita struggled and asked Jordan for his help. Still there are several mysteries about Morita, for example, 1. No physical figure, 2. Little information available in Japan. 3. His family and offspring unknown, 4. How he managed to live financially in the US for almost 30 years, 5. How and where his life ended.

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### Persistent Shallow, Low-Amplitude Diel Vertical Migration (DVM) in Tope Sharks, *Galeorhinus galeus*

*Andrew Nosal, Daniel Cartamil, Lyall Bellquist, Noah Ben-Aderet, Kayla Blincow, Natalie Klinard, Christopher Mull, Brice Semmens, Philip Hastings*

Diel vertical migration (DVM) is a widespread phenomenon among aquatic animal taxa, where 'normal' DVM presents as individuals occupying deeper water during the day than at night, and vice versa for 'reverse' DVM. The amplitude of DVM can range from tens to hundreds of meters. Among marine fishes, DVM is mostly known from pelagic species following the deep scattering layer (DSL); however, coastal benthic species are also known to exhibit DVM. In this study, we investigated DVM in the coastal-pelagic tope shark (*Galeorhinus galeus*) off southern California. Between 2015 and 2017, 13 adult female tope sharks (mean total length  $\pm$  SD = 180.0  $\pm$  10.3 cm) were externally fitted with pop-up satellite archival tags (Microwave X-Tag) at a shallow-water aggregation site off La Jolla, California. Track durations ranged from 9 to 247 d (mean  $\pm$  SD = 88.4  $\pm$  65.9 d), and the distance between tagging and pop-up locations ranged from 0.15 to 84.2 km (mean  $\pm$  SD = 19.7  $\pm$  24.0 km). Mean depth for each shark ranged from 4.3 to 9.7 m (mean  $\pm$  SD = 7.4  $\pm$  1.4 m). A wavelet analysis revealed that, on average, tope sharks exhibited significant DVM (compared to a white-noise null hypothesis) 81.3  $\pm$  14.7% of the time, approximating a sinusoidal wave with a mean amplitude of 9.8  $\pm$  4.6 m. Shark depth was strongly negatively correlated with solar elevation angle, with greatest depths occurring around local noon. In this presentation, we consider various proximate and ultimate causes for this behavior.

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### Effect of Head-starting on Survival and Movement of Mojave Desert Tortoises

*Clay Noss, Brian Henen, Scott Hillard, Robert Johnson, Lynn Sweet, Kenneth Nagy*

Head-starting, in which eggs are hatched and juveniles raised in captivity before being returned to the wild, is one strategy for addressing population declines in turtles. Here, we report the survival and movement of 175 head-started Mojave desert tortoises (*Gopherus agassizi*) at the Marine Corps Air Ground Combat Center (MCAGCC) in Twentynine Palms, California. Juveniles were hatched from eggs laid by wild, gravid females temporarily held in outdoor pens. Tortoises were released 5-13 years after hatching, in the spring and/or fall 2015 to 2019. We radio-tracked individuals following release, recording location and survival at least every month. We used GLMs and GLMMs to explore survival and movement. By December 2019, 65.1 percent of head-started tortoises were alive. Of animals that died, death occurred on average 372.6 days following release ( $\pm$  308.3 days). We found that release season had a significant relationship with survival, with 51.8 percent survival of tortoises released in the Fall compared to 78.9 percent in Spring releases. Movement in the first 6 months post releases was highly variable, with average kernel density estimates (KDE) of 13.8  $\pm$  21.9 hectares. Excluding the year of release, we found no relationships between annual KDE and survival. We also found no relationship between survival and total distance moved in the first 30 or 180 days. Future and ongoing work includes incorporating additional years in our analyses, adding additional variables (e.g. landscape metrics and environmental variables) to models, and comparing survival and movement to tortoises that were not head-started.

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### The Long-Term Effects of Sex Ratio Bias in an Island Population of Tuatara (*Sphenodon punctatus*)

*Linlin Liu, Kristine Grayson, Susan Keall, Nicola Nelson*

Sex ratio bias is an underappreciated threat to population viability, particularly in populations that appear numerically stable. For reptile species with



temperature-dependent sex determination (TSD), increases in temperature under climate change may skew the sex ratio of offspring from nests. Biased sex ratios can have additional consequences for demographic parameters, particularly for small or isolated populations of conservation concern. Here we provide an additional decade of demographic data from a male-biased population of tuatara (*Sphenodon punctatus*) on North Brother Island. This population has experienced a long-term male-biased sex ratio since 1988, alongside declines in survival and individual body condition. Our more recent surveys found a concerning drop in population size and female reproductive rate. However, in recent years the population sex ratio has remained around 62.5% males while survival and body condition have improved in new cohorts under a lower population density. Still, the small population size and potential for over-production of male offspring from warmer nests pose persistent threats to the viability of this isolated population. Our work highlights that long-lived species may have delayed demographic responses and sustained, long-term monitoring is particularly important for species with high longevity. Understanding the mechanisms underlying changes in population demography is only the first step in determining the appropriate conservation measures for a declining population. For North Brother Island tuatara, these decisions are made alongside the Māori kaitiaki (stewardship guardians of the land and environment) and Department of Conservation.

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### Evaluating the Physiological and Environmental Benefits of Creating Naturalistic Enclosures for Federally Threatened Captive Snakes

*Elizabeth Livingston, Karina Cocks, Mariana Jones, Erika Nowak*

The Narrow-headed Gartersnake (*Thamnophis rufipunctatus*) is a federally threatened, riparian obligate, fish-eating snake which lives in cold-water drainages in Arizona and western New Mexico. In 2014, 11 wild juvenile garter-snakes were salvaged from Oak Creek, Arizona, following a large-scale wildfire. They were brought into captivity at Northern Arizona University under the Gartersnake Research Program (<https://in.nau.edu/gartersnake-research-project/>). The program includes unique captive husbandry research facilities managed by undergraduate and graduate students, with the oversight of a faculty advisor, animal care manager, and veterinarians. Narrow-headed Gartersnakes have proven challenging to keep in captivity due to obligate piscivory, apparent proclivity to stress-induced pathologies, heat stress, and intolerance of repeated handling; however, the program produced 34 viable offspring produced between 2017 and 2019. After the discovery of an asymptomatic deceased captive individual carrying *Ophidiomyces ophidiicola* in 2017, breeding and plans for repatriation were suspended by state and federal regulators. Instead, student interns have completed projects designed to improve the operational sustainability of existing facilities and health of the snakes through minimization of handling. Two new outdoor mini-vivaria designed to replicate features of the larger vivarium, and indoor self-feeding tanks were constructed; assessments of gartersnakes' physiological and behavioral responses to the different enclosure types have begun. We will overview results of the improvement projects, including savings in usage of water and expendable supplies, and comparison of body condition indices of the snakes experiencing different husbandry conditions. We will discuss considerations for other programs seeking to improve the health of sensitive captive snakes.

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### Macroecological Drivers of Species Diversity in the Ecologically Variable Garter and Water Snakes (Thamnophiini)

*Leroy Nuñez, Frank Burbrink*

Thamnophiini (garter snakes, water snakes, brown snakes, and swamp snakes) are a clade of colubroid snakes found throughout North America and adjacent Central America. This group composed of 61 species exhibits a high level of ecological variance inhabiting many unique biomes. Additionally, Thamnophiini represents an asymmetric radiation where the garter snakes (Thamnophis) represent 57% of species diversity. Furthermore, the group is geographically unevenly distributed; Thamnophis is the only genus that naturally occurs west of the Western Continental Divide in North America. One potential explanation is that the shift from the mesic forests and prairies of

Eastern North America to the xeric and higher elevation environments along the Western Continental Divide represent impassable barriers for most other lineages within Thamnophiini. However, this hypothesis has yet to be tested using a robust comparative approach. Using a well-resolved phylogeny inferred from whole genomes and an extensive database of occurrence and environmental data, we identify the extrinsic abiotic factors that generate inter-specific diversity. We take a comparative phylogenetic approach to model the evolution of environmental niches in Thamnophiini to identify shifts in adaptive regimes of environmental variables across the phylogeny. We also inferred the impact of westward dispersal on environmental niche evolution. Our results support our hypothesis that aridity and elevation are potential limiting factors for dispersal of non-Thamnophis lineages. This dataset will allow us to identify potential adaptive regions of the genome that allow Thamnophis lineages to disperse westward, yielding ecological opportunity that drives diversification within Thamnophiini.

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### Comparison of Semen Collection Methods in the Texas Horned Lizard (Phrynosoma cornutum)

*Brittany Nunn, Ariana Aronis, Kamryn Richard, Bekky Muscher-Hodges, Andrew Gluesenkamp*

The iconic Texas horned lizard (*Phrynosoma cornutum*) is the state reptile of Texas, and while the species used to be abundant throughout most of the state, populations have significantly declined since the 1960s. Habitat loss and non-native invasive species are among the factors that led to the extirpation of this species in many areas. In response to this species decline, several institutions have begun captive breeding and reintroduction projects. The Center for Conservation and Research (CCR) at San Antonio Zoo began the Texas Horned Lizard Reintroduction Project in 2016. Since then, a total of 379 in-house bred eggs have been laid, and 23% of those eggs have produced viable offspring. We are uncertain how this egg viability rate compares to fertility rates in the wild, but there may be ways to improve production. While there are several possible causes for low egg viability, one potential factor may be low sperm quality in captive males, resulting in low egg fertility. To study sperm quality in our captive population, the first step will be to explore various sperm collection techniques on captive male horned lizards. Our goal is to identify the safest and most reproducible method for obtaining adequate samples. By comparing different approaches, we hope to determine the most reliable technique to ensure the successful collection of sperm while prioritizing the well-being and recovery of these unique reptiles.

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### Analysis of Large Bull Shark (Carcharhinus leucas) Movement and Distribution in the Northwest Atlantic

*Jasmine Nyce, Matthew Smukall, R. Dean Grubbs, Curry Cunningham, Debra Abercrombie, Andrew Seitz*

Understanding movement, distribution, and occupied habitat are integral to managing and conserving large mobile species. Bull sharks (*Carcharhinus leucas*) are a large, upper trophic-level species circumglobally distributed in inshore and offshore habitats. Previous studies have shown that they exhibit some level of site fidelity while also making long-distance seasonal migrations across multiple regions in the northwest Atlantic Ocean. It is currently unknown how the demographic data of individuals and the changing climate have affected the movement of these large sharks over time. Therefore, the objective of this study is to better understand the distribution and movement of large bull sharks in the regions in relation to sex, pregnancy status, seasonality, and abiotic factors. This study analyzes passive acoustic detections of bull sharks ( $n = 69$ ) at multiple sites on the U.S. east coast and The Bahamas from 2009 to 2023. Of the tagged sharks, at least 12 were pregnant during tagging, which was confirmed with ultrasonography. The species distribution determined from predictive modeling will provide additional information on the occurrence and characteristics of occupied habitats of bull sharks, including where females spend time before pupping, which may help inform the management and conservation of this species across multiple jurisdictions.

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## Habitat Factor Impacts on the Sonoma Population of the California Tiger Salamander: Implications for Habitat Conservation Plans

Aidan O'Brien, Dave Cook, Stacy Martinelli, Michael Fawcett, Christopher Searcy

Management of vulnerable amphibian populations requires a better understanding of the habitat factors that will make the greatest difference in their preservation. Here, we study the endangered Sonoma County California tiger salamander (*Ambystoma californiense*), an amphibian threatened by habitat loss and fragmentation in an urban matrix. In 2019, 298 ponds (almost every breeding pond in this endangered Distinct Population Segment) were surveyed to determine *A. californiense* larval densities. We used model selection to identify which factors of pond morphology, pond neighborhood, and upland habitat best predict variation in larval densities. Our best model showed a significant positive relationship between larval SCTS densities and three variables: depth of pools, the number of breeding pools within 500 meters of a focal pool, and the amount of suitable and accessible upland habitat within 1500 meters of a pool. To further refine this model, we then examined interactions and non-linearities using a general additive model. Based on this refined model, we simulated a variety of management scenarios to identify which management practices would most increase larval densities across the endangered Distinct Population Segment and where on the landscape employing them would yield the greatest return. This provides the basis for a county-wide Habitat Conservation Plan.

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## Data-limited Assessment Methods for unassessed Large and Small Coastal Sharks along the US Southeast Atlantic Coast and Gulf of Mexico

Kaitlyn O'Brien, Robert Latour

Stock assessments of the large coastal (LCS) and small coastal shark (SCS) complexes are problematic due to significant differences in vital life history characteristics, difficulties in determining the trends of individual species, and problems determining the status of the complex. Individualized stock assessments for species to infer stock status and thus inform management have been the ultimate goal since the LCS and SCS complexes were formed. This goal was previously impossible due to poor species specification within catch logs, minimal biological data, a lack of attention to shark application, and no readily available programming platforms to apply data-limited assessment methods. Thus, multiple species, especially LCS, have never been formally assessed. However, recent advancements in both methodology and a readily available programming platform enable the application of multiple data-limited assessment tools that can be implemented for several unassessed shark species. This study examined how best to determine the status of several previously unassessed LCS and SCS stocks using data-limited methodologies. First, an index of abundance for each species considered was fit using data from multiple surveys with the Vector Autoregressive Spatio-Temporal (VAST) package, which estimates a vessel or survey effect to produce a single index. Indices were used as the basis for multiple data-limited methods within the *datalimtd* and *datalimtd2* package in R and the Stock Synthesis Data-limited Tool (SS-DL). Results from this study are expected to inform management and provide answers on the status and well-being of multiple previously unassessed shark species.

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## Eastern Hellbender Reproductive Behavior and Responses to Anthropogenic Threats

Rebecca O'Brien, Groffen Jordy, Ashley Dayer, William Hopkins

Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are an evolutionarily unique species of salamander that is of conservation concern. Despite growing interest in their breeding and parental care behavior, there remain significant gaps in our understanding of hellbender reproduction. In this presentation, we discuss our use of custom-built infrared cameras installed in artificial breeding shelters to record pre-breeding behaviors, mating sequences, and parental care behaviors of hellbenders. Using these recordings, we describe the basic mating sequence, the presence of potential alternative mating tactics, and two novel breeding behaviors, including

possible signal production via wave-based communication and a unique egg laying behavior by female hellbenders. We also describe patterns in hellbender parental care and explore the potential for this care to buffer offspring against anthropogenic stream degradation.

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## Relationship of Lateral Line Scale Meristics to Neuromast Patterning and its Variation among Fishes

Jenna O'Del, Jacqueline F Webb

Lateral line (LL) scales, trunk canal segments, are assumed to each contain a neuromast (flow sensing) organ, but few species have been studied in detail. We examined the potential correlation of LL scale meristics and neuromast patterning by comparing neuromast and trunk canal development in two species with contrasting meristics (*Menidia menidia* [44-50 LL scales, 46 vertebrae] and *Salvelinus fontinalis* [≥110 LL scales, 56-62 vertebrae]). Vital fluorescent staining (4-Di-2-ASP), clearing and staining, and SEM revealed that *M. menidia* larvae have one neuromast/myomere and *S. fontinalis* larvae have two neuromasts/myomere before each neuromast is enclosed in a LL scale, establishing contrasting LL scale meristics. To explore this more generally,  $RLLS:V$  ( $= LL\ scale\ \# [neuromast\ \#]:vertebrae\ \# [myomere\ \#]$ ), was calculated from the literature for a geographically limited subset of teleosts - from the US Atlantic coast and freshwaters east of the Mississippi River (459 spp., 37 orders). A  $RLLS:V = 1:1$  was in ~50% of species, across the teleost tree. Marine species had a  $RLLS:V = 2:1$  or  $3:1$ , but some had a  $RLLS:V = up\ to\ 9:1$ . Freshwater fishes had a  $RLLS:V = 1:1$  or  $2:1$ . Variation in  $RLLS:V$  among species among and within genera will help identify taxa for the study of development and evolution of LL scale meristics. Further, if neuromasts are found in each LL scale, then variation in  $RLLS:V$  and inter-pore distance (~ inter-neuromast distance, a function of scale size), a potential basis for functional studies, are likely to be correlated.

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## Assessing metal concentrations in muscle tissue of blacktip sharks (*Carcharhinus limbatus*) from coastal Maryland compared to the Florida Peninsula

Conor O'Meally, Gretchen Bielmyer-Fraser, Bryan Franks, Dean Grubbs

Human activities substantially increase contaminants in marine ecosystems, which presents challenges for various organisms. Metal pollution, in particular, is common in aquatic environments and may bioaccumulate causing toxicity in animals that feed higher in the food chain. Coastal sharks, such as the blacktip (*Carcharhinus limbatus*), are particularly at risk to metal exposure as they utilize coastal environments affected by anthropogenic disturbances. Accumulation and effects exerted by metal contaminants may be exacerbated by the health condition of the animal. For example, disease occurrence in a population can change organismal responses to contaminants. This research will compare metal (mercury [Hg], lead [Pb], copper [Cu], zinc [Zn], silver [Ag], cadmium [Cd], and nickel [Ni]) concentrations in the muscle tissue of blacktip sharks from four regions where these sharks are present: the coast of Maryland, the Florida Atlantic, the Florida Gulf and the Florida Keys. This research will determine if there is a difference in heavy metal concentrations in the muscle tissue of blacktip sharks based on geographical location. To study the effects that metal accumulation has on the health condition in blacktip sharks, the state of each shark's health will be assessed and ranked based on several criteria: presence of lesions and white blood cell (WBC) count and distribution. This will provide needed information about metal contaminant levels, as a result of their environment, and as related to the immune response of the shark. Further, results will provide guidance for consumers of this commercially or recreationally caught meso-predator species.

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## Short Term Environmental DNA Variation Using Active and Passive Sampling Techniques in the Tangipahoa River, Louisiana

Camden Oathout, Kyle Piller

Environmental DNA (eDNA) is a novel sampling technique for evaluating species richness. In aquatic ecosystems, eDNA is being used to detect the presence of invasive and rare taxa, as well as to create assemblage profiles of

fishes in various habitats. Due to its infancy as a sampling technique, there are several unanswered questions about eDNA sampling that remain regarding the temporal resolution of the technique. Environmental DNA sampling is often limited to water samples from a single time-period on a particular day, essentially representing a snapshot in time. Little is known regarding the degree of temporal variation of eDNA across a 24-hr period from a single site. In this study, we examined fine scale temporal variation of eDNA by collecting water samples over a 24-hour period at the Tangipahoa River (Louisiana) using both active and passive sampling approaches. Active sampling involved taking water samples from pre-defined areas every hour for 24 hours. Passive sampling included the deployment of a sampling device that remained stationary in the same area for 24 hours. All of these water samples were filtered on glass microfiber filters, extracted, PCR amplified (12S mtDNA) and Illumina sequenced using a pre-defined protocol. Data collection is on-going, and we anticipate that the species richness estimates for active and passive sampling approaches will be similar and will highlight the benefits of using passive sampling approaches for studying fish assemblages based on eDNA.

## Dispersal of *Carcharodon carcharias* eDNA through space and time in a coastal ecosystem

*Kyle Oliveira, Megan Winton, Damian Brady, Nicholas Record*

Environmental DNA (eDNA) is a rapidly developing tool in environmental monitoring that is praised for being accessible, affordable, and with a quick turnaround time on results. For detecting our target species, the white shark (*Carcharodon carcharias*), eDNA also has the ability to detect individuals that have not been tagged using traditional techniques. However, to better interpret findings from eDNA studies, we set out to examine how eDNA behaves in a coastal ocean environment through space and time. Due to the large size of *C. carcharias*, we are unable to use traditional methods using enclosures to understand how eDNA from our target behaves in water. To address this challenge, we took sequential water samples in both space and time while using bucket drifters to follow surface current patterns off Cape Cod, MA. These samples are then filtered, and eDNA extracted before being subject to quantitative PCR analysis, to quantify the amount of *C. carcharias* specific eDNA in respective samples. Preliminary results indicate an increase and subsequent decrease in the quantity of eDNA with increased time and distance away from the source; this provides foundational information for understanding how eDNA from a megafaunal species moves in a coastal ocean environment. We anticipate this approach will be applicable to other megafaunal species and will provide valuable validating information for using eDNA as a detection method in environmental and ecological sciences.

## Anatomical Survey of the Olfactory Apparatus in the Walking Catfishes (Siluriformes: Clariidae)

*Cameron Olsen, Kevin W. Conway*

The Family Clariidae is a diverse group of facultative airbreathing catfishes (Siluriformes) distributed throughout Africa and Asia, and introduced into the southeastern US. Members of this group are referred to as walking catfishes due to the ability of some species to travel short distances across land. Clariid catfishes exhibit remarkable variation in body shape and length, and exhibit a range of ecological strategies, ranging from stout-bodied “generalist” species to highly elongate, eel-like “specialist” species that inhabit shallow water and make nocturnal excursions onto land to hunt terrestrial prey. Despite this ecological diversity, information on the sensory organs of clariids is limited, available only for a small number of generalist species. To provide new information on clariid sensory organ anatomy, we surveyed the olfactory apparatus of thirty-nine clariid catfishes representing eight genera (including both generalists and specialists). Using a combination of light and scanning microscopy techniques, we document the gross anatomy of the olfactory apparatus, including the olfactory rosette and the accessory chamber, and for the first time document micro-surface structure and cellular composition of the olfactory lamellae. Preliminary results suggest the number of lamellae may differ between species of clariids, though we note a strong positive correlation between body size (standard length) and number of olfactory lamellae. *Gymnallabes typus* and *Platyallabes tihoni* possess a potentially novel organization of the lamellae of the olfactory rosette with posterolateral extensions of

lamellae. The olfactory rosette of *Gymnallabes typus* displays greater vascularization than the olfactory rosette of *Clarias agboyiensis*, as observed via histology.

## The Effect of Meal Type on Specific Dynamic Action in the Western Cottonmouth (*Agkistrodon piscivorus leucostoma*)

*Jason Ortega, Steven Beaupre*

Diet generalists may not be expected to reap the same net energetic benefit from a wide array of prey types. In our continued efforts to examine the interplay between prey preference and digestive performance, we measured specific dynamic action (SDA) in the western cottonmouth (*Agkistrodon piscivorus leucostoma*). Snakes were fed meals of varying prey types (fish, frog, and mouse) and forms (homogenized and whole) and their SDA responses were measured during the full course of digestion (240 h). Resting and post-feeding metabolic rates,  $\text{VCO}_2$  (mL h<sup>-1</sup>) and  $\text{VO}_2$  (mL h<sup>-1</sup>) were measured using open-flow respirometry. The resulting rates were used to calculate total SDA response to feeding,  $\text{CO}_2$  SDA (mL) and  $\text{O}_2$  SDA (mL). The total SDA response to meal type (the interaction of prey type and form) was overall highest for homogenized mouse meals (log-transformed adjusted means  $\pm$  1 SE),  $7,460 \pm 0,037 \text{ CO}_2$  SDA (mL) and lowest for whole frog meals,  $7,009 \pm 0,043 \text{ CO}_2$  SDA (mL). Within each prey form (homogenized versus whole) mouse meals had the highest SDA response, while homogenized meals had collectively higher values. Overall mouse meals had a higher SDA response when compared to fish / frog meals. The results observed may be due to the higher protein content of mice when compared to frog / fish and the potentially higher accessibility of protein due to meal homogenization.

## Development of Educational Resources About the Shark species of Puerto Rico as a Gateway to Inform the Local Public

*Wanda Ortiz*

The sharks of Puerto Rico are considered data-poor in general. Thus, little is known about this group in Puerto Rico. Furthermore, most of the available information is presented technically and in English. Considering that the first language in the commonwealth is Spanish and around half of the Puerto Rican population are non-English speakers, this could present a problem when trying to inform the general public. To address this, with Sea Grant Puerto Rico, we developed a series of educational and informative materials conveyed in a digestible and practical way and written in Spanish. These materials consist of a book about the sharks of Puerto Rico, a pamphlet with the most common species of the archipelago along with their life history traits, and a poster with every shark species recorded to date in Puerto Rico displayed in a series of illustrations. The primary resource from this effort is the book. Sharks of Puerto Rico contains information from sharks' general biology and ecological, economic, and cultural importance to a chapter that debunks many Puerto Ricans' common misconceptions regarding this group of predators. These materials, the book in particular, can serve as the foundation to inform the general public about the sharks of Puerto Rico.

## voluModel: Modelling species distributions in three-dimensional space

*Hannah Owens, Carsten Rahbek*

Many common ecological niche modeling (ENM) workflows organize and analyze occurrence and environmental data based on two-dimensional latitude and longitude coordinates. However, pelagic marine environments can vary strikingly with depth, and extracting environmental data based only on 2D data may result in poorly trained models and subsequent inaccurate prediction of species' geographical distributions. We developed the voluModel R package, presented here, to efficiently extract environmental data at three-dimensional coordinates (i.e. latitude, longitude, and depth) to train more precise ENMs. We present the main features of the voluModel R package and demonstrate a simple modelling workflow for Luminous Hake, *Steindachneria argentea*, as an example. voluModel includes tools for processing 3D environmental data, generating training regions, sampling data for model calibration,

estimating extrapolation risk, and visualizing 3D geographic distributions based on conditions inferred as suitable based on ENMs. In addition to providing more precise models and distribution estimates, 3D niche modeling will more accurately estimate how climate dynamics may have shaped past and future changes in both the horizontal and vertical dimensions of species' geographic ranges.

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## A New View of Marine Biodiversity

*Hannah Owens, Carsten Rahbek*

Manifold studies exist to determine species richness drivers in terrestrial systems, but corresponding work is comparatively rare in the marine realm. However, marine biodiversity studies may be key to understanding broad scale biodiversity patterns. Temperature and primary productivity, commonly invoked explanatory variables for biodiversity despite being tightly correlated on land, are decoupled in the open ocean. The discriminatory power of such an analysis further increases when considering depth, an oft-neglected axis along which species richness varies. Here, we present a first look at depth-structured biodiversity patterns of three families of fishes (i.e. Beloniformes, Scombriformes, and Gadiformes) in the Atlantic Ocean. We collected occurrence data for over 280 species of fishes, generated 3D correlative ecological niche models (ENMs) for each, and projected the ENMs back into geographic space. Finally, we combined resulting distributions to map species richness of each family as a continuous volume in the Atlantic. This analysis was all done using a recently established workflow facilitated by the R package *voluModel*. To better visualize species richness volumes, we created a new tool to map transects of species richness by depth within a given volume. The resulting plots emphasize that while horizontal diversity patterns differ among the three groups, strikingly concordant patterns emerge when considering vertical biodiversity patterns. Finally, we suggest how oceanographic processes beyond temperature and primary productivity may be shaping broad-scale species richness patterns in the open ocean.

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## Genetic connectivity of flatfishes in the Black Sea

*Rafet Çağrı Öztürk, İlhan Aydın, Şirin Fırdin, Melike Alemdağ, Yahya Terzi, Oğuzhan Eroğlu*

Flatfishes such as flounder and turbot are commercially important fish species. In this study, microsatellite loci were used to resolve the population structure of flatfishes collected across the Black Sea. Three mitochondrial genes, COI, 16S rRNA, and Cyt-b, were used for genetic identification and construction of phylogenetic trees. Available mtDNA sequences with known geographic information were retrieved from the GenBank database and included in the phylogenetic analysis to increase resolution. Pairwise comparisons of FST showed significant differences between surveyed sampling sites ( $P < 0.01$ ) and high genetic variability was detected across different geographic regions. Cluster analysis revealed the presence of three genetic clusters within the Black Sea. Significant genetic differentiation between Northern (Sea of Azov and Crimea) and Southern (Turkish Black Sea Coast) Black Sea sampling sites was detected. The Mantel test supported an isolation-by-distance model of population structure. These findings are vital for the long-term sustainable management of flatfishes and for the development of conservation programs.

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## Estimating population reduction with JARA — A decision-support tool for IUCN Red Listing

*Nathan Pacoureau*

Identifying species at risk of extinction is necessary to prioritise conservation efforts. The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species is the global standard for quantifying extinction risk, with many species categorised according to population reduction thresholds. We present the Bayesian state-space R package 'JARA' (Just Another Red-List Assessment) using real-world datasets. Designed as decision-support tool, JARA allows both process error and uncertainty to be incorporated into IUCN Red List assessments under criterion A. JARA is easy to use, rapid and widely applicable for researchers and conservation practitioners looking to analyse count and relative abundance data.

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## Examining the Factors Contributing to the Decline of Range Edge *Ambystoma* in West Virginia

*Morgan Page, Anna McCallum, Jayme Waldron*

The Ohio River and its tributaries have experienced an extensive increase in urbanization and industrialized agriculture over the last century, resulting in the loss of crucial floodplain habitat and contributing to the decline of local peripheral amphibian populations. Small-mouthed salamanders (*Ambystoma texanum*) and Streamside salamanders (*Ambystoma barbouri*) reach their range edge along the Ohio River, and both species have experienced severe population declines despite maintaining relatively stable core populations. I surveyed for *A. texanum* and *A. barbouri* at historical locations and at randomly selected wetlands with suitable habitat in southwestern West Virginia. Using unbaited and light-baited minnow traps, I conducted presence-absence surveys at 16 wetlands in an occupancy framework to examine occupancy covariates and to assess possible causes of extirpation. Occupancy covariates included vegetation structure, composition, contemporary land use, and historical land use. Peripheral populations are ideal for evaluating species' environmental tolerances, and the results of this study can be used to identify remnant populations and prioritize populations in greatest need of conservation.

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## Comparative anatomy of the eye muscles and heater organ of Istiophoridae

*Larson Palmgren, Miguel Montalvo, Eric Hilton*

Billfishes are highly visual predators and are especially remarkable because of specialized muscle tissue that heats the eye and brain. This feature is known to be present in other, usually pelagic fish species, but is believed to have independently evolved in billfishes. This organ presumably protects against rapid cooling of the eye and brain in water at cold temperatures, and hypothetically leads to increased visual acuity. The heating organ has been described previously, with a focus on its function. The goal of this study is to further describe the istiophorid heating organ and surrounding ocular musculoskeletal anatomy in a comparative framework, providing details across a diversity of western North Atlantic species, including Atlantic Blue Marlin (*Makaira nigricans*), White Marlin (*Kajikia albida*), Roundscale Spearfish (*Tetrapturus georgii*), and Atlantic Sailfish (*Istiophorus albicans*). A method for dissection of the ocular region and study of the ocular muscles will be proposed. Preliminary observations suggest that there are differences in the size of the muscles and the heater organ among these species, (e.g., the lateral rectus insertion on the sclerotic ring and overall size in *T. georgii* appear smaller than that of *K. albida*).

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## Recent Advances in Invasive Pentastome Research to Further Characterize the Threat to Native Snake Populations

*Jenna Palmisano, Terence Farrell, Robert Fitak, Anna Savage*

The invasive lung parasite, *Raillietiella orientalis* (Ro), causes severe and often lethal disease in snakes native to the southeastern United States. Seventeen species of native snakes are documented as definitive hosts but pathology and risk of disease development between species is understudied. Precipitous declines of *Sistrurus miliarius* that are spatially and temporally consistent with the initial invasion of Ro indicate population-level consequences of infections. Further investigation of the geographic expansion of Ro and its pathology in hosts is needed to characterize the threat to snake species. The threat of Ro is a conservation concern as its use of numerous hosts and presence in the pet trade increases the likelihood of rapid dispersal. Our controlled laboratory experiments indicate a three-host life cycle with two intermediate hosts (roaches followed by anurans or lizards) leading to the definitive host (snakes). Snakes dependent on ectothermic prey, like *S. miliarius*, are most vulnerable to infection. To facilitate a better understanding of the population impacts of Ro, we will study the dynamics of disease severity, demographics, and genetic diversity in six populations of *S. miliarius* with variable prevalence. Here, we present data on our published genomic resources for Ro and the findings of our monitoring network of over 40 collaborators across six states in the Southeast. Since the network's establishment in 2022, we have documented six new counties in Florida with Ro prevalence. For effective monitoring, we must increase the number of collaborators and geographic coverage.



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## Cyt-b gene investigation into the origins of a Louisiana Cuban treefrog invasion

*Karen A. Paniagua Torres, Erin Brosnan, Matthew S. Atkinson, Katie Martin, Brad M. Glorioso, Hardin Waddle, Anna Savage*

Invasive populations often experience reductions in genetic variation due to the small number of individuals founding the new population, yet multiple introductions from varying sources can conversely increase genetic diversity over time. Cuban treefrogs (*Osteopilus septentrionalis*) established in South Florida in the mid-1900's with genetic evidence indicating that they originated from at least two separate populations in the Caribbean. In 2016, U.S. Geological Survey researchers detected a novel population of *O. septentrionalis* in New Orleans, Louisiana, USA. Whether this population represents a range expansion from Florida or a novel introduction remains unclear. We sought to determine the source of this novel *O. septentrionalis* invasion, using a 717bp fragment of the *cyt-b* mitochondrial gene. We used PCR and subsequent Sanger sequencing to amplify and generate reads for the *cyt-b* gene of populations present in New Orleans and then compared these generated sequences to previously sequenced individuals from Florida and Cuba found in GenBank. We also generated additional sequences to include Central Florida *O. septentrionalis* populations for comparison. There were 168 new sequences generated including 11 new haplotypes from Central Florida and New Orleans. We used PopArt and MrBayes software to construct a haplotype network and a bayesian phylogeny. Phylogenetic analysis and haplotype networks indicate widespread allele sharing across New Orleans, Florida, and Cuba *O. septentrionalis* populations, but haplotype frequencies suggest Florida is the source of the novel invasion. This study is important to understand the invasion pattern of *O. septentrionalis* and prevent future range expansion.

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## Comparative phylogeography reveals contrasting patterns of population structure in Antarctic notothenioid fishes

*Elyse Parker, Carl D. Struthers, Christopher D. Jones, Thomas J. Near*

Identification of the geographic and environmental factors that influence the distribution of marine fish diversity requires comparing patterns of genetic variation across multiple co-distributed taxa. In Antarctica's Southern Ocean, the widespread circum-Antarctic distributions observed for many fishes is attributed to the existence of a fast-flowing circumpolar current, which is hypothesized to play a crucial role in long-distance dispersal of marine organisms and high genetic connectivity among populations across long geographic distances. However, most previous molecular population genetic studies of Antarctic marine fishes are geographically limited, preventing a more detailed understanding of the role played by prevailing ocean currents in shaping patterns of genetic diversity at a continental scale. Furthermore, the limited taxonomic scope of previous studies hinders an understanding of whether different species respond in a concerted or idiosyncratic fashion to the physical features of the Southern Ocean. Using population genetic and demographic analyses of genome-wide SNP data, we compare patterns of population structure and connectivity across the distributions of multiple circum-Antarctic notothenioid fish species. While some notothenioids exhibit virtual panmixia across thousands of kilometers of the Antarctic continental shelf, others exhibit strong geographic structuring of populations. We hypothesize that these contrasting population genetic patterns reflect differences in characteristics that influence dispersal capabilities, such as length of the pelagic larval phase. In revealing the idiosyncratic manner in which different species respond to shared geographic and environmental features, our findings demonstrate the importance of a comparative phylogeographic approach to investigating the factors that modulate genetic variation.

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## The genomic landscape and the evolution of the Viperidae and their venoms with a focus on New World Pitvipers.

*Christopher Parkinson, Rhett Rautsaw, Edward Myers*

The family Viperidae consists of three subfamilies, Viperinae, Azemiopinae, and Crotalinae, with the highest species diversity in the Crotalinae. We have sequenced and assembled the genomes of representative species from all three subfamilies, including *Echis carinatus*, *Azemiops feae*, and multiple New

World pitviper species. We investigate the evolutionary history and biogeography of this family with a specific focus on New World pitvipers. Combining our newly sequenced genomes with previously published genomes, we aim to improve our understanding of chromosomal and venom gene family evolution. With these data, we find that most of the venom genes are found on microchromosomes, in contrast with Elapidae, and that substantial structural evolution has occurred across relatively short timescales. To further address evolutionary and biogeographic questions, we use a multi-omic approach combining multiple data types to infer one of the first phylogenomic inferences of this family. We reveal several novel, well-supported relationships within vipers which help clarify taxonomy as well as a rapid speciation rate upon invasion of the New World. Additionally, using our novel phylogenomic reconstruction and >500 venom gland transcriptomes, we determine that venom phenotypes diverge when multiple species coexist in a given area through time. Furthermore, we hypothesize that pitviper communities have evolved to maximize functional diversity despite comparatively low phylogenetic diversity, suggesting an evolutionary response of venom rather than communities accumulating phylogenetically diverse species. Together, we demonstrate the utility of the next-generation sequencing era in improving our understanding of genome biology, phylogenetics, systematics, and venom evolution within Viperidae.

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## Insights into the biogeography of southwestern Angola: lessons from lizards

*Diogo Parrinha, Aaron Bauer, Luis Ceriáco*

Southwestern Angola is characterized by ecological and altitudinal gradients associated with a biogeographic transition. The interaction between climate and topography creates a diverse landscape that supports a variety of habitats, from coastal desert to Afromontane grasslands. This is reflected in species richness and endemism, and one third of all reptile and amphibian species known from Angola have been recorded in this region. Recognized as a center of endemism by some authors, southwestern Angola assumes particular relevance as a hotspot of diversity for lizards associated with the arid biomes of the Namib Desert. Despite this, distribution ranges and biogeographical boundaries are still poorly known. After decades of armed conflict delayed research in Angola, recent expeditions have resulted in the collection of new material to assess the true diversity and distribution of its herpetofauna. Integrative taxonomic work reveals cryptic diversity in several lizard groups, leading to the description of more than 15 new species endemic or near-endemic to southwestern Angola in the past two decades. Recently published and ongoing works on the taxonomy of Angolan reptiles are used as case studies, unveiling recurrent patterns of diversity and endemism. These findings are contextualized in the growing body of evidence supporting the recognition of southwestern Angola as a hotspot of lizard diversity with complex biogeographic patterns.

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## "How old are you?" and other rhetorical questions: solving the age puzzle in elasmobranch fishery management

*Michelle Passerotti*

Obtaining accurate life history parameters such as growth rates, age at maturity, and longevity has historically posed a challenge for managing elasmobranch populations. While baseline knowledge has been amassed over the past 50 years for most managed stocks, gaps persist creating uncertainty around baseline parameters as well as jeopardizing the ability to detect climate- and fishing-induced changes over time. Traditional age estimation via growth band counts in vertebrae has proven unreliable in many long-lived shark species, and increasingly more data indicate this issue may in fact be widespread. Further, lethally-derived tissues required for traditional life history research are increasingly difficult to obtain as regulatory changes limit fishery-dependent samples. Novel, non-lethal methods for estimating age are sorely needed. This presentation reviews recent advancements in age estimation research, including FT-NIRS, genetic techniques, and modern bomb radiocarbon analyses, and outlines uncertainties surrounding these methods. FT-NIRS has generated interest as a fast, non-destructive method for age prediction based on laser scans of vertebrae but requires a known-age calibration model to inform predictions and hence, is not a standalone method. Non-lethal use of

genetic markers such as DNA methylation are promising, but like any method require true validation via mark-recapture or bomb radiocarbon analysis to be fully vetted. Recent development of modern (i.e. post-1970 14C peak) bomb radiocarbon references has the potential to expand the utility of the  $\Delta 14C$  “gold standard” to validate age in fish born after 1970, and may hold the key to vetting new age estimation methods in the future.

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### **Preliminary Results of Tagged Freckled Guitarfish (*Pseudobatos lentiginosus*) Reveal High Site Fidelity and a Long-distance Movement**

*Jessica Pate*

Globally populations of guitarfish and wedgefish are facing high risk of extinction. The freckled guitarfish (*Pseudobatos lentiginosus*), found in Florida waters in the Atlantic and Gulf of Mexico, is listed as Vulnerable on the IUCN Redlist. Little is known about the habitat use, site fidelity and movement patterns of *P. lentiginosus*. We took advantage of a fine-scale Vemco Positioning System (VPS) array of 15 receivers that was deployed for another study in habitat utilized by guitarfish. Three guitarfish (two females and one male) were internally tagged with V-9 acoustic tags in the summer of 2022. The array received over 72,000 detections from tagged guitarfish throughout the 153-day study period. The guitarfish were detected within the array on 80-90% of days within the study with each guitarfish spending a maximum of 13-19 consecutive days outside of the array. Only one guitarfish was detected while outside the VPS array. This female swam a minimum of 67 km north over 5 days, where she spent ~30 hours being detected by 3 receivers in the area before returning to the VPS array (~80 km) over 9 days. These preliminary results indicate that while guitarfish may show high site fidelity to some areas, they are capable of making long-distance movements. Future research will focus on tagging additional animals to further quantify site fidelity and movement patterns, as well as determining the feasibility of using photo-ID to study *P. lentiginosus* populations.

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### **The Florida Manta Project: Research and Conservation of Florida's Manta Rays**

*Jessica Pate, Vicky Fong*

The giant manta ray (*Mobula birostris*) was listed on the U.S. Endangered Species Act as a “Threatened” species in 2018, yet insufficient data exist on the manta population along the eastern U.S. to designate critical habitat. Taxonomic and genetic evidence suggest that manta rays in the Western Atlantic are a separate species (*M. cf. birostris*) and little is understood about the ecology and life history of this putative species. The Florida Manta Project (FMP) is the first to describe the manta rays along Florida's highly-developed coastlines. Since 2016, we have identified 151 individuals in south Florida with 95% of males being sexually immature. Fifty-two percent of individuals have been re-sighted with number of re-sightings per individual ranging from 1 to 33 (average 6) and 40 individuals (26%) have been sighted over multiple years. Aerial surveys document mantas year-round, but show a clear seasonal trend, with most observed between July and December. Twenty-four percent of mantas are entangled in fishing line and 8% have injuries from boat propellers. The occurrence of juveniles, high site fidelity and extended habitat use suggests the south Florida study area is a nursery habitat. In 2021, FMP began research on a seasonal aggregation of adult mantas off central Atlantic Florida coast. We have identified 17 individuals, documented courtship and foraging behaviors, and observed a maximum of 64 manta rays on a single aerial survey. Current and future research will utilize satellite and acoustic telemetry to elucidate movement patterns and critical habitats of Florida's manta rays.

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### **Systematics of Southern African Ground Agamas (Genus: *Agama*)**

*Elizabeth Patton, Stuart Nielsen, Todd Jackman, Aaron Bauer*

Existing work on southern African ground agamas has historically emphasized patterns within South Africa, but the ranges of two highly widespread taxa (*A. anchietae*, *A. aculeata*) extend well beyond its borders. In this study, we utilize more representative geographic coverage across their ranges (chiefly

by the inclusion of specimens from Angola and Namibia) and increased gene sampling to generate robust molecular phylogenies in maximum likelihood and Bayesian frameworks using a combination of two mitochondrial genes (ND4, 16s) and three nuclear genes (NT3, PNN, SETX). We uncover previously undescribed, genetically distinct clades within each of these taxa. Preliminary species distribution modeling using fine-scale climatic and geophysical variables in MAXENT suggests that patterns of genetic differentiation can be corroborated with potentially disparate ecological requirements in this group. This work will contribute to a broader integrative taxonomic assessment of all members of the southern African ground agama radiation using additional nuclear markers, increased sampling coverage, species distribution modeling, and morphological comparisons to resolve problematic species boundaries within this highly conspicuous group of lizards.

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### **Little difference in personality among different sexes and age-classes of the Little Brown Skink lizard, *Scincella lateralis* (Scincidae)**

*Mark Paulissen*

In lizards, personality refers to consistent behavioral differences among individuals. Research has shown that personality traits such as boldness or exploratory behavior can differ dramatically between males and females as well as between adults and neonates. I attempted to document personality differences between sexes and age-classes of the Little brown Skink lizard, *Scincella lateralis*, a litter cryptozoid common in forests of the southeastern USA. Individual lizards were confined in an observation chamber for a short acclimation period, then were released and their behavior recorded to obtain the following data: latency to move, time spent moving, time spent still, and time spent hidden. Then the lizards were subjected to a second trial which repeated the first except that two novel objects that the lizards have never encountered were placed into the observation chamber. When all data were combined, the latency to move was significantly greater for the second trial than for the first, though most of this effect was due to longer latency to move in trial 2 by neonates. There was little difference in time spent moving, still, or hidden between sexes or age-classes or between trials other than a trend for neonates to spend less time moving and more time hidden than adults in trials 2. The relative lack of personality differences among sexes and age-classes of Little Brown Skinks may reflect the secretive life style of all members of this species.

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### **Evidence for Partitioning? A Comparison of Thermal Niche in Three Species of Sympatric Lizards in North Eastern Utah**

*Kristopher Pedersen, Charles Hanifin*

Maintaining thermal homeostasis is critical for living organisms. Squamates typically maintain a preferred range of body temperatures through active thermoregulation and microhabitat choice. There is good evidence that variation in habitat quality can mediate the success of thermoregulation and that selection can act on thermal niche traits associated with organismal thermal limits. Taken together these facts suggest that competition for thermal resources may drive selection and diversification in squamates. Here we report data from a multi-year study of three sympatric species of lizards in a semi-arid desert in northeastern Utah: *Sceloporus tristichus*, *Sceloporus graciosus*, and *Uta stansburiana*. We compared microhabitat characteristics and thermal niche traits across all species to look for evidence of thermal resource partitioning. Data included a mix of field and lab measurements to assess thermal niche traits and to estimate efficiency of thermoregulation using de-db. Multinomial regression suggests differences in microhabitat selection. Estimates of preferred body temperatures suggest thermal niche differentiation across our species and comparisons of field body temperatures with lab measurements are consistent with competitive exclusion associated with thermoregulation. Specifically, our data suggests *S. tristichus* being warmer and *U. stansburiana* being cooler than preferred. Efficiency of thermoregulation showed seasonal changes in de-db for *S. graciosus* and *S. tristichus*, but not *U. stansburiana*, as well as a difference in de-db between *S. graciosus* and *U. stansburiana* during part of the summer. Together this shows differentiation in thermal niche, as well as microhabitat selection.

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## Male-male Combat in Free-ranging *Sistrurus* Rattlesnakes

Zander Perelman, Terence Farrell

Although male-male combat is documented in numerous snake species, reports of combat behaviors are seldom more detailed than simple descriptions. Combat between snakes in the genus *Sistrurus* is rarely observed and only one previous account, focused on captive specimens, offered behavioral descriptions and analyses. Here, we discuss combat events between free-ranging Eastern Massasaugas (*S. catenatus*) in Pennsylvania, and free-ranging Pygmy Rattlesnakes (*S. miliarius*) in Florida. We used video recordings to describe and quantify observed combat behaviors. Additional behavioral contexts, specifically observed copulation (*S. catenatus*) and active defeat (*S. miliarius*), allowed for quantified behaviors to be analyzed and compared with knowledge of the winning male in both combat events. Quantifying and analyzing these two events has provided insight into important behavioral determinants of combat success in *Sistrurus* rattlesnakes. Observation of snake combat is opportunistic, and consequently, detailed behavioral descriptions and analyses as performed here are rare in natural settings. Such social behaviors have clear implications for reproductive success and fitness, and comprehensive analyses of combat behavior, as accomplished here, may prove useful in understanding the evolution of these reproductive behaviors.

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## Beyond the Visual Field: Identifying Shifts in Selective Pressures on Anuran Phototransduction Genes across Differing Photic Environments

Taegan Perez, Camille Lavoie, Rayna C Bell, Matthew K Fujita, David J Gower, Jeffery W Streicher, Kate N Thomas, Ryan K Schott

The phototransduction cascade of vertebrates comprises the initial stages of vision, involving over 35 genes and proteins. These genes can be important for adaptation to different light environments as demonstrated through studies across vertebrate groups. Overall, though, few studies have investigated evolutionary and functional variation in vertebrate phototransduction genes. Anurans (frogs and toads) are a very diverse group that has adapted to a wide range of spectrally and optically distinct terrestrial and aquatic environments, making them an interesting group to investigate the molecular evolution of phototransduction. We hypothesized that the phototransduction cascades of anurans with different ecologies are adapted to their corresponding environments and predicted that this is reflected in patterns of positive selection and shifts in selective pressure in vision genes. To test this, eye transcriptomes from 82 species were sequenced, and supplemented with available genomic data (101 species, 34 families). We found that anurans possess all known vertebrate phototransduction genes. To test for evidence of positive selection and shifts in selective pressure across species' differing photic environments we implemented models of molecular evolution (PAML and HYPHY). We found evidence for shifts in selection between aquatic and terrestrial species that appears to result from variation in spectral composition across these environments. Further work is required to assess the impact of other ecological factors (e.g., diel activity pattern). This first assessment of molecular evolution of phototransduction in anurans provides an important contribution to increased understanding of the evolution of visual systems in this group.

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## Biotic factors affecting habitat use in multiple life stages of sympatric coastal sharks

Cheston Peterson, Blake Hamilton, Ashley Dawdy, Dean Grubbs

The objective of the present research was to use fishery-independent surveys using multiple gear types to assess the distribution and habitat overlap of each life stage (i.e. YOY, juvenile, adult) of a group of three sympatric coastal sharks and explore the relationship of size-specific prey group density with habitat use in those species. We conducted fishery-independent gillnet and longline surveys in Apalachicola Bay, Florida, from 2018 to 2020 during summer to study habitat overlap in multiple life stages of a group of sympatric coastal sharks. We investigated the relationship of habitat use and prey distribution/density in coastal sharks by estimating prey density through space using fishery-independent otter trawl and seine survey data shared by the Florida Fish and Wildlife Conservation Commission. Our results suggested the importance of prey density and predation risk in habitat use of coastal sharks may

vary among species with different life histories and degrees of dietary specialization. Differences in habitat and diet selectivity may mediate competition at some life stages, though habitat use appeared to converge over ontogeny as dietary overlap would also be expected to increase. This study is among the first to compare shark species-life stage distribution with size-appropriate prey density.

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## High Parasitism on Amazonian Diving Lizards *Uranoscodon superciliosus* (Linnaeus, 1758) is Influenced by Stream Characteristics

Amanda Picelli, Giuliana Appel, Igor Kaefer, Aaron Bauer

Diving Lizards (*Uranoscodon superciliosus*) are commonly found perching over the vegetation above Amazonian streams and frequently dive into the water. Given that the proximity of water bodies also exposes vertebrates to vector-borne parasites, we tested the hypothesis that stream characteristics affect blood parasitism in Amazonian Diving Lizards, thus predicting that habitat-related variables should influence lizard infection susceptibility. We also tested the roles of individual-related traits on lizard parasitism. Therefore, we accessed the effect of both environmental factors (river basin, stream width and perch height) and intrinsic variables (sex, age, body size, and body condition index) on individual hemoparasite parameters (parasite occurrence and co-infection index) along streams inside Central Amazonian forests. The hemoparasite prevalence among individuals was very high (87%;  $n = 105$ ), as well as diverse, including haemosporidians, haemococcidians, piroplasms, trypanosomes and microfilariae. Over half of the positive lizards (60%;  $n = 92$ ) showed coinfection by two or more hemoparasite taxa. Based on GLMM results, there was a positive relationship between stream width and prevalence, suggesting that lizards from larger streams are more susceptible to hemoparasite infections. We also observed that age group positively affected parasite prevalence and coinfection, indicating that adult lizards (SVL > 110 mm) harbor more hemoparasite infections than younger lizards (SVL < 110 mm). In general, our results show that both intrinsic and environmental factors affect hemoparasite infection in Diving Lizards. Our work calls attention to the ecology-parasitology interface in Amazonian lizards, an understudied field of research.

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## Advancing eDNA Sampling to Monitor Neotropical Fish Communities within a Natural History Museum Context

Sophie Picq, Caleb McMahan, Lesley de Souza

Neotropical rivers harbor the most diverse freshwater fish fauna on Earth, representing close to 45% of all known freshwater fish species and about 10% of all vertebrates. Ichthyological surveys have traditionally been conducted using capture-based methods, which can result in low capture rates in terrains that are hard to access. This potential undersampling may in turn lead to uncertainty in species range and underestimation of species diversity, both of which challenge conservation priorities. Recently, environmental DNA (eDNA) has emerged as a complementary or alternative technique that relies on extracting the genetic material shed by organisms into the water, amplifying species "barcodes", and assigning taxon information using DNA reference databases. Here, I will share our recent efforts in developing eDNA sampling as a tool to survey fish communities and to track rare freshwater species in remote locations of the Neotropics. I will present eDNA metabarcoding results from our proof-of-concept study in an Illinois watershed, the Kankakee River, as well as from a recent expedition to Guyana. I will also highlight the importance of archiving eDNA samples within Natural History museums for future studies, associating these types of samples with voucher specimens, tissue samples, photographs, and ecological data, and distributing those data out freely to aggregators across the globe. We hope our work solidifies the role of Natural History museums in understanding biodiversity and our changing planet and in yielding conservation action results on the ground.

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## The Many Grains of the Sand shiner: Comparing Traditional and High-Throughput Analyses of the *Notropis stramineus* (Cope, 1865) Species Complex

Amanda Pinion, Daemin Kim, Elizabeth Hunt, David Portnoy, Kevin Conway

The Sand Shiner *Notropis stramineus* (Cope, 1865) is a small-bodied minnow distributed widely east of the continental divide. Though previous taxonomic work on *N. stramineus* resulted in the designation of two subspecies based on meristics, the Sand Shiner is still considered to be one widespread species. However, analysis using Sanger-sequencing has provided evidence that the Sand Shiner may represent a species complex (i.e., comprising multiple species). Though major clade membership was consistent, mito-nuclear discordance existed between the three loci likely due to introgression, hybridization, incomplete lineage sorting or a combination of the three. In hopes of resolving this conflict, we collected ultra-conserved elements (UCEs) to construct a phylogenomic hypothesis for the species complex. Results from maximum likelihood (RAxML) and species tree analyses (ASTRAL-III) at all thresholds of data filtering recover the same major clades. Therefore, our UCE dataset strongly supports the existence of a species complex. UCE analyses also support a surprising sister-group relationship between *N. stramineus* from the Devil's River and Colorado drainage of Texas with another species of *Notropis* from the Rio Grande drainage well outside of the major Sand Shiner clade, a relationship also recovered using one mitochondrial gene. We compare the topologies resulting from the individual loci as well as that of various levels of filtering of UCE data, including "complete" (88 loci) and "incomplete" (429 loci) datasets. Finally, we discuss morphological traits useful for distinguishing between putative members of the *N. stramineus* species complex.

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## Tuberculation in the North American chub genus *Macrhybopsis*

Amanda Pinion, Kevin Conway

The genus *Macrhybopsis* contains 12 species of benthic, barbeled cyprinids that are distributed throughout central and eastern North America (including Canada, Mexico and the US). In a recent published study, we documented a novel tubercle type (unculiferous tubercle, type I, non-sexually dimorphic) on the surface of the head, body, and fins of the Shoal Chub, *Macrhybopsis hyostoma*. Unculiferous tubercles are roughly ovoid aggregations of keratinized epidermal cells, each with a keratinized projection (unculus) at the surface. Unculiferous tubercles are present in juveniles of at least 18 mm SL and are present also in adults of both sexes of *M. hyostoma*. In addition to unculiferous tubercles, male shoal chubs also possess typical conical ("nuptial", type II) tubercles on the dorsal surface of the pectoral fin in association with the anterior rays. Based on this information, we hypothesized a non-reproductive (potentially hydro-dynamic) function for unculiferous tubercles and predicted that they would be present also in other members of the genus, though tuberculation that is not sexually dimorphic may be uncommon in minnows. To determine if this condition is shared with other members of *Macrhybopsis*, we used scanning electron microscopy (SEM) and histology to investigate tuberculation in other members of the genus. All species of *Macrhybopsis* examined possessed type I tubercles, though there were four distinct morphological variations of this type of tubercle in the genus.

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## Snake Hemipenes Delineate *Heterodon* Species

Theodora Pinou, Panayiota Kotsakiozi, Melina Kohilakis, Aristeidis Parmakelis

Snake hemipenes have been used to define snake clades (Dowling, 1988), and most clades have been affirmed by molecular data (Pinou et al., 2004). Little, however, is known about the variation of this reproductive organ within a species and/or genus. A total of 78 *Heterodon* hemipenes, representing all extant species (*H. nasicus*, *H. simus*, and *H. platirhinos*) were examined for five hemipenal traits. Preliminary results suggest that spine number and bifurcation lengths can discriminate species, and geographical populations of species.

Speculation into the evolution of the snake hemipenis is discussed, and how these results corroborate reported species relationships and biogeographical history for *Heterodon* are explored.

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## Selenium:Mercury Molar Ratios in Blacktip Sharks (*Carcharhinus limbatus*) and Bonnethead Sharks (*Sphyrna tiburo*) in the Lower Laguna Madre, Texas

Natalie Pitman, Weston Nowlin, Faye Grubbs, Brian Bartram, Brian Jackson, Jessica Dutton

Mercury (Hg) can accumulate to high concentrations in sharks, potentially resulting in adverse health effects. Selenium (Se) has an antagonistic relationship with Hg and may have a protective effect against Hg toxicity if the Se:Hg molar ratio is >1:1. This study measured the Hg and Se concentrations, and calculated the Se:Hg molar ratios in dorsal muscle, heart, brain, kidney, and liver of young-of-the-year and juvenile blacktip sharks (*Carcharhinus limbatus*) and bonnethead sharks (*Sphyrna tiburo*), in the Lower Laguna Madre, Texas using a direct mercury analyzer and ICP-MS analysis. Overall, mean Hg concentrations were greatest in muscle of bonnethead sharks ( $0.491 \pm 0.441$   $\mu\text{g/g}$  dry weight) and heart of blacktip sharks ( $1.04 \pm 0.300$   $\mu\text{g/g}$  dry weight). In all tissues, the Se:Hg molar ratio was greater in bonnethead sharks compared to blacktip sharks, and all ratios were >1:1, indicating that Se may have a protective effect against Hg toxicity. There was no relationship between the Se:Hg molar ratio and body length for all tissues in both species. There was a significant inverse relationship between the Se:Hg molar ratio and Hg concentration in each tissue for both species. High Se:Hg molar ratios (e.g., >300:1 and >80:1 in the brain and >150:1 and >40:1 in the heart of bonnethead sharks and blacktip sharks, respectively) could reduce the adverse effects of Hg exposure during growth and development. However, the Se:Hg molar ratio at which Se is protective against Hg toxicity (e.g., 1:1, 5:1, or greater) is not known, highlighting the need for further research.

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## Investigating Sex Differences in Skin Lipid Synthesis Genes in Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*)

Emily Plant, Rocky Parker

Pheromones are a crucial mode of communication for squamates and aid in identifying conspecifics and initiating courtship. The pheromones identified to date in snakes are sex-specific blends of long-chain lipids produced by the skin. In garter snakes, lipid-based methyl ketones serve as the attractive components used in sexual signaling (*Thamnophis sirtalis*), are produced by the skin, and are the primary driver of male sexual behavior. The methyl ketones become more desaturated across winter brumation preceding the breeding season, which occurs as total skin lipid production increases. Production and modification of lipid pheromones is likely mediated through modulation of specific lipid synthesis genes in males and females, yet much remains elusive regarding such gene expression and any sex differences that may exist. In my research, I am investigating sex differences in expression of lipid synthesis and desaturase genes in the skin of male and female red-sided garter snakes (*T. s. parietalis*). I am comparing expression of lipid synthesis (*FASN*) and lipid desaturase (*FADS1*) genes between males and females within skin using quantitative PCR. I predict that lipid synthesis genes will be upregulated in males given that males generally have a higher proportion of skin lipids. Additionally, I anticipate higher expression of desaturase genes in females, as long chain, unsaturated methyl ketones are responsible for producing an attractive pheromone blend that drives female pheromone quality. My research will help increase our understanding of how sex-specific traits are regulated at the molecular level in organisms that utilize pheromones in the context of reproduction.

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## Stiff-legged Behavior in the Green and Black Poison Frog (*Dendrobates auratus*) in Response to Simulated Predation

Katherine Porras-Brenes, Gabriella Church, Elizabeth Clark, Anna Fellenstein, Ralph Saporito

Predation pressure has led to the evolution of a diversity of antipredator strategies in nature. Stiff-legged behavior is a defensive strategy that has



been described in several species of cryptically colored leaf-litter frogs in the neotropics, and is thought to reduce (or avoid) detection by visual predators. The behavior is characterized by frogs flattening their body and fully extending their forelimbs and back limbs into a stiff position. To further study the defensive behaviors in aposematically colored dendrobatid poison frogs, we conducted an experiment to measure the responses of captive raised, juvenile *Dendrobates auratus* to low (N=4) and high (N=4) levels of simulated predation. Simulated predation consisted of picking up and releasing individual frogs with 3" pressure-sensitive forceps. Surprisingly, all individuals exposed to simulated predation exhibited a stiff-legged behavior. Individuals exposed to high predation exhibited the behavior between 5-6 days after the start of the experiment, whereas individuals exposed to low predation began the same behavior between 6-22 days. Stiff-legged behavior in *D. auratus* consisted of frogs keeping their eyes open and assuming a stiff- and cross-legged position. The behavior lasted on average for five minutes, after which frogs returned to a relaxed posture. Our study provides evidence of predation-induced stiff-legged behavior, in naïve captive-raised *D. auratus*. Stiff-legged behavior may act as a complementary defense to aposematism in deterring the attack of visually oriented predators; however, additional studies will be required to determine if this behavior also occurs in wild individuals, and if so, its potential defensive function.

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## The Genomic Toolbox for Fisheries Management

*David S. Portnoy, William F. Patterson III, Christopher M. Hollenbeck*

The advent of massively parallel DNA sequencing has brought the field of genetics into the genomics age. By allowing the assay of many thousands of genetic markers across hundreds of individuals in population genetic assessments, the resolution to detect population structure has increased substantially and localized adaptive genetic variation can be more readily characterized. However, access to a variety of types of genomic data have also offered the means to provide information for fisheries assessment and management beyond population structure, including age structure, estimates of census size and population trends, and stock mixing rates. This talk will provide a brief background on some of these new and promising techniques, such as epigenetic ageing and close-kin mark-recapture, with examples of current and future applications in elasmobranchs and bony fishes.

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## Conservation Genomics of the Great Hammerhead in the Western North Atlantic

*David S. Portnoy, Dominic G. Swift, Christopher M. Hollenbeck, R. Dean Grubbs, Neil Hammerschlag, Tristan L. Guttridge*

The great hammerhead (*Sphyrna mokarran*) has experienced global population declines due in large part to its susceptibility to fisheries. In U.S. waters of the western North Atlantic Ocean, rates of bycatch and recreational catch-and-release mortality are thought to be high, but the species status is undetermined. To better understand potential genetic structuring and population condition in U.S. waters, patterns of genetic variation were assessed among 273 individuals sampled throughout the Gulf of Mexico, off the U.S. East Coast, and in The Bahamas, using reduced representation genomic data. Genetic heterogeneity was detected among samples with subtle differences between The Bahamas and elsewhere, suggesting the presence of two genetic units. In the total dataset, 19 pairs of kin were identified, with two pairs between The Bahamas and Florida Keys, suggesting movement between units. Within the U.S. Atlantic and Gulf of Mexico, kin were captured three days to nine years apart at distances ranging from 30-965 km, and two animals were recaptured at intervals of two and five years at liberty. Estimates of contemporary effective population size for the entire U.S. Atlantic and Gulf of Mexico were ~4,000. The random sampling of 17 pairs of kin and recapture of two individuals in a study with modest sample size, along with the relatively small estimate of contemporary effective size, suggest that the population of great hammerheads in the U.S. western North Atlantic is relatively small compared with other large coastal shark species managed in the region.

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## A Preliminary Census of Copperheads (*Agkistrodon contortrix*) in Connecticut, USA.

*Andrew Powers, Theodora Pinou*

The eastern copperhead (*Agkistrodon contortrix*) is a medium-sized viper native to the eastern coast of the United States of America. Within New England, the copperhead has experienced land-use changes within their range and is considered a sensitive species in Connecticut in need of study (Klemens et al. 2021). Within the last three decades there has been an increase in urbanization and land-use change in central Connecticut resulting in the destruction of copperhead habitat. Our study uses mark-and-recapture data to revisit known dens, and record population demographics for a documented population (Smith et al. 2009) in Meriden, CT living in 1,2000 acres of a town protected land parcel. Our study examines if demographic change has occurred in this copperhead population over 22 years. This study is the only current long-term monitoring project on Connecticut copperheads that may inform future management of the species.

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## Characterizing Anglers' Responses to Reported Increases in Shark Depredation Across the Gulf of Mexico

*Evan Prasky, Marcus Drymon, Alena Anderson, Sarah Gibbs, Amanda Jargowsky, Danielle McAree, Ana Osowski, Savannah Swinea, Steven Scyphers*

Since the onset of industrialized fishing, shark populations have seen declines in abundance throughout the world. However, in the United States Gulf of Mexico (GoM), several large coastal shark species are showing preliminary signs of recovery following decades of successful regulations, enforcement, and monitoring. The stabilization of coastal shark populations, coupled with increases in recreational fishing efforts, escalates the potential for human-wildlife conflicts. The most often reported conflict is shark depredation, where a shark partially or wholly consumes an angler's catch while in the process of landing. Reported increases in shark depredation within the last several years have begun to challenge angler norms and have the possibility of undermining decades of shark conservation. To address these concerns, we implemented a Gulf-wide survey to characterize the impact of shark depredation on recreational anglers' fishing satisfaction. Our study results showed that the majority of recreational anglers in the GoM reported encountering or seeing depredation, yet have not changed their fishing behaviors. In contrast, anglers' viewpoints on how shark populations should be managed were split between a reduction in population sizes and maintaining current population levels. As coastal shark populations in the GoM continue to recover, shark depredation may increase, which may impact recreational anglers' fishing satisfaction and may adversely affect future shark conservation initiatives.

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## Long-term Use of a Shark Breeding Ground: Three Decades of Mating Site Fidelity in the Nurse Shark, *Ginglymostoma cirratum*

*Harold Pratt Jr., Theo Pratt, Ryan Knotek, Jeffrey Carrier, Nick Whitney*

The Dry Tortugas courtship and mating ground (DTCMG) has been known as a mating site for nurse sharks, *Ginglymostoma cirratum*, since 1895. In a 30-yr (1992–2021) study we documented long-term site fidelity to this area with data from 137 adult sharks (89 female, 48 male) tagged with PIT, fin, and acoustic tags. Of 118 sharks tagged from 1993 to 2014, at least 80 (68%) returned to the DTCMG in subsequent years during the June-July mating season. Known individuals returned in up to 16 different mating seasons and over periods of up to 28 years, indicating that life span extends well into the forties for this species. Of all returning sharks, 59% (N = 47) have been monitored for over 10 years and 13% (N = 10) have been monitored for over 20 years. Males arrive annually in May and June and depart in July, whereas females arrive biennially or triennially in June, with a secondary peak in site use in September and August, likely associated with thermoregulation during gestation. Females typically mated biennially but showed a triennial cycle in 32% of cases, with many females switching cycles at least once. This pattern would reduce the potential reproductive lifetime output of a female by 11% compared to what would be

projected from a strict biennial cycle. The long-term mating site fidelity of this shark population reveals the importance of identifying and protecting mating sites for this and other elasmobranch species.

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### **The Gustatory Filament of the Remo Flounder (Pleuronectiformes: Oncopteridae: *Oncopterus darwinii*)**

*Paulo Presti, Murilo Nogueira de Lima Pastana, G. David Johnson, Alessio Datovo*

Pleuronectiformes present one of the most intriguing morphological modifications of vertebrates: the ontogenetic migration of one eye towards the opposite side of the head. Most previous morphological studies on flatfishes have focused on their cranial skeleton, poorly exploring other informational systems. Our study describes an extreme modification in the first ray of the dorsal fin of *Oncopterus darwinii* (Oncopteridae). This ray is completely independent of the others (free of interradiation membrane) and is restricted to the blind side of the body. When at rest, it is housed in a groove in the head. The ray has several fimbriae and its distal tip is globular. It is innervated by the ramus lateralis accessorius (RLA), which is a nerve exclusively dedicated to taste perception and has the common muscles of a dorsal fin (depressor, erector and inclinor dorsalis), but this musculature originates from the neurocranium and is arranged differently from the common dorsal fin musculature. Such morphology suggests that the first ray of the dorsal fin is modified into a gustatory filament that is probably used in the active search for food buried in the substrate. A structure with such characteristics has never been reported for other flatfishes. The only known similar modification is that of *Asterorhombus fijiensis* (Bothidae), which presents the first ray of the dorsal fin modified in a lure.

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### **Is chytrid avoidance an innate or learned behavior in the strawberry poison frog?**

*Rachel Prokopijs, Alessandro Catenazzi*

Batrachochytrium dendrobatidis (Bd) is deadly fungus implicated in the massive decline of the world's amphibian populations. A research avenue that has received little attention is a frog's ability to avoid Bd. Learned avoidance of Bd has been documented, but no frog is known to exhibit innate avoidance, and avoidance has never been studied in an individual from the poison frog family. This study tested the ability of strawberry poison frogs (*Oophaga pumilio*) to avoid Bd through chemosensory choice trials. In each trial frogs were allowed to smell but not touch the fungus, and separate trials were run with live and dead Bd. The proportion of trial time spent in the control (broth media) and experiment (Bd) areas of the trial chambers was recorded for each frog. Trials were repeated after frogs were successfully infected and cleared of Bd to determine if avoidance could be learned. Models were generated to determine under which circumstances, if any, Bd avoidance occurred. These results will provide information as to how frogs may use avoidance tactics to live in a world with a deadly fungal pathogen.

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### **Does fear of humans filter animals from urban areas? A case study of Southern California lizards**

*Bree Putman, Daniel Blumstein, Greg Pauly*

Urbanization is occurring worldwide and is one of the greatest threats to biodiversity. How animals assess and respond to novel threats will predict who survives such rapid environmental change. Human activity affects animal behavior and physiology, and individuals may need to modify responses to humans in order to co-exist with them in urban areas. We have categorized various lizard species in Southern California as urban-intolerant, urban-tolerant, or urban-exploitive, and are quantifying behavioral and physiological fear responses to determine if anthropophobia (fear of humans) associates with interspecific differences in urban tolerance. Thus far, measures of boldness do not scale linearly with the level of urban tolerance in lizards. For instance, nonnative urban-exploitive species are not bold risk-takers as we might expect. Overall, our results show that urban life does not necessarily promote habituation-like processes to humans and most species experience at least some negative health effects due to urbanization.

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### **Floodplain inundation mediates the abundance, behavior, and size-class distribution of the associated fish community**

*Adam Quade, Kelly Boyle, Guillaume Rieucou*

Anthropogenic modifications to the Mississippi River have decoupled the surrounding floodplain from the annual flood-pulse, disrupting trophic stability and biogeochemical cycles. Louisiana's emblematic fish species that use the floodplains of the Mississippi River rely on the timing, magnitude, and duration of the flood-pulse to cue aspects of their life history (e.g. spawning and foraging). We utilized adaptive-resolution imaging sonar technology to test whether floodplain inundation and proximity to the main-river channel influences the abundance, behavior, and size-class distribution of the fish community. Since summer 2021, we conducted sonar monitoring at four sites across a latitudinal gradient of decreasing hydrologic connectivity. Acoustic monitoring reveals the dynamic nature of the fish community, highlighting an unsuspected degree of spatiotemporal variability in abundance, behavior, and size-class distribution driven by the inundation level. Shifts in abundance of small-bodied fish (20 – 40 cm such as *Dorosoma cepedianum*) through time were observed, supporting the function of an inundated floodplain as an important foraging opportunity for large piscivorous (> 40 cm) fishes such as Gar (*Lepisosteidae*). Observed changes in Gar abundance and size-class distribution supports the idea that floodplain inundation provides both spawning opportunities and nursery habitat depending on size-class. Additionally, an inundated floodplain supports larger schools than when disconnected, suggesting the behaviors of smaller-bodied (<20cm) fishes within the floodplain are mediated by the inundation level. Ongoing acoustic monitoring continues to provide valuable information regarding the complex interactions between floodplain-associated fishes and how they perceive and utilize the floodplain in order to guide conservation and management strategies.

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### **Assessing population structure at the range edge of a regionally threatened anuran, *Acris blanchardi***

*Travis Rainey, Kirsten Nicholson, Brad Swanson*

The current biodiversity crisis, marked in part by a disproportionate loss of amphibian diversity, is affecting all parts of the globe, including Michigan's Lower Peninsula, USA. Blanchard's Cricket Frog (*Acris blanchardi*), a wide ranging hylid once common across its northern range edge, has been declining across its northern range since the 1970s. Understanding how the remaining Michigan metapopulation is structured, and how landscape metrics in highly modified regions like southern Michigan can lead to informed management decisions for species recovery. We collected non-lethal genetic samples from known populations spanning across Michigan. We used microsatellite loci to determine allele frequencies, then used frequencies to characterize population structure across these Michigan populations using Bayesian likelihoods. We found a high degree of structuring across the Michigan metapopulation and evidence that the highly fragmented landscape of the study area has led to isolated populations and hindered gene flow. Our results can provide support for land and wildlife managers to better facilitate increased connectivity and gene flow between populations and prioritize particularly isolated populations.

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### **An Investigation of Low-coverage Whole Genome Sequencing as a Tool for Studying Osmoregulatory Evolution in Potamotrygonid Rays**

*Anthony Rajkumar, Belinda Chang, Nathan Lovejoy*

Osmoregulation refers to the processes by which organisms maintain a stable internal cellular balance of water and salts, and affects environmental tolerance. Fishes provide an excellent osmoregulation study system, since they must maintain a specific ion concentration, distinct from their environment. In marine species, a lower salt concentration than the environment must be maintained by excreting excess ions and uptake of large volumes of water, while in freshwater fishes, this is reversed. Thus, environmental salinity determines the necessary form of osmoregulation. While previous studies have investigated osmoregulatory activity in fishes which can tolerate varying salinity, less focus has been devoted to macroevolutionary transitions between habitat types. Osmoregulation studies generally concern physiological processes

or regulation of gene expression, but lack focus on molecular evolution over large timescales. South American freshwater stingrays (Potamotrygonidae) provide an ideal study group to investigate molecular evolution of osmoregulatory genes, due to their Miocene era marine to freshwater transition. Potamotrygonids have diversified within fresh water and currently inhabit environments of varying pH, a possible additional influence on osmoregulatory gene evolution. To assess selected genes across numerous species in a cost-effective manner, we investigated the effectiveness of low-coverage genome sequencing for studying osmoregulatory genes in Potamotrygonidae. We sequenced the genomes of four potamotrygonid species and used a custom pipeline to obtain consensus sequences of selected osmoregulatory genes. Simulations of different sequencing coverages were also used to assess the impact on downstream analyses. Here, we present the efficacy of our sequencing methodology in recovering coding sequences from ethanol-preserved specimens.

### Intraspecific Priority Effects in Smallmouth Salamanders: Effects on Larval Body Size and Survival

*Trevor Rallo, Thomas Anderson*

The timing of biological events (i.e., phenology) is shifting for many taxa worldwide, which can have strong consequences for species demography. One way in which demography is altered is through changes to the magnitude or outcome of intraspecific interactions. In particular, body size differences generated by variation in phenology may provide advantages to early-arriving individuals in antagonistic interactions (i.e., size-mediated priority effects) over later-arriving individuals. Here we examined how variation in the timing of hatching influenced intraspecific priority effects in a pond-breeding salamander, *Ambystoma texanum* (smallmouth salamander). We manipulated the timing of hatching of eggs and then reared larvae in outdoor mesocosms until the initiation of metamorphosis. Treatments included timing differences of 0, 7, 11, 14, and 17 days differences in timing of addition between cohorts of larvae. We found that survival was unaffected by differences in hatch timing, and remained relatively constant. Similarly, both average larval body size and variation in body size did not differ across hatching treatments, and instead only varied in response to larval survival—an indication of intraspecific competition. These results indicate that shifts in an organisms' phenology may not always result in strong negative demographic consequences. Future research is underway to explore whether greater temporal variation in hatch timing, representative of larger phenological shifts, may have a larger impact on salamander demography.

### Conservation of Gharial (*Gavialis gangeticus*) in National Chambal Sanctuary, India

*RJ Rao*

The Chambal River is considered a hot spot for biodiversity and aquatic wildlife conservation in India. The Gharial (*Gavialis gangeticus*) occur in the Chambal River is among the threatened crocodilian species in India. Although this species is distributed in different rivers in India and Nepal, its IUCN endangered category is of concern. A review of literature dealing with the biology and conservation status of gharial revealed that relatively few reports of abundance values and the lack of long-term projects make it difficult to establish the conservation status of this species. Research studies on habitat assessment of gharial are a good parameter to estimate the amount of suitable habitat in the Chambal River. Distributed along the 400 km stretch of the Chambal River, protected as National Chambal Sanctuary, the gharial selected sandy habitats on both the banks as their nesting grounds and basking areas. Knowledge on long-term population trends in crocodilians is essential to assess the effectiveness of conservation areas and to guide sustainable management practices. Research studies have been extensively carried out in the National Chambal Sanctuary on the ecology, biology, conservation and management of mugger and gharial crocodiles and freshwater turtles in the National Chambal Sanctuary by researchers from different Universities and National Institutes in collaboration with sanctuary management. Ecological studies strive to identify factors that explain patterns of species distribution and abundance. In crocodiles, habitat suitability is the major force influencing distribution and abundance, but there is also increasing evidence pointing at the influence of

habitat structure and prey abundance. The research studies indicated that the gharial population in the Chambal River has been relatively stable in the past few years, mainly due to reintroduction of captive raised gharial under 'grow and release' programme and stringent conservation measures. However, it is important to accurately estimate the population size of *G. gangeticus* and protect the nesting habitats. Therefore, it is recommended for continuation of the population studies and involvement of environmental organizations to regulate anthropogenic activities in the Chambal River. Spatial information gaps, combined with the absence of knowledge about the population ecology and habitat reduction are indeed the principal threats to the gharial in India.

### Novel genomes from Coral Triangle fishes reveal correlates of past demographic processes in the epicenter of marine biodiversity

*Brendan Reid, Allison Fink, Abigail Ethridge, Eryka Molino, Eric Garcia, Rene Clark, John Whalen, Jemelyn Baldisimo, Roy Roberts, Chandry Jablonski, Marial Malabag, Abner Bucol, Chris Bird, Kent Carpenter, Malin Pinsky*

The Coral Triangle represents the apex of marine species diversity. Characterizing the demographic history of species inhabiting the Coral Triangle can help illuminate the processes that have generated this treasure trove of biodiversity as well as inform ongoing efforts to manage and preserve tropical marine species. As part of an ongoing temporal genomics study, we generated shotgun whole-genome sequencing data for over twenty Philippine fish species exhibiting a variety of habitat preferences, range sizes, and reproductive modes. We used de novo genome assembly methods to produce draft genomes for a single individual from each species, and we used a pairwise sequentially Markovian coalescent (PSMC) methodology to infer the past population trajectory for each species. PSMC analyses identified a number of distinct demographic modes, including long-term stability, long-term expansion, and late Pleistocene expansion followed by recent decline. Reproductive mode showed the strongest associations with demography, and mouth- and tail-brooding species exhibited lower estimated effective population sizes over time compared to species with demersal or pelagic eggs. These results provide useful baselines for often data-poor tropical species and will lay the foundation for studies of recent demography using historical and contemporary specimens.

### Patterns of Overlapping Habitat Use of Juvenile White Shark and Human Recreational Water Users

*Patrick Rex, Christopher G. Lowe*

Juvenile white sharks (JWS) of the Northeastern Pacific population are present in nearshore, southern California waters and form mixed size class (~1.5-3 m) long-term aggregations, often close to shore. These nearshore beach habitats are heavily used for human recreation and the amount of spatio-temporal overlap between JWS and humans is currently unknown. Increases in human population and this population of white sharks have raised concern over human beach safety. To determine spatio-temporal JWS-human overlap at various spatial scales, 1644 aerial drone surveys were conducted monthly from January 2019 to March 2021 at 26 beach locations. Thirteen environmental variables were also assessed to predict when spatio-temporal JWS-human overlap was highest. JWS southern California coast-wide distribution was clumped, limiting human-JWS overlap to specific JWS aggregation locations. Nearshore distribution indicated JWS are often close enough to the wave break to interact with some water users (median=101 m, range=2-702 m), although JWS had the most overlap with stand-up paddlers. Daily human-JWS co-occurrence was 97% at beaches where aggregations had formed, and human-JWS activity showed high spatial overlap at shark aggregation sites. Although there is higher seasonal human-JWS spatio-temporal overlap where aggregations form in southern California, the risk of unprovoked shark bite across southern California is low. Because of high daily encounter rates, it is possible JWS in these areas have habituated to humans as non-prey items. Lastly, this study shows that drones may be a powerful tool for quantifying shark-human distributions, encounter rates, and provide a cost-effective means for addressing human safety concerns.

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## Competitive Aggression in Cheoah Bald Salamanders

Carter Ricks, Joseph Pechmann

In the southern Appalachian Mountains, multiple large species of salamanders in the genus *Plethodon* occur within the same niche, with different species dominating high and low elevations and engaging in interspecific competition where they overlap. The high-elevation species are usually more aggressive than their low-elevation competitors, which may prevent the latter from ascending the mountain and pushing the high-elevation species to the top, potentially eradicating the high-elevation species over time. In the Cheoah Mountains, this interaction may occur between the Cheoah Bald salamander (*Plethodon cheoah*) as the high-elevation species and the southern Appalachian salamander (*P. teyahalee*) as the low-elevation species. *Plethodon teyahalee* is not limited by physiological restrictions to low elevations, so we wanted to examine if interspecific aggression may be contributing to the dominance of *P. cheoah* at high elevations. We ran aggression trials with *P. cheoah* and *P. teyahalee* in a lab setting to compare both species' interspecific and intraspecific aggression. We did 30 interspecific aggression trials and 15 intraspecific trials for both species. Aggression was defined as acts of a salamander looking towards, approaching, snapping, lunging, chasing, or biting the other salamander. *Plethodon cheoah* was found to be more aggressive than *P. teyahalee* in both intraspecific and interspecific trials, and larger individuals of both species committed more aggressive acts. However, smaller *P. cheoah* did show aggression toward larger *P. teyahalee*. These results suggest that aggressive interactions help maintain the elevational distributions of *P. teyahalee* and *P. cheoah* as in other *Plethodon*.

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## Assessing Species Boundaries in Antillean Four-lined Skinks (*Spondylurus*) Using Whole Genome Data

Danielle Rivera, Nathan Hostetter, Jaime Collazo, Rayna Bell

The taxonomic status and species boundaries of skinks from the genus *Spondylurus* have been under debate for the last decade. A contemporary redescription of the group, which was primarily based on morphological characters from historical museum specimens, only included genetic information for about half of the species described. We conducted multiple surveys across Puerto Rico and the US Virgin Islands to assess presence, document ecology, and collect genetic data with the aim of evaluating species boundaries and resolving taxonomic uncertainty within this group. In addition to the fresh tissue collected on these surveys, we also obtained fresh tissue samples from collaborators and historical tissue samples from museum specimens resulting in a fully represented phylogeny for the genus *Spondylurus*. Focusing on species within the US, we comment on the status of each species, and the future utility of having a reference genome from the Puerto Rican species, *Spondylurus nitidus*. These data will help assess the conservation status of the species in the US Caribbean.

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## Femoral Gland Gene Expression in Parthenogenetic and Sexually Reproducing Lizards of the Genus *Aspidoscelis*

Joshua Rivera, Joseph Rangel, André Carvalho, Adam Leaché, Matthew Fujita

Chemical communication, facilitated by the secretion and recognition of pheromones, plays a vital role in the behavior of squamate reptiles. These chemical signals are linked to various intraspecific and interspecific interactions, such as kin recognition, mate choice, and aggression. In some cases, pheromone composition may even be linked to speciation. For example, some morphologically conservative lizard radiations exhibit divergence in pheromone composition. Whiptail lizards of the genus *Aspidoscelis* possess femoral glands, which likely play a key role in their chemical communication. However, the composition of the pheromones and the genetic mechanisms regulating expression of the glandular content are not well understood. In this study, we compared the expression of pheromone-related genes between parthenogenetic and sexually reproducing whiptail lizards, anticipating minimal differences due to the presence of pseudocopulatory behavior in all-female parthenogens. We collected lizards in the field, extracted gland chemicals, and excised femoral gland and forelimb tissues for transcriptomic analysis. We used gene ontology and combined pathway analyses to investigate

the diversity of proteins associated with glandular tissue and infer potential biochemical pathways. Finally, we performed differential gene expression analyses to compare glandular gene expression between male and female *A. gularis*. Our findings shed light of the genetic machinery that maintains the evolutionary ubiquitous femoral glands and contributes to our understanding of chemical communication in reptiles, paving the way for future research in this field.

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## Whole Genome Sequencing of Century-Old Philippine Reef Fishes

Roy Roberts, Sharon F Magnuson, Christopher E Bird

The genomes of organisms stored in museums hold a wealth of information that is challenging to access. Recent success has been reported in sequencing desiccated museum insects involved using whole genome amplification (WGA) and enzymatic repair (NEBNext FFPE Repair Mix) of DNA damage, but these techniques have not been tested on historical EtOH preserved fishes. Here, we use factorial treatment combinations to test for the effects of WGA, repair, and amount of starting DNA on whole genome sequencing of historical (1907 - 1909) and contemporary (2017) Philippine marine fishes. A total of 192 extractions were performed, 171 (93 +WGA, 78 -WGA) of which were put through each treatment combination before library prep. Contrary to expectation, WGA had a negative effect on success of libraries and repair had no consistent effect at all. A greater percentage of historical libraries treated with WGA failed than those that weren't (35.6% +WGA, 72.5% -WGA). Within the +WGA treatment group there was a much lower yield of DNA in the historical specimens compared to the contemporary. For the contemporary samples, there was a positive relationship between DNA yield and starting amount of DNA, however this was not the case in historical samples. In total we successfully sequenced 128 libraries (45 Historical, 83 Contemporary; Illumina). The success of these methods means that these museum specimens can be used to test for the effects of human activities on the evolution of fishes.

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## larval fish ingress and vertical distribution in the Aransas Pass Inlet system (TX, USA)

Olivia Robson, Simon Geist, David Portnoy

In the Coastal Bend region of Texas several proposals for desalination plants are currently underway. These plants would take in water from the adjacent bay and coastal inlet and dispose of the brine using a diffuser creating a plume of higher salinity and temperature in the coastal inlet. The proposed developments have caused concern that especially vulnerable larval fish and crustaceans would be harmed when passing through the inlet from their Gulf of Mexico spawning grounds to their estuarine nursery habitats both in the public and scientific communities. Changing vertical position is a key behavior of larvae during selective tidal stream transport, which is considered one of the two main mechanisms to pass a coastal inlet together with passive transport. By sampling the Aransas Pass Inlet and its three tributaries, in the vicinity of one of the proposed desalination plans, during day and nighttime and during both incoming and outgoing tides at three different depth strata we aimed to improve our knowledge of the vertical position of larval fish and crustaceans during their passage through the channel. Preliminary data analysis revealed that larval densities occurred throughout the whole water column but were greater in the deepest stratum and nighttime hours. There may be an adverse effect on the larvae's development and survival depending on the larvae's sensitivity to abrupt changes in salinity and temperature which differs by species and developmental stage.

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## Trophic Ecology and Environmental Conditions Affect Mercury Concentrations in Immature Sharks in Texas Bays.

Jasmine Rodriguez, Jessica Dutton, Faye Grubbs, Brian Bartram, Weston Nowlin

Sharks are among some of the most threatened coastal taxa due to overfishing, climate change, and exposure to contaminants. Because many shark species are higher trophic level consumers, they are prone to mercury (Hg) exposure primarily through their diet. There is limited understanding of how



relationships between Hg concentrations in young-of-the-year (YOY) and juvenile sharks are affected by spatial variation in Hg in their prey. We measured Hg concentrations in YOY and juveniles of three shark species [blacktip (*Carcharhinus limbatus*), bull (*Carcharhinus leucas*), and bonnethead (*Sphyrna tiburo*)] in four Texas bays (Sabine Lake, Aransas Bay, Corpus Christi Bay, Lower Laguna Madre). Shark dorsal muscle tissue and prey were assessed for Hg and stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ ) to determine how variation in inter-species and inter-site trophic ecology influenced shark Hg exposure risk. There were inter-species Hg differences in sharks and potential prey within bays as well as differences in overall Hg in biota among bays. Shark  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  varied among species and niche modeling indicated that dietary specialization and degree of dietary overlap between species differed among bays. Our findings indicate that bay-specific environmental conditions that influence Hg methylation rates and biotic Hg accumulation (e.g., salinity, water temperature, Hg deposition) may play a role in spatial Hg differences and that inter-species differences in trophic ecology of immature sharks also influence Hg concentrations. However, we also found that YOY and juvenile shark trophic ecology can exhibit substantial variation, which likely influences spatial patterns of Hg exposure risk.

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### Incomplete Lineage Sorting and Reticulate Evolution Obscure Major Diversification Events Within Poeciliidae (Teleostei: Cyprinodontiformes)

*Sheila Rodríguez Machado, Diego J. Elias, Kyle R. Piller, Caleb McMahan, Prosanta Chakrabarty*

The Fish Tree of Life has benefitted from the advent of next-generation sequencing by solving contentious phylogenetic relationships. Nevertheless, a combination of complex evolutionary histories and our inability to approach genealogical discordance prevents us from fully understanding the evolutionary history of recalcitrant taxa. The family Poeciliidae ("live bearers") is widespread in the Neotropics and harbors the highest number of freshwater fish species in Middle America and the Caribbean (~273 spp.). Although poeciliids have been the center of heated debates about their origin and colonization, their systematics and biogeography need evaluation in the genomics era. Here, we re-examined phylogenetic relationships within Poeciliidae using 1,018 ultraconserved element loci. We inferred relationships among genera using both concatenation and coalescence-based methods and explored sources of gene tree discordance. All genera but one was recovered as monophyletic. In contrast, most recognized tribes were not supported. Although we found topological discordance between the concatenated and species trees, both topologies were equally likely. We also found branches that deviate significantly from a pure incomplete lineage sorting model, and branches that potentially are in the anomaly zone. Our results provide evidence of both rapid diversification and reticulate evolution. Such processes may explain genealogical discordance and make it difficult to unambiguously solve the phylogenetic placement of some taxa. While further exploration is needed to address the remaining conflict, our study shows some mechanisms underlying the systematics of Poeciliidae based on genomic data.

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### Movement Patterns and Home Ranges of the Puerto Rican Racer (*Borikenophis portoricensis*)

*Adolfo Rodríguez-Velázquez, Alberto Sabat*

Most of the research in snakes related to movement and home ranges (HR) have been done in temperate and continental regions. More studies should be done with species from tropical zones and islands due to how vulnerable they can be to global warming, human activities, invasive species, among others. Here we report the results of a field study with the objective to document movement patterns and HR in the Puerto Rican racer (*Borikenophis portoricensis*), in two seasons. This species has a wide distribution in the Puerto Rican archipelago and shares similarities with other Antilles snakes. External transmitters were placed on 33 snakes in the Cambalache State Forest, 16 for the dry/cold season and 17 for the wet/warm season. They were relocated every day until the transmitters came off. In each relocation, GPS points, data on environmental parameters, activity of the specimen, topographic habitat, and microhabitat were taken. We found that male and female snakes exhibited temporal variability in movement. Males from the dry/cold months exhibited

a significant increase in HR per day, compared to males from the wet/warm months and females from both seasons. Also, female snake was found most of the time hiding on crest and slopes, while male was either moving or basking on valleys, suggesting that sexes differ in topographic habitat. In terms of microhabitats, males were more commonly found buried in tree roots and dray branches and females prefer to hide under leaf litter. The differences presented could be related to reproduction and courtship factors.

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### Movement and survival of adfluvial Bull Trout in the Yakima Basin, Washington

*Jason Romine, Craig Haskell, Connor Cunningham, Todd Newsome, Russ Byington, Aimee Taylor*

Bull Trout *Salvelinus confluentus* is a char native to the river systems of north-western North America. They require clean, cold, and connected habitats. However, overfishing, habitat fragmentation, introduction of non-native Brook Trout, and over-obligated water resources have all contributed to their decline and ultimate listing under the US Endangered Species Act in 1998. Historically, the Yakima River Basin was home to 15 genetically distinct populations of Bull Trout, three of which have been extirpated. Although Yakima Basin Bull Trout exhibit the three generally accepted life history types (adfluvial, fluvial, resident), most populations are considered adfluvial. Within the Yakima Basin, the US Fish and Wildlife Service works with state, federal, tribal, and non-governmental partners to monitor the movement and survival of Bull Trout using a combination of Passive Integrated Transponder (PIT) tags and acoustic telemetry. We used detections from our PIT and telemetry systems to model movement and estimate survival trends of juvenile and adult Bull Trout using a multi-state mark recapture model in a Bayesian framework.

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### Long-term Demographic Comparison of Head-start vs. Natural Release Diamond-backed Terrapin Hatchlings

*Willem Roosenburg, Julia Joos, David Jenkins, Alayna Tokash, Paul Converse, Nicholas Smeenk*

We conducted a multi-year demographic study to evaluate the life history difference that may occur by head-starting. We released almost 3000 head-starts over 17 years and more than 16,000 natural release hatchlings over 20 years on the Poplar Island Ecosystem Restoration Project. All hatchlings were marked with coded wire tags prior to release and the head-starts were marked with PIT tags. Additionally, all wild hatchlings were cohort notched to determine age upon recapture. All head-starts were reared in K-12 classrooms throughout Maryland, USA. Trapping using fyke nets, crab pots, and other non-lethal techniques was done from mid-May through early August every field season since 2008. We compare demographic parameters of these two groups including age of first reproduction, effect of size at release on survival, and stage specific survival. Head-starting accelerates age of first reproduction by 2-3 years initiated by accelerated growth during their first winter. However, size of head-starts at release does not influence recapture probability. Initial survival of head-starts is higher than wild individuals but overall lower than expected relative to the survival of natural release hatchling groups. We interpret our results analyzing the effectiveness of head-starting as a management tool to restore and supplement turtle populations that have declined.

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### A new species of deep-sea lantern shark genus *Etmopterus* Rafinesque 1810 (Etmopteridae: Squaliformes) from the Western South Atlantic

*Pollyana Roque, Otto Gadig, Claudio Oliveira, Marcelo Melo*

*Etmopterus Rafinesque 1810* is a genus of small to medium-sized (<800 mm TL), bioluminescent, deep-sea sharks, commonly known as lantern shark. Among the 42 valid species recognized nowadays, five were previously recorded in the western South Atlantic (WSA), *E. bigelowi* Shirai & Tachikawa, 1993; *E. gracilispinis* Krefft 1968; *E. granulosus* (Günther, 1880); *E. hillebrandi* (Poey, 1861); and *E. lucifer* Jordan & Snyder, 1902. Nevertheless, recent taxonomic revisions demonstrated that *E. lucifer* is restricted to the western North Pacific. The material used in this description includes 32 specimens (158–470 mm TL) collected on the Brazilian continental slope, on board the R/V Alpha

Crucis, as part of the DEEP-OCEAN Project, and two specimens from other museums (180–398 mm TL). *Etmopterus* sp. n. is a member of the *E. lucifer* group, diagnosed among its congeners by the patterns of photophores on flank, dermal denticles pattern, and number of teeth cupids on palatoquadrate. The new species is only known from southern Brazil, between São Paulo and Paraná States, in depths between 655 and 900 m. Prospective work includes sequencing of mitochondrial genes (COI, NADH2, and 12S) as part of an integrative, taxonomic of the WSA species.

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## Jack Musick and his Ties to Elasmobranch Research in Brazil

*Ricardo Rosa, Otto Gadig, Rafael Menezes*

The late John A. Musick, Professor Emeritus of the Virginia Institute of Marine Science (VIMS), was a versatile scientist whose influence came in many publications and through the graduate students he prepared for academic and research life. As a professor, Jack communicated with passion, such in the ichthyology course he taught at VIMS and in the books he wrote. He also showed inspiring scientific curiosity and generosity. More relevant was his strong bond with nature, vividly expressed along the Appalachian trails, during the many days at sea and in daily life. This characteristic, intrinsic to his personality, certainly influenced his students, who also developed deep respect and interest in biodiversity, many of them also studying elasmobranchs and working for their conservation. Jack supervised 88 graduate students (nine as co-chair), 22 of whom (25%) worked with elasmobranchs. Four of his PhD students (8%) came from Brazil, revealing Jack's strong ties to the country. For the first author (RSR), he steered a career towards elasmobranchs, suggesting a revision of the South American freshwater stingrays (*Potamotrygonidae*) - a group previously neglected by taxonomists. A preliminary data mining indicates that 373 Brazilian researchers have worked with elasmobranchs over the last decade (2013-2022), 355 of which published journal articles on the group. Thirty-two researchers have ties to Jack, either directly as alumni (2) or as alumni of the latter (15-academic grandchildren, 14 great-grandchildren and 1 great-great-grandchild). The completion of this ongoing survey will enable tracing all branches of Jack's contribution to elasmobranch studies in Brazil.

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## Accounting for lipid and urea content when analyzing elasmobranch plasma for stable isotope analysis

*Alexander Rubin, Aaron Carlisle, Sunita Shah Walter, John Mohan*

Stable isotope analysis (SIA), particularly of carbon and nitrogen stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively) is a valuable tool for studying aquatic species and ecosystems as it can provide insight on trophic ecology, food web dynamics, physiological processes, and movements and habitat use. However, to effectively use SIA to address ecological questions, aspects of the chemical composition of an animal's tissues that may bias measurements of its stable isotope composition must be accounted for. In elasmobranch fishes, the primary tissue constituents that may bias SIA values are urea and lipids, which are enriched in  $^{14}\text{N}$  and  $^{12}\text{C}$  respectively. While lipid and urea effects on muscle and liver tissue are understood, how to account for these tissue constituents in blood, in particular plasma, remains unknown. As a high turnover rate tissue, plasma can provide important insight into recent diet of animals, hence an understanding of how to effectively analyze this tissue is necessary. In this study, we adapted protein extraction methods used in metabolomics and human hematology to remove proteins (the primary tissue constituent of interest in most SIA studies) from blood plasma, as opposed to using traditional techniques of removing lipid and urea from protein. We test this approach across a range of elasmobranchs and use bovine serum albumin (BSA) as a control protein. We found that removing protein from blood plasma significantly changes the carbon and nitrogen isotope ratio of the serum ( $p < 0.05$ ) but does not significantly change the isotope ratio of BSA, demonstrating that removing protein from plasma is effective at addressing the issues associated with lipid and urea content. Through protein precipitation,  $\delta^{15}\text{N}$  increases, indicating removal of  $^{14}\text{N}$  enriched urea and nitrogenous salts from the protein. Unexpectedly,  $\delta^{13}\text{C}$  decreases, indicating the removal of isotopically heavy carbon compounds, opposite to the expected increase in  $\delta^{13}\text{C}$  due to the removal of  $^{12}\text{C}$  enriched lipids. Our results demonstrate that

this approach is an easy way to process blood plasma for SIA while obtaining reliable SIA data, and provides a new approach for effectively using plasma to study ecological questions over short temporal scales.

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## Using FT-NIRS as a tool to age blue shark *Prionace glauca* from the northwest Atlantic Ocean

*Alexander Rubin, Michelle Passerotti, John Carlson*

Fourier transform near infrared spectroscopy (FT-NIRS) is an emerging method to estimate age of marine fishes. We assessed the efficacy of FT-NIRS to predict age and fork length (FL) of blue sharks, *Prionace glauca*, from laser scans of vertebrae collected in the northwest Atlantic Ocean. Spectral data from 188 vertebrae were collected via scanning with a FT-NIR spectrometer using two different sample presentations - whole centra placed rostro-caudal side down and half centra placed cut side down - to determine the best technique for enhancing signal to noise. Traditionally estimated ages and specimen FL (cm) were assigned to resulting spectra, and partial least squares (PLS) regression was used to create predictive models of age and FL based on spectral data. FT-NIRS models using whole centra estimated age to within 1.6 years ( $R^2 = 74.42$ , Root Mean Square Error (RMSE) = 1.63 years) of traditional ages, and estimated FL to within 15 cm ( $R^2 = 93.7$ , RMSE = 14.7 cm) of measured lengths. However, accuracy of FT-NIRS ages using whole centra declined after about age 8, at which point FT-NIRS predicted ages severely underestimated traditional age, likely due to centrum diameter exceeding the spectrometer window. Results from half centra are pending. Scanning time averaged 1.1 minutes per centrum, hence FT-NIRS ages can be produced in a fraction of the time required to generate traditional ages. However, sample presentation using whole centra may reduce accuracy of age predictions, potentially limiting the utility of this technique for larger-bodied sharks.

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## Response of the Western Fence Lizard (*Sceloporus occidentalis*) to Post-Fire Disturbances

*Stephanie Ruck, Breanna Putman*

Wildfire activity is growing globally with increased frequency, intensity, and duration. After a fire occurs, organisms across a range of taxonomic levels are impacted by altered habitats, including reduced resources and increased predation risk. Although there are studies assessing post-fire effects on organisms, these typically address population abundances and densities after a fire. To better understand how organisms are affected at the individual level after a wildfire, we collected male and female Western Fence Lizards (*Sceloporus occidentalis*) from unburned (> 100 years since last known burn), recently burned (< 1 year since last burn), and older burned habitats (< 2 years since last burn) within Southern California. We conducted Flight Initiation Distance (FID) trials to better understand how animals assess risk in fire-disturbed habitats. Habitat type did not significantly affect FID. However, lizards were significantly easier to spot higher up in burned habitats as well as at farther distances. Additionally, we collected morphological data and ectoparasite counts in order to assess the effects of habitat type on lizard health. We found that lizards from older burned habitats had significantly reduced body condition compared to lizards from unburned habitats. We also found that recently burned habitats had a higher proportion of lizards with less ticks compared to unburned and older burned habitats. Conducting post-fire studies at the individual organismal level can be used to inform conservation management decisions for species that are found in fire-prone areas across the globe.

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## Morphological Study of the Competitive Relationships between Native and Introduced Freshwater Fishes in Florida

*Khalil Russell, Peter Wainwright*

Studying invasion biology is crucial to our ability to effectively respond to the modern biodiversity crisis. Introduced species have the potential to not only impact the native species they encounter, but also the very characteristics of their invaded communities. This study utilized the relationship between the morphology of a species and its ecological position within a community to explore the theoretical competitive relationships between native and introduced freshwater fishes across Florida. We combined the position of each

species in a body shape morphospace with diet information to calculate competitive stress scores for each species with respect to its community membership. Compared to native species, we found that introduced species occupy more sparsely populated regions of morphospace and are therefore inferred to experience less-intense competition from other members of their community. However, this relationship varied across drainages and at different hydrologic scales and was stronger in generalized invertivores and piscivores than herbivores and molluscivores. There was also a geographic signature to the pattern. In the peninsular drainages, native members of a diet category tend to experience less intense competitive stress from other species; this relationship is reversed in the panhandle and North-Central drainages. This study establishes hypotheses about the competitive relationships between native and introduced species, as well as how these relationships vary across hydrologic scales, establishing a potential foundation for future work. It will be necessary to clarify the complex competitive relationships between native and introduced species and explore the effects of that competition on both native and introduced species.

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## Climate change impacts on sea turtles

*Vincent Saba*

In this presentation, I will review the large body of research on the impacts of contemporary and future climate change on sea turtle populations around the globe. Global climate or Earth system models must be used to assess future climate change impacts and I will first discuss how these models can be used for marine species research. Given that sea turtle sex determination is temperature-dependent, much of this work has focused on the impacts of warming temperatures at nesting beaches on sea turtle hatchling sex ratios. Most sea turtle populations are assumed to have a larger proportion of females but a warmer climate may skew population sex ratios to become even more female, potentially leading to negative impacts on recruitment and genetic diversity. However, other research suggests that the warming of nesting beaches will also lead to a reduction in nest success through changes in hatching success and that these impacts will have a greater impact on recruitment than changes in sex ratios. I will also discuss the impacts of warming oceans on sea turtle habitat and how some of these changes could expand habitat distribution poleward.

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## The impact of carbon nanoparticles on the growth, development, and telomere length of tadpoles

*Anne Sabol, Yiqun Zhou, Alessandro Catenazzi*

Carbon nanoparticles, or carbon dots, can have many beneficial uses. However, we must consider whether they may have any potential negative side effects on wildlife or the ecosystem when these particles end up in wastewater. Early-development stages of amphibians are particularly sensitive to contaminants, and exposure to carbon dots could disrupt their development and cause morbidity or death. Past studies have investigated short-term exposure to certain types of nanoparticles, but if these particles get into wastewater exposure may not be short term. Therefore, we tested whether exposure to different concentrations of carbon dots affects the growth, metamorphosis, and telomere length of Cuban tree frog (*Osteopilus septentrionalis*) tadpoles. To test this we exposed 12 groups of five tadpoles each to different concentrations of carbon dots and a control for three months and tracked survival, growth and metamorphosis. We used carbon nitride dots approximately 2 nm in size at concentrations of 0.01mg/ml and 0.02mg/ml. After three months, we measured telomere length from tissue samples. We found no difference in tadpole survivorship, growth, development rate, or telomere length among any of the groups, suggesting that carbon dots at these concentrations do not disrupt tadpole development.

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## Calling Phenology of High-Andean Anurans at the Edge of the Cryosphere

*Daniel Saenz, Kelsey Reider, Cory Adams, Lee Fitzgerald*

Increasing temperatures, changing precipitation patterns and hydrology alter prospects of persistence of species in high-elevation montane environments. In the Cordillera Vilcanota of southern Peru, some anuran species have

expanded their ranges into high-elevation habitats created by glacial recession. These sites are among the highest known to be occupied by amphibians on Earth. Occurrence at these elevations presents a rare opportunity to examine the breeding ecology of colonizing anuran species in the extreme conditions of the High Andes. In early December 2018 we initiated a long-term autonomous audio sampling program to record two species of calling anurans at breeding sites, ranging in elevation from 4,882m to 5,362m. Preliminary results indicate that marbled four-eyed frogs (*Pleurodema marmoratum*) are present at all our survey sites and we observed noticeable variation in the seasonal phenology of calling activity, as lower-elevation populations tended to initiate calling earlier and have a longer calling period than higher-elevation frogs. Marbled water frogs (*Telmatobius marmoratus*) were recorded calling underwater with an ARU hydrophone at only one site in our study, therefore we were unable to make inferences about the effects of elevation on calling phenology. Though results are preliminary, clear patterns of seasonal calling activity are emerging and appear to be related to elevation. As more data become available, we will explore interactions among temperature and hydrology with breeding activity of the advancing anuran species. Research on this system should reveal significant insight into the effects of climate change on biota at the absolute edge of the retreating cryosphere.

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## Temporary Noise-Induced Underwater Hearing Loss in Freshwater Turtles (*Trachemys scripta elegans* and *Chrysemys picta picta*)

*Andria K. Salas, Alyssa M. Capuano, Michele A. Sims, Craig A. Harms, Wendy E.D. Piniak, T. Aran Mooney*

Aquatic turtles use underwater sound for environmental awareness and communication. Increasing noise pollution in both marine and freshwater habitats may compromise detection of acoustic cues and signals by inducing temporary hearing loss in noise-exposed animals. Data on temporary hearing loss (or temporary threshold shifts; TTS) in reptiles is extremely limited (aerial or underwater), and there have been no data collected in Testudines. To address this knowledge gap, we exposed *Trachemys scripta elegans* (Red-eared slider; N=3) and *Chrysemys picta picta* (Eastern painted turtle; N=3) to underwater continuous broadband noise (50–1000 Hz) varying in amplitude and duration (5–30 min). We used auditory evoked potential methodology to measure underwater hearing thresholds after noise exposures, and compared these thresholds to those measured during control trials (ambient noise only). We observed temporary hearing loss in all individuals of both species at relatively low sound exposure levels, suggesting Testudines may be more noise sensitive than previously considered. After exposure to broadband noise, auditory thresholds in both species were elevated up to 40 dB re 1  $\mu$ Pa above baseline hearing at frequencies of highest auditory sensitivity (400 or 600 Hz). Threshold shift magnitude was frequency dependent, with higher TTS levels at more sensitive frequencies. Hearing sensitivity always recovered, either within the approximate hour of testing post exposure or by the next control trial two days later. These data suggest that even short duration noise exposures (e.g., 5 min) can impair turtle hearing, underscoring concern for acoustic habitat management and conservation of Testudine species.

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## Determining Relatedness of Juvenile Atlantic Striped Bass (*Morone saxatilis*) in Chesapeake Bay Using Microsatellite Markers.

*Amanda Salmoiraghi, Ellen Biesack, Jan McDowell*

This study used genotyping of 16 microsatellite loci to investigate relatedness among 362 young-of-year (YOY) striped bass sampled over a range of roughly 50-miles in the Rappahannock River, VA in 2016. Ranges of expected relatedness values for full-sibling, half-sibling and unrelated pairs were generated based on simulations using allele frequencies estimated from adult samples. Due to the limited number of loci used, strict boundaries between half-siblings and unrelated pairs could not be determined. In total, 5 full-sibling pairs were identified with high-confidence, and 562 putative half-sibling pairs were identified but with lower confidence. To further investigate relatedness patterns, estimates of average pairwise relatedness values were calculated and compared by partitioning YOY samples using three criteria: sampling month, fork length (FL), and capture location. For sampling month, there were

significantly higher levels of average relatedness among June samples than among August samples. For FL comparisons, larger FL (55mm-70mm) samples were significantly more related than smaller FL (20mm-35mm) samples. Finally, comparison of samples based on capture location found significantly higher average relatedness in pairs caught within the lower (<45 river miles from the mouth) river than within the upper river (>45 river miles from the mouth). Decreasing levels of average relatedness among YOY samples as the spawning season continued may be attributed to more spawners entering the river over time. Increasing the number of genetic markers and sampling other sub-estuaries could be used to better understand connectivity among sub-estuaries of Chesapeake Bay, enabling better management.

### Intraspecific Dietary Differences among Juvenile White Sharks (*Carcharodon carcharias*) in Southern California

*Yamilla Samara, Christopher G. Lowe*

In the Southern California Bight (SCB), juvenile white sharks (JWS) have been reported to form loose aggregations at coastal beaches (<500m from shoreline) but can also be found offshore (>4km from shoreline) where they are incidentally caught in commercial gillnet fisheries. JWS aggregating inshore and incidentally caught offshore, were tagged with acoustic transmitters to passively track their coastal movements. In addition, to describe the dietary composition of JWS, tissues samples (muscle & blood) from 50 nearshore tagged sharks and 24 offshore-caught sharks were collected for stable isotope analysis (SIA). Inshore and offshore isotopic prey baselines were built by collecting muscle samples from potential prey species found at nearshore nursery habitats, and by taking isotopes values from published peer-reviewed literature, from offshore prey. JWS sampled at different habitat types (nearshore vs. offshore) showed different  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (pseudo- $F=55.95$ ,  $P(\text{perm})=0.001$ ), and distinct isotopic niches. Bayesian mixing models revealed that nearshore sharks fed mainly on coastal prey (e.g., benthic elasmobranchs and inshore demersal fishes), while offshore sharks fed more on offshore food sources (e.g., small pelagic fish, squids). In addition, coastal movement data showed that nearshore sharks spend more time close to the shoreline than sharks caught offshore. Diet and movement data suggests two subpopulations of JWS with different behavioral patterns, which may also expose them to differential conservation risks.

### Global Network of Women in Ichthyology

*Paula Sánchez Duarte, Ángela Gutiérrez, Ana Clara Sampaio Franco, Vanessa Correa, Sandra Correa, Juliana Herrera, Andrea Polanco, Juliana M. Wingert, Daniela Nunez Rodriguez, Carla Pavanelli, Alicia Álvarez, Gladys Garrido, Nivia Sousa, Cacilda Rocha, Luisa Maria Sarmento-Souares, Luz Fernanda Jiménez S., Malu Araújo*

According to UNESCO, in 2019, only 28% of researchers were women receiving lower salaries and poorly represented in scientific academic spaces worldwide. This scenario is also reflected in ichthyology, where women comprise less than 50% and are underrepresented in scientific events and higher positions. Among the factors that have perpetuated the gender gap are the lack of role models, stereotypes about the intellectual capabilities of women, patriarchy, and misogyny. The creation of the Global Network of Women in Ichthyology started with a pilot project in Latin America that now has ichthyologists from 13 countries, ranging in ages from 21 to 65 and in different career stages.

The Global Network of Women in Ichthyology intends to create a collaborative and horizontal participation model to promote the generation of equitable and inclusive scientific spaces. The initiative also seeks to build networks of trust where female ichthyologists at different career stages can connect, and serve as role models through opportunities to sponsor, mentor, and coach others. In this talk, we will present survey data collected since launching the pilot project in Latin America early in 2023. The survey results reflect the necessity of promoting and consolidating initiatives like the Global Network of Women in Ichthyology. We will also discuss some of the most critical aspects that must be addressed for the transition into a more supportive, productive, and equitable field. Through the talk, we aim to generate a space for discussion and exchange of ideas on concrete actions necessary to foster inclusion and gender equity in ichthyology.

### Biotic and Abiotic Drivers of Phenotypic Variation in a Paedomorphic Salamander

*Jessica Sandoval, Thomas Anderson*

Phenotypic variation is ubiquitous among organisms and is thought to provide an evolutionary advantage for species living in variable environments. Paedomorphosis, or the retention of juvenile traits in sexually mature adults, is a model system for understanding the ecological causes of phenotypic variation. In the facultatively paedomorphic mole salamander (*Ambystoma talpoideum*), the species' complex life cycle can follow three pathways that ultimately lead to the expression of two discrete adult phenotypes. An individual may undergo traditional development from egg to aquatic larva but will (1) remain in the water as a reproductive, gilled adult (i.e., paedomorph), (2) metamorphose into a terrestrial adult, or (3) delay metamorphosis and overwinter in an immature state in ponds, eventually developing into either the terrestrial or aquatic phenotype the subsequent year. We examined the influence of biotic and abiotic variables on phenotypic variation by assessing how larval salamander densities, predator densities, and a suite of habitat characteristics impacted life history outcomes in mole salamanders. In May and December of 2013-2015 and 2022, we sampled twenty ponds in western Kentucky using minnow traps and dip-nets. Paedomorph and OWL occupancy and abundance were modeled while accounting for imperfect detection using the package unmarked. The top models for paedomorph occupancy and abundance included both larval (*Ambystoma maculatum* and *A. talpoideum*) and predator densities (*Anax* dragonflies and *Notophthalmus viridescens*). The top model for OWL occupancy included water depth, canopy cover, and dissolved oxygen and OWL abundance was best explained by larval salamander, newt, and larval dragonfly densities.

### Lack of country-wide systematic Herpetology Collections threatens future research and conservation

*Bruna S. Santos, Luis M. P. Ceríaco*

Natural History Collections (NHCs) represent the world's biggest repositories of long-term biodiversity datasets. Specimen collection and voucher deposition has been the backbone of NHCs since their inception, but recent decades have seen a drastic decline in their rate. Amphibians and reptiles are amongst the most threatened zoological groups on the planet and are historically underrepresented in most worldwide NHCs. As part of an ongoing project to review the Portuguese zoological collections in the country's NHCs, herpetological data from its three major museums and smaller collections was gathered and used to test the coverage and representation of the different taxa extant in Portugal. These collections are not taxonomically, geographically or temporally complete. Approximately 90% of the Portuguese herpetological taxa are extant in the assessed NHCs. The large majority are housed in the Museu Nacional de História Natural e da Ciência, in Lisbon, with half of the taxa represented by less than 50 specimens. Institutional instability prevented consistent collecting efforts and significant new entries. Geographically, the collections cover less than 30% of the country's territory and many regions are represented by a sole locality. The disconnect between researchers and museums needs to be repaired if we want to safeguard the existence and quality of non replicable data provided by NHCs. New technologies and approaches have brought them to the forefront of scientific research, but without new depositions of present data, how can we prepare for the challenges of the future and ensure species conservation?

### Museum Specimens Reveal Novel Lateral Line Pattern in Sciaenids

*Jonas Santos, Lucas Garcia, Marcelo Britto*

Highly specialized sensory systems are one of the most recognizable traits of Sciaenidae. A complete lateral line (LL), extending to the end of the caudal fin, occurs in most members of this fish family. To our knowledge, there is no known case of multiple LL in sciaenids. Here, we describe a novel pattern based on museum specimens. We examined and photographed a specimen of *Stellifer nasus* (Jordan, 1889) (MZFS 4466: Mucuri River, Bahia, Brazil; 123 mm - SL) and examined a specimen of *Stellifer microps* (Steindachner, 1864) (MZUSP 98657: Marajó island, Pará, Brazil; 48.32 mm - SL). They both present



a normal count of scales above and below the LL, and pored scales on the LL. However, we observed an additional line of pored scales below the first dorsal fin (on the left side), fourteen pored scales in the case of *S. naso*, and twenty-one in the case of *S. microps*. It was located right over the insertion of both depressor and erector dorsalis with the epaxial muscles. Additionally, we analyzed over 120 specimens from nearby or more distant locations in an attempt to sample this variation. We failed to find another specimen bearing a similar pattern. Most likely this resulted from mispression during the differentiation of scales and a rare mutation. Although we could not establish whether there is an innervated connection between likely hair cells and the brain, we stress the fact that this asymmetrical condition may cancel potential adaptive advantages to this mutation.

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## Reappraisal of *Stellifer punctatissimus* reveals trends in species complex misidentification

Jonas Santos, Ricardo Rosa, Telton Ramos

Well-studied species are only a small fraction of all species described. This falls into the Linnean shortfall, that is, an unsuitable level of basic taxonomic knowledge. It is a significant concern for cryptic species, which require integrated approaches to evaluate their diversity. Such shortage of knowledge also hinders species conservation efforts. In this study, we conducted morphological analyses using over 300 specimens of *Stellifer punctatissimus*, *S. gomezi*, and *S. menezesi* to reassess their taxonomic identity. We also evaluated the allometric and sexual components of morphology. Our results support the current morphological hypothesis of three species included in this complex. However, as cryptic species, they have overlapping traits. This is particularly evident in females and juveniles. *Stellifer punctatissimus* can be distinguished from *S. gomezi* and *S. menezesi* by its deeper body, eye diameter, nostril shape/position, and larger interorbital width. However, body depth varies greatly by sex and size and no longer can be used as the main diagnostic character for *S. punctatissimus*. This demonstrates a trend in allometry and sexual dimorphism, which explains past and current taxonomic errors in this complex. It suggests that taxonomical studies require better assessment of allometry and sexual dimorphism, especially for cryptic species. Differences in growth patterns suggest distinct habitat use and provide further evidence for our hypothesis. This also highlights that species could be facing different threats depending on their distinct habitat use. Overall, this study emphasizes the need for this type of research to fill taxonomic gaps.

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## Alkaloid-based Chemical Defenses and Diet in Six Species of Australian Poison Frogs in the genus *Pseudophryne*

Mikayla Sague, Vilma Dudaitis, Lilja Plumert, Kate D.L. Umbers, Ralph A. Saporito, J.P. Lawrence

Poison frogs are well known for their ability to sequester alkaloids from their diet of leaf-litter arthropods for use in defense against predators and pathogens. Australian frogs in the genus *Pseudophryne* represent an understudied lineage of poison frogs, which have the unique ability to both sequester dietary alkaloids and synthesize pseudophrynamine alkaloids. Herein, we describe the alkaloid profiles and diet of six species of *Pseudophryne* (*P. guentheri*, *P. occidentalis*, *P. coriacea*, *P. dendyi*, *P. semimarmorata*, and *P. bibronii*) to gain a better understanding of how alkaloid defenses and diet are related within and among species. We characterized and quantified alkaloids using Gas Chromatography-Mass Spectrometry (GC-MS), and assessed diet by way of dissection and examination of stomach contents using light microscopy. Our results found that alkaloid profiles varied significantly among species, with pumiliotoxins (dietary derived) and pseudophrynamines (synthesized) being the most abundant alkaloids. *P. dendyi* contained mostly dietary derived alkaloids, whereas *P. coriacea*, *P. guentheri*, and *P. occidentalis* possessed mostly synthesized alkaloids. *P. bibronii* and *P. semimarmorata* possessed a mixture of sequestered and synthesized alkaloids. We dissected the stomachs of same individuals used in the alkaloid analyses to observe their arthropod diet. The stomachs were largely empty, containing mostly soil and few partially digested insects. This indicates that the male frogs may reduce consumption of arthropods during the breeding season. Our data suggest

that the decrease in dietary alkaloids is therefore compensated by the biosynthesized pseudophrynamines, which allows these poison frogs to remain defended during the breeding season.

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## Nesting Ecology of the Rio Grande Cooter (*Pseudemys gorzugi*) in Southeast New Mexico

Ana Grace Sapp, Sierra Shoemaker, Thanchira Suriyamongkol, Ivana Mali

The Rio Grande Cooter (*Pseudemys gorzugi*) is a medium size freshwater turtle native to the lower Rio Grande River basin. The Rio Grande Cooter recently underwent a Species Status Assessment for potential federal protection under the Endangered Species Act, but in 2022 the decision was made not to list the species. Nonetheless, *P. gorzugi* is still considered state threatened in New Mexico and a species of conservation concern in Texas. Historically, this has been one of the least studied freshwater turtle species in the United States and their life history is still poorly understood. In this study, we aimed to characterize the nesting seasonality, nesting habitat, and incubation period of *P. gorzugi* on the Black River in New Mexico. To locate the nests, we used a combination of pedestrian surveys, game camera surveys, and GPS tracking from 2020 to 2022. Over this three-year period, we found 47 nest remnants (predated or hatched eggshells) and five intact nests. The nesting season began in mid-June, and turtles constructed their nests one to thirteen meters from the water's edge. Of five intact nests, three suffered egg mortality, while two successfully hatched with an incubation period of 78 and 91 days. Overall, this is the first study aimed to specifically assess the nesting ecology and nesting habitat of the Rio Grande Cooter. Knowing the timing of nesting and identifying nesting grounds can guide future conservation efforts (e.g., nesting habitat protection) for this state-threatened species.

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## Use of Coelomic Fluid as a Noninvasive Indicator of Reproductive Status in Elasmobranchs

Emily Sapp, Jim Gelsleichter

Information on reproduction in elasmobranchs is important for the management and conservation of populations. There has been progress in the field of elasmobranch reproduction; however, our ability to obtain reliable data without animal euthanasia is still extremely limited. Therefore, there has been a call for nonlethal approaches for identifying reproductive status, such as ultrasonography and blood analyses. Although useful, these techniques can be limited with respect to access to equipment and reliability of blood analyses. This study will investigate whether a new noninvasive approach, examination of coelomic fluid (CF), can provide a reliable indicator of reproductive status. CF is fluid that bathes the internal organs of the abdominal cavity, including ovaries, and can be sampled noninvasively through catheter. Recent studies on CF have demonstrated the presence of yolk platelets in the fluid, an indicator of follicular development. This suggests that CF may provide a more detailed representation of reproductive state, than other nonlethal approaches, such as plasma hormone levels. We are examining CF collected from Atlantic stingrays (*Hypanus sabinus*) and bonnethead sharks (*Sphyrna tiburo*) which serve as model elasmobranchs for this work due to their well-described pattern of reproduction, abundance, and easily accessible coelomic pores. In this presentation, we report on preliminary findings regarding differences in CF content in relation to reproductive stage in these species, as well as variations in gonadal steroid concentrations in CF and those in plasma.

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## What inner ear anatomy can tell us about hearing in elasmobranchs

Derek Sauer

Fishes possess the largest diversity in auditory anatomy among any vertebrate group. However, data come largely from bony fishes, while comparatively little is known about inner ear morphological variation in elasmobranchs (sharks, rays, and skates). Elasmobranchs are thought to exhibit high interspecific variation in inner ear morphology, although comparative assessment across a range of species is rare. In the present study, we used magnetic resonance imaging (MRI) to characterize the inner ears of elasmobranchs, and analyzed morphological variation in the inner ears of 26 species, including nine batoids

and 17 sharks. Inner ears were imaged in situ on either a 9.4T Bruker Biospec small animal scanner or a Siemens Magnetom 7T whole body MR scanner, using contrast-enhanced, T1-weighted MR imaging. The inner ear end organs (sacculle, lagena, utricle, macula neglecta), semi-circular canals (horizontal, anterior, and posterior), and endolymphatic duct were segmented from MR data. Using phylogenetically informed multivariate analyses, we assessed inner ear structure as it relates to body size, diet, and primary habitat, and found that variation in inner ear organization correlates with ecological parameters, and may reflect differences in the hearing capabilities of elasmobranchs. Findings establish a morphological foundation for comparative studies on the inner ears of elasmobranchs, and suggest that, similar to other sensory systems in elasmobranchs, variation in morphology of the auditory system may reflect differences in auditory specialization.

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## Observations on the Soft Tissues and Vasculature of Sea Snakes (Hydrophiinae)

*Alan Savitzky, Helen Bond Plylar, Takumi Yamamoto, Mamoru Toda*

The elapid subfamily Hydrophiinae includes two lineages of marine snakes, the Laticaudini and the Hydrophiini. Both are well-studied with respect to their ecology and physiology, and recently a number of detailed morphological studies have also appeared. We examined several species, belonging to both lineages, that occur in the waters of Okinawa, Japan, using microCT augmented with Lugol's stain (for soft tissues) and Microfil (for vascular casting), in addition to dissection. Our observations confirm many published details of sea snake morphology and extend those studies in several respects. In particular, casting with Microfil, a radio-opaque latex compound, reveals much greater detail of the vasculature, including extensive cutaneous vascular networks and details of the pulmonary vessels. Staining of soft tissues reveals complex modifications of cardiac valves. We interpret some of these morphological features of sea snakes as likely adaptations to cutaneous gas exchange, resistance to hydrostatic pressure, or the control of buoyancy. Our studies suggest the benefits of using several anatomical methods to obtain a more comprehensive picture of the snakes' morphology, while also revealing some limitations to the use of vascular casting with Microfil.

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## Is Intranasal GnRH Administration an Effective Alternative Method for Hormone Delivery to Stimulate Ovulation and Oviposition in Female Anurans?

*Erin Saylor, Andy Kouba, Carrie Kouba*

Hormone therapy regimens to induce natural breeding is a common practice for many anuran conservation breeding programs and facilitates gamete collection from captive or wild individuals for research, biobanking, and in vitro fertilization. Hormones are often administered via intraperitoneal or muscular injection. While non-invasive methods such as intranasal administration have been explored in male anurans, this alternative delivery route has not been explored in female anurans and may provide a more effective, less costly, and simpler technique for hormone delivery. Here, we attempt to induce final follicular maturation, ovulation, and oviposition in female Fowler's toads (*Anaxyrus fowleri*) (n=6) with intranasal administration of a gonadotropin releasing hormone analog (GnRHa) at a dosage of 0.4ug/g body weight. The GnRHa was administered in a volume of 30 µl, with half the volume provided to each nare via a micropipette. Ultrasonography was used to track individual follicular maturation over time between 0- and 96-hours post-hormone administration. Our results showed that intranasal administration of GnRHa to female Fowler's toads did not stimulate follicular maturation, ovulation, or oviposition. Considering intranasal administration of GnRHa has been proven to elicit spermiation in male *A. fowleri*, this study highlights the importance of considering sex-specific differences in response to hormone therapy regimens when developing alternative non-invasive routes of delivery.

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## Molecular evolution of visual opsins across anurans: duplication, loss, and adaptation to distinct light environments

*Ryan Schott, Matthew Fujita, David Gower, Jeffrey Streicher, Kate Thomas, Leah Perez, Matthew Kwiatkowski, Vance Imhoff, Jennifer Gumm, Rayna Bell*

Visual systems can adapt to different light environments through several avenues including optical changes to the eye and neurological changes that modify how light signals are processed and interpreted. At the molecular level, spectral sensitivity can evolve through changes to the light-sensitive visual pigments housed in the retinal photoreceptors through several, often complementary, mechanisms including gene loss and duplication, differential and co-expression, and sequence evolution. Frogs provide an excellent system for studies of visual evolution due to the convergent evolution of similar activity patterns, lifestyles, and behaviors that are likely to influence the evolution of visual function. Here, we have sequenced eye transcriptomes from 82 species and obtained additional sequences from available genomes to produce a dataset of 101 species spanning 34 families. Using this dataset, we analyzed the evolution of visual opsin genes, which encode the protein component of visual pigments. We found that most species express the four visual opsins previously identified in anurans, but find evidence for independent gene loss in two lineages, and gene duplication in one species. We identify new, potentially functional, variation in the opsins that suggests frogs may have unique spectral tuning pathways not used by other vertebrates. Further, we found evidence of positive selection in three of the opsins and significant shifts in selective pressures associated with differences in habitat and life history, but not activity pattern. Overall, these results suggest the potential for adaptive evolution across the frog tree of life and further expand our growing understanding of frog visual evolution.

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## Ultraviolet Light and Body Temperature Regulation Trade-Offs in Tree Lizards along an Elevation Gradient

*Andreanna Schultz, Matthew Lattanzio*

Ectotherms rely on regulatory behaviors, such as basking and shuttling, to maintain physiological homeostasis in the face of variable external conditions. Historically, this process has long been understood mainly through the lens of body temperature regulation alone. However, other solar outputs, such as ultraviolet (UV) light, drive vitamin D synthesis, which is critical for organ function, bone development, and reproductive fitness, to name a few. Recent work in our lab relying on Arduino electronics has demonstrated lizards behaviorally regulate their exposure to UV and can prioritize UV over thermal needs. However, the extent regulation effectiveness varies among populations, as well as any trade-offs between UV and thermal needs, remains unknown. Here, we combine field surveys and laboratory behavioral data to evaluate the extent that tree lizards (*Urosaurus ornatus*) effectively regulate their UV exposure and/or body temperature across an elevation gradient. Currently, we are assembling data loggers and expect data collection to be completed by June 2023. Based on known temperature and UV relationships with elevation, as well as preliminary findings on *U. ornatus*, lizards should overall prefer low UV exposures (~1.5 UVI) and moderately high temperatures (~36 °C). Thus, because of the divergent but extreme UV and thermal conditions at low and high elevations, we predict that lizards at mid-elevation localities will be the most effective at regulating both temperature and UV. Our findings should provide novel insight into how a greatly underappreciated but critical physiological process (UV regulation), and its trade-offs with thermal needs, manifest within and among populations.

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## At-vessel and Post-release Mortality of Shortfin Mako Sharks in the US Atlantic Pelagic Longline Fishery

*Mischa Schultz, Eric R. Hoffmayer, James A. Sulikowski, Michael Byrne*

To help address steep declines of shortfin mako sharks (*Isurus oxyrinchus*) in the North Atlantic Ocean, retention bans have been instituted to reduce fishing mortality and aid population recovery. However, recovery may be slowed by high at-vessel mortality (AVM), or post-release mortality (PRM) of mako sharks caught as bycatch. We used 20 years (2000 – 2020) of fisheries

observer data to estimate AVM, and pop-up archival satellite transmitting tags (PSATs) deployed during commercial fishing activities in 2022 - 2023 to estimate PRM in the US Atlantic pelagic longline fishery. At-vessel mortality rate was 0.27 (95% CI: 0.25 – 0.29) in the Western North Atlantic (WNA), and 0.32 (95% CI: 0.28 – 0.36) in the Gulf of Mexico (GOM). Soak time, hook depth, and sea surface temperature (SST) were important in the WNA, whereas hook depth and shark size were important in the GOM. Preliminary estimated PRM rate based on tags deployed to date ( $n = 24$ ) is 0.10 (95% CI: 0.02 - 0.32); lower than other recent estimates that combine data across fleets, however data collection is still ongoing. Combining AVM and PRM in a Monte Carlo simulation, estimated survival probability of a mako shark hooked in a pelagic longline fishery is 0.65 (95% CI: 0.56 – 0.74) and 0.61 (95% CI: 0.51 – 0.70) in the WNA and GOM, respectively. Given retention bans, AVM currently represents the greatest source of fishing mortality for mako sharks that interact with the US longline fishery, and future management strategies focused on bycatch reduction may be most effective in stock recovery.

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## Evaluating Long-Term Shifts in Diet in Harvested Chesapeake Bay Fishes

*Matthew Schumm, Katherine Bemis, Dan Okamoto, Lynne Parenti*

Size-specific diet and whether it varies across time and space can inform conservation of focal populations and of co-occurring species. Museum collections are an archive of specimens from fish populations that often predate fisheries-independent scientific surveys of such populations. We sampled tissues from museum collections to assess stable isotope ratios of nitrogen as a proxy for trophic variation and to determine how isotope enrichment changes with time or tracks other environmental covariates in fishes of the Chesapeake Bay. Comparing three high-trophic-level species (*Morone saxatilis*, *Pomatomus saltatrix*, and *Paralichthys dentatus*) and two lower-trophic-level species (*Anchoa mitchilli* and *Brevoortia tyrannus*) allowed us to distinguish if isotopic patterns applied across trophic levels and are therefore caused by variation at the base of food webs, or if isotopic trends differed in higher-trophic-level fishes, thus indicating spatiotemporal change in trophic level of these predators. We found that nitrogen-15 trends are similar across species, indicating change in the base of the food web consistent with more anthropogenic nitrogen input closer to present and nearer to dense human settlement, with evidence of a change in trophic level of *M. saxatilis*, which we will further examine with compound-specific isotope analyses. We also conducted an experiment estimating effects of collection preservatives on isotopes, X-rayed all predator specimens to confirm that small fishes, such as *A. mitchilli*, were important prey items for these individuals in their lives.

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## Environmental DNA Assessment for Freshwater Fishes in the Guayas River Basin using New 12s, 16s, and COI Metabarcoding Reference Library

*Olivia Schweikart, Sophie Picq, Hannah Willis, Mario Landa, Katlin Jacoby, Paola Calle, Caleb McMahan, Windsor Aguirre*

As a result of rising sea levels, economically and ecologically vital species are threatened by coastal incursion events across the Guayas River Basin, Western Ecuador's lowest-elevation flood plain. This study provides a new genetic reference library at three metabarcoding loci: 12s, 16s, and COI, for neotropical freshwater fish surveys within Western Ecuador. When used in combination, the chosen loci minimize loss in species detection by increasing potential for genetic capture. This genetic reference library was tested in an environmental DNA survey of five taxonomic fish species (*Brachyhyopomus palenque*, *Ichthyoelephas humeralis*, *Hoplias microlepis*, *Leporinus equadoriensis*, *Mesoheros festae*) using water samples from three rivers in the Guayas River Basin (el Chimbo, la Taura, el Milagro). Adjusted primer-bias in the data allowed for target-species detection, as well as estimation of population abundance throughout sampling locations. The Oxford MinION, a nanopore sequencing technology, was used to process amplified environmental DNA. The results of this study, coupled with the genetic reference library, make a leap toward closing taxonomic knowledge gaps at popular metabarcoding loci. This provides accessible data and bioinformatic pathways to

aid in governmental conservation-workshop practices, integrating minimally-invasive data collection, cost-effective sequencing solutions, and swift and processing methods.

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## Characterizing the ecological role of sharks in Delaware Bay, USA

*Devon Scott, Edward Hale, Dewayne Fox, Camilla McCandless, Suni Shah-Walter, Aaron Carlisle*

As upper trophic level predators, Sandbar (*Carcharhinus plumbeus*) and Sand Tiger (*Carcharias taurus*) Sharks play an important role in the Delaware Bay ecosystem, impacting food web structure and function directly through feeding and indirectly through changing the behaviors of potential prey. These ecological effects will influence the abundance and fitness of managed species with economic implications for commercial and recreational fisheries, many of which use Delaware Bay as a nursery area. Currently little is known about the diet and ecological role of these predators in the Delaware Bay, but information is needed as populations of both species are recovering after being historically depleted due to overfishing. To address this lack of information, and better facilitate ecosystem based management by the state, we used stable isotope analysis of multiple tissues (plasma, red blood cells, muscle) with varying turnover rates to characterize the trophic ecology of these species across size classes. We used Bayesian isotope mixing models to estimate relative importance of different prey groups and coupled these estimates with bioenergetic models to quantify daily consumption of prey species or groups. We characterized major prey groups (which included managed species such as Blue crab, *Callinectes sapidus*, Weakfish, *Cynoscion regalis*, and Menhaden, *Brevoortia tyrannus*) and identified clear niche partitioning between species and ontogenetic stages in the Delaware Bay. This information enhances our understanding of the ecological role of sharks in Delaware Bay and will be used to inform the management of marine resources of the Mid-Atlantic Bight as shark populations recover from historic overfishing.

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## Investigating the impacts of stress and Bd on Raleigh, North Carolina amphibian populations

*Megan Serr, Kara Solomon, Sophia Bogan*

Stress is an important factor in overall amphibian health as it impacts infection risk with the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Pathogenesis with Bd is negatively impacting amphibian populations around the world and in North Carolina. For this study, we have nine field sites including Wake County Parks and the Meredith College campus in Raleigh, North Carolina. At each location, we are dermally swabbing individual amphibians for Corticosterone (CORT), and Bd infection. We also record biometric data – such as weight and snout-to-vent-length – as well as the location where each individual was captured. CORT is used as a correlate of stress, and we will analyze it using Enzyme-Linked Immunosorbent Assays (ELISA). Elevated CORT concentrations have been found to suppress amphibian immune systems and thus increase Bd infection susceptibility. At this time, we are refining CORT analysis by performing practice ELISAs to see how we can best quantify CORT from dermal swabs. We will also test to see whether the individuals we swabbed are infected with Bd. Correlating CORT stress levels against Bd is critical for overall amphibian survival. Measuring CORT for Wake County amphibian populations is an important first step to understanding the overall health of local amphibians.

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## The Effects of Wear on the Morphology and Puncture Force of Shark Teeth

*Ryan L. Sesler, Lisa B. Whitenack*

Sharks frequently replace their teeth throughout their lives from once per week to roughly every three months. Past studies on the biomechanics of shark teeth indicate that teeth are structurally and mechanically strong, indicating that frequent tooth replacement is not due to tooth breakage. Instead, it has been hypothesized that teeth are replaced due to wear and its effects on tooth function. Teeth from five species of shark (*Carcharhinus limbatus*, *C. leucas*, *C. plumbeus*, *Sphyrna mokkaran*, and *I. oxyrinchus*) were

subjected to 400 punctures each in teleost prey; tooth wear and puncture force were measured throughout the series of punctures. Measurable tooth wear happens within 30 punctures, although the rate of wear differs among species. Puncture forces steadily increase as the teeth wear, with significant differences in puncture force. These results together indicate that tooth wear may affect the ability of teeth to function efficiently. However, data on tooth replacement rates or how many uses each tooth undergoes before it is shed do not exist for the vast majority of shark species. Expanding this study to include lateral head shaking would also be helpful for understanding the link between wear, tooth performance, and tooth replacement.

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### **Establishment of Aquatic Caecilians (Gymnophiona, Typhlonectidae, *Typhlonectes natans*) in Florida, with Notes on their Relative Abundance, Distribution, and Natural History**

*Coleman Sheehy III, Robert Robins, David Blackburn, Jaimi Gray, Marcel Kouete, Magalie Zoungana*

The state of Florida contains more established species of non-native reptiles and amphibians than any other region worldwide. In 2021, the first occurrence of an aquatic caecilian in the Tamiami (C-4) Canal was documented and identified as *Typhlonectes natans*. However, it was not clear at the time whether this species was established. We subsequently trapped for caecilians at nine different sites along the C-4 Canal between 2021 and 2022. These sites are located north, south, and southwest of the original 2021 location along connected branches of the main C-4 Canal. We captured 93 caecilians and made additional visual observations at three of the sites. Caecilians of both sexes and all age classes were collected. Thirty-three preserved specimens were X-rayed to check for pregnancy and stomach contents and nine specimens were CT-scanned, showing three specimens containing late-stage fetuses and one specimen containing stomach contents identified as fish bones. We screened 78 specimens for the presence of the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd). However, we did not detect Bd on any specimens. Preliminary results suggest that *T. natans* is well established and relatively abundant in some branches of the C-4 Canal. Their diet appears to consist primarily of soft-bodied prey and scavenged material. The caecilians do not appear to be a reservoir for transmission of Bd, though further testing is required. Efforts are needed to assess the viability of eradication and to understand how the establishment of aquatic caecilians might affect aquatic ecosystems in south Florida.

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### **ddRADSeq bridges Population Genetics and Deep Phylogeny in an Ancient Amphibian Radiation.**

*Miranda Sherlock, Jeffrey Streicher, David Gower, Simon Maddock, Ronald Nussbaum, Oommen V Oommen, Ana Serra-Silva, Julia Day, Mark Wilkinson*

Unusually for oceanic islands, the granitic islands of the Seychelles host multiple lineages of endemic amphibians. This includes a clade of eight species of caecilians, most of which occur on several islands. Historic fluctuations in sea-level periodically connected and isolated the islands; the most recent connections likely occurred in the last 10ka. The islands vary in size, proximity and the species they host. A resolved phylogeny of Seychelles caecilians would allow a clearer understanding of how historical patterns of vicariance and gene-flow shaped their current biodiversity. Resolving the phylogeny of Seychelles caecilians has been attempted with several nuclear genes and mitochondrial genomes, but with limited success, yielding conflicting topologies and some branches with (very) low support. To address this deficit, and interrogate population dynamics, we obtained ddRADseq data from all species in the radiation, and additionally focused intraspecific sampling on the most widely distributed species, *Hypogeophis rostratus*. ddRADseq phylogenomics has successfully resolved recent divergences of various organismal groups, but is applied less frequently to older relationships. Using ddRADseq data we obtained a fully resolved species-level tree with high branch support, and suggest that previous difficulties are partly explained by mitonuclear discordance. Although our results support previous findings that identified a broad north-south inter-island divide in *H. rostratus*, we also detected much finer-scale inter-island diversity and intra-island divergence on

the largest island (Mahé). Our results corroborate the view that ddRADseq data are highly versatile and that sampling from many loci across the genome is informative at many levels of evolutionary relatedness.

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### **This research is important for the conservation and management of sharks: A framework for making that actually true**

*David Shiffman*

Many chondrichthyan scientists report that they want to perform conservation-relevant work, but that they do not know how to effectively do this. Here we present advice in the form of survey responses from natural resources managers, environmental advocates, and scientists with experience performing policy-relevant work. We also present a novel, simple checklist for determining if a given research project is indeed relevant to the conservation and management of sharks.

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### **A Review of the Biology, Ecology, and Conservation of the Sandbar Shark (*Carcharhinus plumbeus*)**

*David Shiffman, Amani Webber-Schultz, Toby Daly-Engel, Dean Grubbs, Lara Ferry*

The sandbar shark (*Carcharhinus plumbeus*) is one of the best-studied species of chondrichthyan fishes, and as the subject of at least 855 papers, has contributed significantly to scientific understanding of coastal ecology, sensory biology, habitat usage, and life history, among many other disciplines. They have recently been assessed as Endangered by the IUCN Red List, and recently were listed under CITES Appendix II, but population recoveries have been reported in parts of their range. Here we present the first multi-disciplinary, systematic review of the biology, ecology, and conservation of the sandbar shark. They are the focus of the US research fishery, a unique partnership between industry, management, and academic research that could serve as a model for data gathering elsewhere. They are one of the most-common species kept in captivity and have been found in the coastal waters of at least 119 countries across all major oceans and the Mediterranean and Black seas. As generalist predators, they have been shown to consume at least 53 species of teleost fishes across 50 families, as well as at least 20 species of crustaceans, as well as smaller chondrichthyan fishes, and marine mammals. They are one of the most-commonly caught shark species by recreational anglers in the United States, with nearly 36,000 tagged animals in the National Marine Fisheries Service cooperative angler database—and they are the subject of illegal fishing and growing controversies surrounding depredation. We also make recommendations for future studies.

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### **Energetic Connectivity of Diverse Elasmobranch Populations – Implications for Ecological Resilience**

*Oliver Shipley*

Understanding the factors shaping patterns of ecological resilience is critical for mitigating the loss of global biodiversity. Throughout aquatic environments, highly mobile predators are thought to serve as important vectors of energy between ecosystems thereby promoting stability and resilience. However, the role these predators play in connecting food webs and promoting energy flow remains poorly understood in most contexts. Using carbon and nitrogen isotopes, we quantified the use of several prey resource pools (small oceanic forage, large oceanic prey, coral reef, and seagrass) by 17 species of elasmobranch fishes ( $n = 351$  individuals) in The Bahamas to determine their functional diversity and roles as ecosystem links. We observed remarkable functional diversity across species and identified four major groups responsible for connecting discrete regions of the seascape. Elasmobranchs were responsible for promoting energetic connectivity between neritic, oceanic, and deep-sea ecosystems. Our findings illustrate how mobile predators promote ecosystem connectivity, underscoring their functional significance and role in supporting ecological resilience. More broadly, strong predator conservation efforts in developing island nations, such as The Bahamas, are likely to yield ecological benefits that enhance the resilience of marine ecosystems to combat imminent threats such as habitat degradation and climate change.



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## Effects of Flash Drought during Incubation on Common Snapping Turtle (*Chelydra serpentina*) Phenotype and Fitness

Ayley Shortridge, Fredric Janzen

The frequency and intensity of flash drought are increasing across the central United States, with peak occurrence in the summer months. The associated decrease in soil moisture could have broad ecological impacts. Many turtle species, including common snapping turtles (*Chelydra serpentina*), have flexible-shelled eggs that are highly sensitive to hydric conditions in the nest. Moreover, the high rate of predation on hatchling turtles means that phenotypic differences at this early life stage are particularly important. Here, we present the results of an experimental study on the effects of flash drought on eggs and hatchlings of *C. serpentina*. First, we manipulated soil water potential during egg incubation in the laboratory. Second, we conducted an experimental release in the field to evaluate hatchling survivorship during dispersal from the nest. As predicted, we found that eggs decreased in mass during flash drought events. We also observed significantly shorter incubation times and decreased mass in hatchlings that experienced flash drought during mid-to late incubation. Finally, flash drought during late incubation was associated with higher hatchling mortality during dispersal from the nest. These results suggest that turtle populations may be significantly impacted by increased frequency of flash drought.

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## Effects of dietary lipid level and environmental temperature on lipid metabolism and choline requirement in Atlantic salmon parr

Daphne Siciliani

Choline was recently established as an essential nutrient for Atlantic salmon at all life stages. Choline deficiency is manifested as an excessive accumulation of dietary fat within the intestinal enterocytes, a condition known as steatosis. Most of today's plant-based salmon feeds will be choline deficient unless choline is supplemented. Choline's role in lipid transport suggests that choline requirement may depend on factors such as dietary lipid level and environmental temperature. This study was therefore conducted to investigate whether lipid level and water temperature can affect steatosis symptoms, and thereby choline requirement in Atlantic salmon. Four choline deficient plant-based diets were formulated differing in lipid level of 16, 20, 25 and 28% and fed to salmon of 25g initial weight in duplicate tanks per diet at two different environmental temperatures: 8 and 15°. After 8 weeks of feeding, samples of blood, tissue and gut content from six fish per tank were collected, for analyses of histomorphological, biochemical and molecular biomarkers of steatosis and choline requirement. Increasing lipid level did not affect growth rate but increased relative weight and lipid content of the pyloric caeca and histological symptoms of intestinal steatosis, and decreased fish yield. Elevation of the water temperature from 8 to 15°, increased growth rate, relative weight of the pyloric caeca, and the histological symptoms of steatosis seemed to become more severe. We conclude that dietary lipid level, as well as environmental temperature affect choline requirement to a magnitude of importance for fish biology and health, and for fish yield.

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## Bioaccumulation of Imidacloprid in Hemlock Leaf Litter in the Hocking Hills of Southeast Ohio

Courtney Silver-Peavey, Ola Bataineh

Pesticides pose a significant threat to amphibians, particularly during their embryonic and larval stages. Imidacloprid, a systemic neonicotinoid pesticide, is commonly used in agriculture, but also to fight against the spread of a forest pest, the Hemlock Woolly Adelgid. Through the application of this pesticide, Imidacloprid enters streams and ponds through runoff water, causing sublethal effects on amphibians. Most notable in my research is Imidacloprid's effects on increased body size and time to metamorphosis, as well as increased predator avoidance activity seen in mid-Gosner stage (25-28) wood frog tadpoles. These phenomena are well studied, however, there is little information on the accumulation of this pesticide, which can persist in organic matter and soils for a year or more post-application, and the breakdown and possible release from leaf litter surrounding the application sites. This is important because the

widespread application of Imidacloprid in forests has the potential to affect forest and freshwater biota for many years post-application. For this study, leaf litter and soil samples consisting of the organic layers of soil (Oi to Oa horizons) in Hemlock stands treated with Imidacloprid in both the Cantwell Cliff and Cedar Falls/Old Man's Creek areas within the Hocking Hills of Southeast Ohio were collected. Quantification of Imidacloprid in the leaf litter of these Hemlock stands is underway using coupled liquid chromatography-mass spectrometry (HPLC-MS). Preliminary results suggest Imidacloprid does accumulate in the leaf litter going back to our 2013 samples with large variations of persisting quantities varying by site.

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## Australia's Shark and Ray Report Card: a unique national approach to sustainability assessment that informs decision making

Colin Simpfendorfer, Cassandra Rigby

With sharks and rays under growing pressure from fishing developing processes that enable managers and policy makers to make informed decisions is critical to recovery of depleted populations. Such processes are mostly focused on target species, often neglecting the many species that are caught incidentally but which are none-the-less effected. To address this deficiency Australia has developed the Shark and Ray Report Card, a sustainability assessment for all 331 species of sharks rays and chimaeras occurring in its waters. The Report Card process relies on stock assessments, national Red List Assessments, expert knowledge and a national sustainability framework. An approach that allowed equivalency between Red List Assessments and the national sustainability framework was developed to assess the status of species for which stock assessments were not available. Since few species have stock assessments this approach allowed the sustainability of hundreds of species, which would normally be considered data limited, to be assessed. All assessment outcomes were vetted by expert workshops to ensure consistent and peer-reviewed results. The draft results of the first full Report Card released in 2023 found 14% of stocks rarely if ever encountered fisheries, 68% we sustainable, 3% recovering (ie previously overfished but overfishing not occurring), 4% depleting (ie overfishing occurring but not overfished), 6% depleted (overfished) and 5% had insufficient information to determine their status. The outputs of this work are being used to inform ecological risk assessments, fisheries management plans, CITES non-detriment findings and threatened species assessments.

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## Do You See What I See? Cryptic snakes and the accuracy of citizen scientists

Ryan Singer, Emily Taylor, Scott Boback, Eddie Tu, Brandon Kong

Citizen science is an effective way of providing opportunities for the public to engage in authentic scientific research by assisting scientists in collecting and analyzing large datasets. We created Project RattleCam, the first snake-based project on the citizen science platform Zooniverse, which asked users to annotate time-lapse images of rattlesnake rookeries (communal nesting sites). Prairie Rattlesnakes (*Crotalus viridis*) pose a particular challenge for a community science project because analyzing images of cryptic animals can be challenging. Therefore, we aimed to determine how accurate Zooniverse users are at identifying Prairie Rattlesnake presence, abundance, and behaviors from time-lapse images. Each photograph was presented to multiple users along with a series of annotative tasks. Citizen scientists completed a total of 375,618 annotations for the 60,355 photos in the image set. We utilized agreement indices to assess users' annotations of the various tasks. We found that users were able to determine whether snakes were present or absent with a high level of agreement. However, disagreement increased when determining how many adult or baby snakes were present. We also found that counting baby rattlesnakes incited more disagreement than counting adults. Users disagreed most when asked to identify behaviors such as rain harvesting. Our findings can help to inform the evaluation and implementation of future community science.

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## Re-Evaluating Species Richness of an Ancient Fish Lineage (Lepisosteidae)

*Daniel Sinopoli, Prosanta Chakrabarty, Yahya Terzi, Rafet Ozturk, Dr. Solomon David*

Spotted Gar (*Lepisosteus oculatus*, Winchell 1864) is widespread with a disjunct population in the Great Lakes Basin found from Michigan south to Louisiana, Texas, and the western Florida panhandle. The primary area of its range comprises the Mississippi River Basin and neighboring drainages. A disjunct northern and southern population sets *L. oculatus* apart from other gar species which have more contiguous ranges. Despite their external appearances being little changed since the Jurassic, there appears to be more diversity within gars than once expected. Spotted Gar fossils have been recovered in strata dating back to the upper Miocene/lower Pliocene (~5 mya). Here we compared differences in body shape using traditional and geometric morphometrics of northern and southern populations of Spotted Gar. We also examined genetic differences at both deep and shallow time scales to update the relevant taxonomy of this species.

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## Impact of Urbanization on the Escape Behavior and Ecomorphology of Tree Lizards

*Ke'alani Sison, Matthew Lattanzio*

Understanding the impacts of urbanization on wildlife ecology and evolution has become increasingly important as human population growth continues around the world. In general, the ecological and evolutionary impacts of urbanization on wildlife have mainly been addressed via assessments of spatial variation in antipredator responses between urbanized and natural localities. However, the degree that concomitant shifts in other underlying physiological traits linked to the escape response (and thus important for fitness) occur remains unclear. Here, we assess the extent that shifts in escape behavior of adult ornate tree lizards (*Urosaurus ornatus*) along a gradient of urbanization corresponds to concomitant shifts in morphology (body size, limb lengths) and performance capacity (sprint speed). We have completed preliminary analyses at this point and will collect the main data for this study May-June. Based on our preliminary data and prior studies, we expect that flight initiation distance (distance between approaching researcher and lizard at the initiation of escape) will be shorter for lizards in urban localities due to habituation to human presence. We further expect lizards that flee sooner to exhibit longer limbs and faster sprint speeds. Overall, our findings should provide new insight into the extent of ecophysiological impacts of urbanization on *U. ornatus*, as well as lizards in general. Our study should also be useful for other researchers seeking to understand how human presence impacts patterns of trait integration within a species.

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## The Limits and Relationships of the Deep-water Basses (Percomorpha: Acropomatiformes)

*Leo Smith, Michael Ghedotti, Matthew Davis*

During the last 35 years, ichthyologists have made tremendous improvements in our understanding of the relationships among the percomorph radiation using explicit phylogenetic analyses of either morphological or molecular data. These studies have often resulted in the polyphyly of many traditionally recognized groups. Here we use a high-throughput DNA dataset to revise the relationships among the recently recognized Acropomatiformes including the addition of several shallow water fishes and the recognition of a new family of fishes for the Giant Sea Basses.

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## The influence of micro-scale habitat on the movements of juvenile white sharks in their Southern California aggregation sites

*Emily Spurgeon, Marten Thompson, James Anderson, Kevin Abbott, Mitchell Alexander, Patrick Rex, Brian Stirling, Christopher Lowe*

While juvenile white sharks (JWS) can display regional endothermy, the need to maintain internal temperatures within an energetically favorable range likely drives thermoregulatory movements to maximize growth and foraging

efficiency. Juvenile white sharks from the northeastern Pacific population aggregate seasonally in nursery habitats throughout the Southern California Bight and historic data show that these inter-seasonal movement patterns may be heavily dependent on ambient water temperature. However, the degree to which water temperature influences JWS three-dimensional movement within their long-standing nearshore aggregation sites is unknown. We used high-resolution temperature and acoustic-telemetry positional data to quantify and predict how temperature and vertical thermal stratification influenced JWS movement over space and time using several modeling approaches. JWS selected for water temperatures between 16 – 22° and depths above 2 m. JWS depth distribution indicated activity deeper during dawn and dusk periods and their distance from the seafloor and geolocations of deeper distributions was significantly related to the thermal structure of the water column across the area monitored. The sharks remained significantly above a 16° thermal threshold and altered their horizontal and vertical distributions accordingly. JWS 3D distribution within the monitored region was most predictive based on time of day and water temperature across years. While high-resolution movement and environmental data provide improved micro-scale habitat use and distribution predictability, other variables such as prey distribution, behavior, and competition would likely further improve habitat use models for this highly mobile species.

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## Seasonal variation in salamander abundance and species diversity in relation to distance from a trail.

*Sydney Stahl, Jennifer Deitloff*

Amphibian populations are shown to decline when humans are present. Human influence affects food and refuge availability, and habitat quality. Urbanization is one of the biggest anthropogenic drivers for habitat fragmentation. Habitat fragmentation and urbanization affect genetic variation through creating barriers between populations and altering environmental conditions. The most prominent form of fragmentation that humans cause is the creation of roads. Population size of amphibians are shown to decrease as density of roads increases. Lower soil moisture and limited refuge are linked to this decrease. Road effects on amphibians are observed to be negative, whereas the effects of trail are relatively unknown. Creation of trails is less intrusive than roads, but could impact habitats similarly. This study focuses on the abundance and variation of salamander species observed through transects off a trail. The trail represents human influence within salamander communities as a form of urbanization. We examined transects perpendicular to the trail, searching leaf litter and cover objects, including rocks and logs, for salamanders. We also examined the data for any anthropogenic sources on salamander populations in regard to habitat fragmentation, as well as the significance of it. To account for seasonal variation, this study will be conducted in both the fall and spring seasons. We examined and analyzed any species observed to determine population variation as well as abundance.

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## An Examination of Spawning Season Sex Ratios and Weight-Length Relationships in 83 Collections of *Etheostoma caeruleum*, the Rainbow Darter

*Bruce Stallsmith*

*Etheostoma caeruleum*, the Rainbow Darter, has a wide range in the Mississippi, Ohio and Tennessee River drainages in the midwestern and southern United States. Using meristic and morphometric characters, within *E. caeruleum* three subspecies were recognized by Knapp (1964) and four separate species (subspecies here) by McCormick (1991). My goal was to characterize spawning season sex ratio and weight-length relationship (WLR in the form  $W=aL^b$ ), across the species' range with reference to this earlier work. I examined 83 lots with 6583 individuals of *E. caeruleum* borrowed from four museum collections. These lots were of at least 20 adults in the February–June spawning season. Most lots were found to have moderate female skew. In the three subspecies found in the central and northern Mississippi River drainage, mean male SL was 45–50 mm and female SL was 33–43 mm. In three large lots of 294–479 individuals from the disjunct southern Mississippi River subspecies, females comprised 64–71% of adult population and mean male SL was 33–37 mm, mean female SL 33.5–35.5 mm. Outside Mississippi, WLRs had a  $b$ , or slope, >3.30 showing positive allometric growth is typical. Little difference

exists between male and female slope in the three more northerly subspecies. In the southernmost subspecies female slope often exceeds male slope, for example in Silver Creek, Mississippi, the slope is 3.29 for 228 females, 3.04 for 120 males. The southern Mississippi River subspecies exhibits very different population parameters from the other three subspecies.

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## Disc-functional Families: Vertebral Evolution Across Squamates

*Natasha Stepanova, Xinchun Liu, Alison Davis Rabosky, Daniel Rabosky*

The evolution of elongate body forms is a common theme in squamate evolution, achieved primarily through the addition of vertebrae rather than their lengthening. Squamates vary widely in number of presacral vertebrae from 14 in the chameleon genus *Brookesia* to over 400 in some sea snakes. In addition to interspecific variation, there is variation within species. Using previously published data and newly collected data from x-rayed museum specimens, from over 2,000 species, we tested how rates of vertebral evolution change across the tree and how vertebral number relates to microhabitat in lizards and snakes. With a smaller subset of species, we also assessed if and how intra-specific variation is related to clade-level interspecific variation. We predicted that species in clades with greater variation would also show greater intraspecific variation. We found a relationship between number of presacral vertebrae and microhabitat in lizards, with grass-swimming and fossorial lizards having more vertebrae. Snakes do not show as clear a relationship, although many of the species with the highest vertebral counts occupy marine or arboreal habitats. Although species with higher vertebral counts do show wider ranges, this amount is not much greater than the intraspecific variation for species with low counts and it does not come close to the substantial differences found among species even within the same genus. These results suggest that intraspecific variation does not contribute to evolutionary lability in vertebral number in a clear way.

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## Evaluating the Use of Unmanned Aerial Systems to Study Diamond-backed Terrapin (*Malaclemys terrapin*) Populations

*Sean Sterrett, Rebecca Berzins, Rachel Katz*

Diamond-backed terrapin (*Malaclemys terrapin*; DT) is the only brackish-adapted turtle in North America and a species of greatest conservation need throughout its range. The biology of DT, including seasonal breeding aggregations, presents a temporally- and geographically-constrained opportunity for transforming the way DT are monitored with novel methods, which may be attractive to wildlife managers, who are resource limited. Unlike traditional approaches, drones offer a non-invasive and potentially unbiased sampling method. However, we know little about DT detection from drones. We used 3D printed DT shells in three biologically realistic sizes and randomly selected numbers of different DT sizes into trial sets. For each trial, we set 3D printed DT in a standardized sampling arena and flew the drone from 18 to 60m, taking standardized images at 5m intervals. Images were presented randomly to independent observers, who identified and marked DT of various sizes. Using images with 3D printed and real DT, we used the program ImageJ to measure DT by standardizing a scale of measurement using the ground sampling distance. Unsurprisingly, we found that drone height and DT size were important factors for accurately counting DT in trials sets. Unsurprisingly, we found that drone height and DT size were important factors for accurately counting DT in trials sets and image glare impacted an observers' ability to correctly label by size. All measurements of 3D-printed DT were within 10mm of actual size and we were able to measure 53% of the total DT in images ( $N = 97$ ).

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## Phylogenetics of African characiforms – resolved – but where's the morphology at?

*Melanie Stiassny, Bruno Melo*

Recent DNA-based analyses utilizing both UCE loci and whole mitogenomes converge on a novel, but well-supported scheme of relationships among a historically problematical group of African characiforms previously included in the families Alestidae and Hepsetidae. While molecular support for the

composition and relationships among the resolved clades (families, subfamilies, genera) is strong, morphological homoplasy appears rampant and congruent support in the form of discrete, diagnostic characters at each level is currently far less abundant. Previous studies, most notably a monumental work published nearly 20 years ago, provides much information on morphological variation among alestids, however refinement and reinterpretation of these comparative data in the reciprocally illuminating context of the current topology is needed. While ongoing, we discuss some novel morphological character data in support of the current molecular phylogeny of African characiform relationships.

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## Exploring anuran photoreceptor diversity with unsupervised machine learning

*Jeffrey Streicher, Rayna Bell, Matthew Fujita, David Gower, Ellis Loew, Ryan Schott, Kate Thomas*

Microspectrophotometry (MSP) can be used to measure the absorbance spectra of individual cells and their pigments. When applied to retinal photoreceptors (rods and cones), this methodology is a cornerstone of comparative vision research. Downstream analyses of MSP data are typically preceded by time-consuming, manual curve fitting and adjustment of individual measurements. Here we explored the viability of using multivariate analyses and Gaussian Mixture Models (GMM) to batch process large numbers of MSP measurements and categorize them with unsupervised machine learning. Using an empirical dataset of 4,000+ MSP measurements from anurans (frogs and toads), we tested how well GMM could (1) sort MSP traces into correct photoreceptor categories, and (2) assign traces to either a vitamin A1 or A2 chromophore group. Unsupervised machine learning predicted clusters that were near-perfectly matched to the three included photoreceptor classes. Results were more mixed for the chromophore assignment, however, this may be related to caveats associated with how MSP data were taken. Thus, unsupervised learning with cluster prediction represents a viable methodology for increasing the analytical throughput of MSP-derived absorbance spectra. This new approach may also be useful for quantitative comparisons of spectral variation in relation to phylogenetic and ecological groupings.

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## Does Behavior Complement the Chemical Defenses of Cryptic and Aposematic Poison Frogs?

*Jennifer L. Stynoski, Francesca Protti-Sánchez, Jeremy Klank, Hannah Rowland*

To avoid predation, animals should rely on the suite of physical, chemical, and behavioral defenses that are best adapted to their context. Some antipredator strategies can be categorized on a continuum, from aposematic (conspicuous, unpalatable, chemically defended) to cryptic (camouflaged, palatable). Behavior should evolve to complement other antipredator traits: aposematic animals should be bold, visible, and slow moving, whereas cryptic animals should be timid, hide more, and move quickly. However, empirical data are lacking to test that hypothesis, especially with vertebrate prey and from an interspecific focus. Here, we tested whether behavior can be reliably placed along an aposematic-cryptic continuum by quantifying antipredator defenses in six species of dendrobatoid poison frogs (*Oophaga pumilio*, *O. granulifera*, *Dendrobates auratus*, *Phyllobates vittatus*, *Allobates talamancae*, and *Silverstoneia flotator*). Using non-invasive sampling methods, we measured flight initiation distance from a bird model, latency to exit a refuge, time budget in an arena, and movement trajectory, as well as visual color models and chemical profiles employing UHPLC-MS/MS and GC-MS. We will share results of the integrated behavioral, chemical, and color profiles of aposematic and cryptic species, with the aim of disentangling the evidence for co-evolution among antipredator traits.

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## Uncovering Developmental and Transcriptional Mechanisms of the Early Synthesis of Toad Chemical Defenses

*Jennifer L. Stynoski, Noé Chaves-Chaves, Katherine Porras-Brenes*

Toads in the family Bufonidae synthesize a diverse cocktail of chemical defenses in the granular glands distributed throughout their skin. One type

of toad chemical defense – the bufadienolides – are cardiotoxic steroid compounds with striking potential for biomedical applications due to their widely demonstrated cytotoxic, anti-inflammatory, anti-bacterial, and anti-fungal properties. Their biosynthesis is thought to involve cholesterol as a precursor, thyroid hormone, 3 beta-hydroxysteroid dehydrogenase, a few key cytochrome P450 enzymes, and direct or co-opted components of the acidic bile acid pathway. However, the developmental and transcriptional mechanisms that allow toads to shift from being vulnerable to well chemically defended at a critical point in ontogeny is poorly understood. Using histochemistry, chemical analysis, and RNA-seq across developmental stages of toads (*Rhinella horribilis*, *Incilius luetkenii*, and *I. melanochlorus*), we aim to draw a more complete picture of the comparative ontogeny of poison synthesis in bufonid toads. Our results suggest that granular gland morphogenesis is well conserved, and that differences in gene expression likely underlie the diversity of bufadienolide chemical defenses stored in the glands as well as the relative palatability seen in distinct developmental stages and lineages of bufonid toads.

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### **Armored fishes - plate design offers insight into dangers.**

*Adam Summers, Matt Kolmann, Cassandra Donatelli, Meg Vandenberg*

Among the more than 30 lineages of armored fishes, if the armor is largely defensive the diverse manifestations must reflect the quality and magnitude of the expected assault. For example, in poachers (Agonidae) the flat, unornamented, imbricate plates in the Smooth Alligatorfish (*Anoplogonus inermis*), are in stark contrast to the rough surfaced plates of the Northern Spearnose Poacher, *Agonopsis vulsa*, most of which have a single, large, curved spine. Performance measures, including crushing resistance, bending stiffness, and body bending during escape, indicate that the latter is moderately effective against gross assault with very little decrease in locomotor performance. A closer look at the role of this armor offers three areas of performance research: function of the spine, function of the rough surface, and function of patterning on the overlapping surfaces. If the assault on *Agonopsis* comes from a narrow, sharp probe, for example, from a crustacean maxilliped or peripod, a central spine will prevent the sharp tip from finding the gap between plates. Similarly, the rough surface will catch a sharp probe, preventing it from sliding into a gap between plates. The plates move easily over one another, as evidenced by swimming speed, but the interplate patterning ensures that a plate under pressure will stick to the plates it lays over. The critical mass of data on the morphology of armor in fishes points towards interesting experiments with potential technical applications.

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### **In Search of... Mesopelagic Boundary Communities. What Are They, Why Just Hawaii, and Could They Occur in the Atlantic?**

*Tracey Sutton, Kevin Boswell, April Cook, Tamara Frank, Jon Moore, Heather Bracken-Grissom, Rosanna Milligan*

Mesopelagic biogeographic boundaries in the open ocean are generally diffuse and coincident with meso-to-basin-scale physical oceanography. A notable exception occurs in certain topographic boundary regions, where spatially compressed midwater areas are constrained by 200-m and 700-m isobaths that may harbor 'indicator' mesopelagic species that are rare offshore and that exhibit behaviors influenced by the bottom. The presence of indicator species differentiates these 'mesopelagic boundary communities' (MBCs) from areas where oceanic deep-scattering layers impinge upon the continental slopes, but where the species composition is similar to that found offshore (although sometimes in high densities). MBCs have been well-documented over the upper slope of the Hawaiian Islands but have rarely been reported elsewhere. The possible reasons for the lack of MBC ubiquity are many, ranging from environmental to ecological to methodological. The last reason may be particularly important; for an MBC to be detected, a well-established offshore faunal inventory and/or behavioral catalog is necessary, against which uniqueness of a potential MBC fauna can be determined. Such inventories are lacking for much of the World Ocean. Here we will take a deep dive into the MBC phenomenon, comparing MBCs to other forms of topographic association (e.g., canyons, ridges), discussing requirements for maintenance of MBCs in a high dispersal environment, and presenting preliminary evidence

of a possible MBC over a large deep-water coral complex in the Gulf of Mexico. We will finish by discussing the importance of MBCs for managed and/or protected marine species and for planning protected areas.

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### **An Ongoing Mission of DEEPEND research: an "Oceanic Fishes of the Gulf of Mexico" Compendium for Stakeholders and Resource Managers**

*Tracey Sutton, Jon Moore, April Cook, Andrea Bernard, Mahmood Shivji*

The DEEPEND (Deep Pelagic Nekton Dynamics) program was originally created to determine what, where, and how many water-column fishes and invertebrates resided in the region of the Gulf of Mexico impacted by the Deepwater Horizon disaster. NOAA needed this information to assess natural resource damage. This was in every sense a "Census of Marine Life"-type project, as no data existed for much of the fauna before the disaster. DEEPEND's follow-on iteration, funded by the Gulf of Mexico Research Initiative (2015-2020), had a primary goal of quantifying oil spill effects on various taxa, fishes being prominent. DEEPEND's ongoing mission, funded by the NOAA RESTORE Science Program (2020-current), aims to continue research on population trends and drivers, with the added goal of transferring products that can be used by Gulf stakeholders and resource managers. One such product is a taxonomic compendium of fishes occurring in the oceanic Gulf. DEEPEND work has identified nearly 900 species of pelagic fishes seaward of the 1000-m isobath, with nearly 190 of these being new records. To create this list, the authors had to compile over 100 taxonomic keys, in addition to establishing a robust genetic barcoding program to supplement morphological identifications. Here we describe our ongoing goal to produce a resource that can be used by current and future assessors of pelagic fish diversity in this extraordinary oceanic ecosystem.

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### **Evaluating Ecosystem Connectivity of Three Common Shark Species in a National Marine Sanctuary**

*Brett Sweezey, Jay Rooker, Brett Falterman, R. J. David Wells*

The spatial and temporal movement patterns of upper trophic-level marine predators is central to guiding successful and efficient fisheries management decisions. Large-bodied and highly migratory elasmobranch species play an important role in shaping marine ecosystems by exhibiting top-down population control through direct predation and indirect non-consumptive risk effects. Three globally threatened elasmobranch species listed under the IUCN Red List, silky (*Carcharhinus falciformis*) [threatened], sandbar (*Carcharhinus plumbeus*) [vulnerable], and scalloped hammerhead sharks (*Sphyrna lewini*) [critically endangered] can be observed within the Flower Garden Banks National Marine Sanctuary. The Flower Garden Banks is a remote marine protected area in the Gulf of Mexico located approximately 100 km offshore and provides unique critical hard bottom habitat for a variety of marine organisms. This productive region attracts highly migratory marine predators and forms suitable aggregation sites for spawning and foraging behaviors. However, despite the regional sanctuary expansion in 2021 (increasing the total area coverage from 145 km<sup>2</sup> to 415 km<sup>2</sup>), our understanding of how elasmobranch species utilize this unique system remains surprisingly limited. To assess the habitat requirements and ecological connectivity of silky, sandbar, and scalloped hammerhead sharks within the Flower Garden Banks, long term movement patterns will be monitored through the implementation of acoustic (V16) and satellite (SPOT6) telemetry techniques. Combining tagging techniques to observe the movement patterns of benthic and pelagic shark species will provide critical information necessary to evaluate the efficacy of the recent perimeter expansion for these highly migratory elasmobranch species on a sanctuary-wide scale.



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## Accounting for Population Growth, Intermittent Breeding, and Aging Error in Close-Kin Mark-Recapture Assessments for Long-Lived Elasmobranchs

*John Swenson, Elizabeth Brooks, Dovi Kacev, Charlotte Boyd, Michael Kinney, Benjamin Marcy-Quay, Anthony Sévêque, Kevin Feldheim, Lisa Komoroske*

Obtaining robust estimates of population abundance is a central challenge hindering the conservation and management of many threatened and exploited elasmobranch species. Close-kin mark-recapture (CKMR) is a genetics-based approach that has strong potential to improve monitoring of these species by enabling estimates of abundance, survival, and other parameters for populations that are challenging to assess. However, CKMR models have received limited sensitivity testing under realistic population dynamics and sampling scenarios, impeding application of the method in population monitoring programs and stock assessments. Here, we use individual-based simulation to examine how unmodeled population dynamics and sampling strategy affect the accuracy and precision of CKMR parameter estimates for long-lived elasmobranch species, and present adapted models that correct the biases that arise from model misspecification. Our results demonstrate that if strong population declines or non-annual breeding dynamics are present, a CKMR model must account for these factors to avoid biased parameter estimates. As long as an appropriate model is specified, our simulations show that CKMR can generate reliable abundance estimates for adults from a variety of sampling strategies, including juvenile-focused sampling where adults are never directly observed. Finally, we apply our adapted CKMR model to two decades of real genetic data from juvenile lemon sharks (*Negaprion brevirostris*) in Bimini, Bahamas to demonstrate how CKMR can be used to expand monitoring efforts for highly mobile elasmobranch populations via targeted sampling of nursery areas.

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## Assessing for MHC-Associated Mate Choice in the Blacktip Shark (*Carcharhinus limbatus*)

*Dominic Swift, Andrew Fields, Dean Grubbs, Kevin Feldheim, Jayne Gardiner, Marcus Drymon, David Portnoy*

A series of studies have demonstrated that female elasmobranchs frequently mate with multiple males within breeding seasons but very few studies have focused on mechanisms by which females might exert mate choice. This is despite studies of other vertebrates indicating females can exert mate choice via multiple mechanisms involving products of the major histocompatibility complex (MHC), a cluster of highly variable genes encoding proteins with immune functions. MHC-based mate choice can confer genetic benefits to females that mate multiply by facilitating inbreeding avoidance and providing offspring with allelic combinations that optimize immune system function. Therefore, evidence of MHC-associated mate choice was assessed for the blacktip shark (*Carcharhinus limbatus*) by genotyping six mothers, each of their offspring (four to six pups per litter), and >50 breeding adults at four microsatellites and two MHC genes (b2m and mhc1a). Each individual displayed one or two alleles for each microsatellite and b2m. By contrast, one to seven alleles were observed for mhc1a, indicating up to four locus copies per individual. For each litter, the minimum number of sires and evidence of inbreeding/outbreeding were assessed using microsatellite and b2m genotypes. Evidence of MHC-associated mate choice was assessed for each litter by comparing similarities between maternal and paternal mhc1a alleles to those expected under random mating. The study is the first population-level assessment of MHC in an elasmobranch and provides insights into the diversity of MHC among vertebrates.

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## Informing silky shark management in the western Atlantic and eastern Pacific Oceans

*Brendan Talwar, John Carlson, Lyall Bellquist, Brice Semmens, Michael Heithaus*

The silky shark *Carcharhinus falciformis* is poorly studied in the western Atlantic and eastern Pacific Oceans, where it often dominates shark bycatch in tuna longline and purse seine fisheries. Information on the spatial ecology of silky sharks through ontogeny is required to improve local, regional, and

ocean-wide conservation and bycatch reduction efforts. In The Bahamas, we used targeted baiting and longline fishing to identify silky shark aggregation sites and habitat associations. Across the western Atlantic, we deployed satellite tags to characterize silky shark horizontal and vertical movements. Lastly, across the eastern Pacific, we developed a conceptual model of silky shark life history to inform future stock assessments and management decisions. We provide valuable new information and summarize prior knowledge about silky shark spatial ecology to facilitate silky shark management at multiple spatial scales.

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## Species Diversity Coverboard Assessment in Western Connecticut Restored Ecosystem

*Michael Tambascio, John Michael Arnett, Theodora Pinou*

Herpetology Club students from Western Connecticut State University (WCSU) conducted a cover-board survey from March 20th – November 19th, 2022. Sixty 4ft–by–4ft plywood coverboards were placed in three habitats (grass shrubland, upland forest, and lowland forest) comprising of an approximately 3-kilometer area to observe and record species diversity and abundance. The Great Hollow Nature Preserve was founded in 2015 and spans a total of 825 acres. The study area bisects Pembroke Rd. Surrounding the preserve are thousands of acres of additional protected land in Connecticut and New York, including the Pootatuck State Forest, Squantz Pond State Park, Michael Cialola Conservation Area, and Cranberry Mountain Wildlife Management Area. We surveyed the cover-boards 20 times and collected habitat and microclimate data during each survey. We report the temporal spatial changes of the observed vertebrates and noticed distinct differences in abundance and occupancy of reptile and amphibian species throughout the field season. This data indicates that different habitat types are preferred by reptile and amphibian species depending on the season.

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## Ecology and Biogeography of the Diversification of Minnows (Cypriniformes: Leuciscidae) of the Holarctic

*Milton Tan, Jonathan Armbruster*

Differences among lineages in biodiversity may be explained by differences in historical diversification patterns. Diversification rates may be influenced by biogeographic processes, such as transcontinental dispersal, or ecology, such as habitat. The Holarctic Leuciscidae minnows are exceptionally diverse (>600 spp., 6th most diverse family of fishes), and hence represent an excellent system to study factors that drive diversification. The diverse leuciscids are also an excellent group to study the ecological and biogeographic factors influencing diversification because a breadth of available distributional and ecological knowledge from long histories of ichthyology in their range in North America and Europe that are unavailable for many other clades. We reconstruct a time-calibrated phylogeny for minnows incorporating phylogenomic and phylogenetic data. Then, we used this tree to test for biogeographic and ecological influences on diversification rates using modern state-dependent speciation and extinction (SSE) models, which jointly consider trait evolution and their effect on diversification for robust inferences. We identified support for continental-scale differences in diversification rate among leuciscids, but not for habitat type. While this suggests a greater importance of biogeography over ecology in leuciscid diversification, other unexplored ecological factors may also be important in leuciscid diversification.

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## Phylogeny of Rabbitfishes (Siganidae)

*Kevin Tang*

The family Siganidae is a widespread group of marine fishes (rabbitfishes) found in the Indo-Pacific Ocean and, more recently, the Mediterranean Sea, where they often occur in association with coral reefs. Of the 32 recognized rabbitfish species, 24 were examined in this analysis. Additional acanthomorph taxa were included as outgroup taxa.

Sequence data from mitochondrial (12S, 16S, ATPase 8/6, cytochrome b, cytochrome c oxidase I) and nuclear (ectoderm-neural cortex protein 1, histone 3,

recombination activating gene 1, and zic family member 1) genes were used for analysis. Analyses recovered strong support for the monophyly of the genus and family.

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### Loss and Impending Recovery of Blunt-nosed Leopard Lizards (*Gambelia sila*) in California's Panoche Hills.

*Rory Telemeco, Lynn Myers, Mark Halvorsen, Steven Sharp, Michael Westphal*

Panoche Plateau in central California supported a robust, genetically distinct population of federal- and CA-endangered Blunt-nosed Leopard Lizards (*Gambelia sila*) prior to 2014. However, following a drought in 2013-2014, the population declined and was extirpated by 2021. In 2020, our collaborative team from Fresno Chaffee Zoo, Fresno State University, and the US Bureau of Land Management began intensive monitoring of *G. sila* in the Panoche Hills, investigating multiple potential contributors to *G. sila* decline such as warming temperatures, predation pressure, pathogen pressure, and water availability. The bulk of evidence suggests that extirpation resulted from drought-induced reproductive failure in 2014 followed by demographic collapse. Delayed collapse following an extreme climate event could be common and suggests *G. sila* populations should be carefully observed both during and following drought. Otherwise, Panoche Plateau appears to still represent high-quality habitat for *G. sila*, with low predation pressure and parasite load paired with high-quality thermoregulatory habitat. In spring 2023, we began repatriating *G. sila* produced by the captive colony maintained by Fresno Chaffee Zoo to Panoche Plateau. Animals will be radio-monitored for their entire lives to estimate survival and reproductive output, and these data will be used to better understand habitat features that facilitate population persistence in *G. sila*.

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### Who's at the Zoo?: A Study of Unaccessioned Freshwater Turtles at the Memphis Zoo

*Jennifer Terry, Alexia Vanoven, Courtney Janney, Virginie Rolland, Sinlan Poo, Lorin Neuman-Lee*

Wildlife living in urbanized spaces are likely to have unique pressures, challenges, and potential benefits that may result in physiological strategies (e.g., immune function) associated with the specific qualities of the habitat. The unaccessioned wild freshwater turtle population at the Memphis Zoo, a living collection institution, presents an ideal opportunity to examine immune function in a managed, urbanized habitat. These turtles inhabit water features of exhibits across the zoo and are managed by zoo staff when needed, but not on a regular basis. Environmental pressures, like predation and resource limitation, are likely partially alleviated in a zoo space. In June 2022, we initiated a capture-mark-recapture program of the wild freshwater turtles inhabiting zoo property. We collected blood samples from 12 adults and assessed innate immune function using bacterial killing assays and hemagglutination-hemolysis assays. We found individual variation in baseline immunocompetence and apparent immune component prioritization across and within species. Future sampling will further investigate immune function and monitor movement, survival, and recruitment of the population to understand how zoo spaces function as habitat for wild freshwater turtles. This research will inform how wildlife use living collection spaces and provide opportunities to inspire the public and foster appreciation for the conservation mission of zoos and urban wildlife at large.

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### Life-History Transitions and the Molecular Stress Response of Garter Snakes

*Rysa Thomas, Rocky Parker*

Life-history transitions are energetically demanding, and the molecular mechanisms regulating energy homeostasis in peripheral tissues are key in determining patterns of stress resilience versus susceptibility. In vertebrates, glucocorticoid hormones (GCs) act on metabolic tissues via tightly regulated activation of glucocorticoid (GR; low-affinity) and mineralocorticoid (MR; high-affinity) receptors. The relative expression of these receptors can inform whether maintenance or emergency conditions are active in a given individual; further, when analyzed within specific life-history contexts (e.g., mating

versus migrating), significant insight can be gained into the genetic regulation of homeostatic control. Red-sided garter snakes (*Thamnophis sirtalis parietalis*) emerge from an eight-month dormancy every spring and immediately engage in a brief-but-explosive mating season directly followed by a long-distance migration. Plasma GCs fluctuate to balance opposing energetic demands: they are maximal during mating and decrease across migration into summer. Because GCs have their effects via receptor activation, we measured expression (qPCR) of GR and MR in energy storage tissues of snakes while either mating or migrating in spring. We observed sex differences in the liver of mating snakes, and MR expression was positively correlated with GR. However, we also observed little influence of body condition on receptor expression; instead, the ratio of MR:GR expression appears indicative of the life-history transition in spring, with MR dominating in spring while GR dominates during migration. More surprising, we discovered strong patterns of overall resilience of receptors to widely variable GC availability, including no effects of GC treatment even at very high physiological doses. We describe this as molecular stress hardness which, to our knowledge, has not been documented but could be a highly adaptive mechanism for buffering critical tissues from the deleterious effects of stress.

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### Molecular systematics and species identification of *Dendropsophus* (Anura: Hylidae) in Colombia

*Brandon Thomason, Holland Galloway, João Tonini, Mariela Osorno Muñoz, Rafael de Sá*

Biological surveys in unexplored areas increase knowledge on local biodiversity by confirming the presence of known species, extending the distribution of species previously not known to occur in each area, or identifying populations as new species to science. Colombia is the South American country with the second highest species diversity of frogs and still new species continue to be described. The frog genus *Dendropsophus* has 109 described species widely distributed throughout Central and South America, east of the Andes and south of Mexico, with species occurring in all South American biomes. In Colombia, it is reported 37 species of *Dendropsophus*; however, the last described species of *Dendropsophus* for the country was ten years ago, which shows how *Dendropsophus* in Colombia have been understudied. To evaluate the species diversity of populations of *Dendropsophus*, we amplified and sequenced the 16S genetic marker. We use Maximum Likelihood to estimate phylogenetic trees and test species identity and phylogenetic positions. The molecular matrix includes our own data and data available on GenBank comprising 33 out of the 37 species of *Dendropsophus* known to occur in Colombia. The preliminary phylogenetic tree shows that the newly sampled populations from Colombia confirmed the presence in those communities of *D. parviceps*, *D. bokermanni*, *D. subocularis*, *D. marmoratus*, *D. minusculus*, *D. mathiassoni*, *D. sarayacuensis*, *D. bifurcus*, and *D. reticulatus*. In addition, our data include a potential new species related to *D. stingi* and *D. sarayacuensis*. Our future goal is to increase genetic data to include more mitochondrial and nuclear genes.

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### C-Metaphase Karyotypes of *Lepomis punctatus* and *L. miniatus* (Teleostei, Centrarchidae).

*Kenneth Thompson*

Karyotypic surveys of the sister species-pair, *Lepomis miniatus* (red spotted sunfish) and *L. punctatus* (spotted sunfish) were carried out on 28 specimens from seven geographically separate populations in the southern United States. Air dried, C-metaphase, diploid karyotypes of both species were shown to consist of 48 one-armed chromosomes. Karyotypes are often designated as having a chromosome formula which is the total diploid number of chromosomes (2N) and the total number of arms in the karyotype (NF). This is expressed as 2N/NF or in this case as 48/48. The chromosome formula of the presumed ancestral karyotype of the Centrarchidae is considered to be 48/48. Karyotypic evolution in which diploid numbers are reduced while the total arm numbers remain constant are indicative of evolution by Robertsonian fusions. Twelve of the seventeen species of *Lepomis* have been karyotyped and all have 2N of 46 – 48 and all twelve have NF values of 48 indicating that karyotypic change in this genus has been both conservative and Robertsonian.

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## Thermal Adaptation of Streamside Salamanders (*Ambystoma barbouri*) across a Latitudinal Gradient

*Julia Thulander, Tatyana Natal, Jason Bracken, Joshua Hall*

Climate change will have impacts on all animals but especially vertebrate ectotherms as they rely on environmental temperature for a wide range of behavioral and physiological activities. The effects of a warming climate on non-avian reptile development have been studied extensively; however, there is comparatively less known about how it will impact amphibian embryo development. Streamside Salamanders (*Ambystoma barbouri*), a Tennessee state endangered species, have a broad latitudinal range which can indicate how thermally sensitive traits adapt to temperature. We collected eggs from across the latitudinal range of the Streamside Salamander to determine how thermally sensitive traits vary across populations. At each location, we deployed temperature loggers to characterize the thermal environment of nests and collected eggs to incubate across a range of temperatures. We measured developmental rates and recorded resultant survival and morphology of larvae and metamorphs. Our objectives for this ongoing research are 1) to use field data to determine how environmental temperature varies across latitudes and habitats and 2) to use laboratory data to create physiological models that generate predictions of growth and survival across latitudes. We report nest temperatures across the species range and the effects of temperature during embryonic development on survival, developmental rate, and larval morphology and highlight the trends of these variables across the latitudinal range of the Streamside Salamander. These results will aid in targeted conservation efforts as they will demonstrate if and how endangered Tennessee populations differ phenotypically from more robust populations in the northern portion of the range.

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## Assessing multi-scale habitat relationships and responses to forest management for cryptic herpetofauna in the Missouri Ozarks, USA.

*Shelby Timm, Alexander Wolf, Xiaoming Gao, Kenneth Kellner*

Cryptic or uncommon herpetofauna are often understudied due to the extensive effort it requires to obtain adequate data for statistical analysis. Additionally, potential impacts from forest management on these already small or difficult to study populations may have a dissimilar effect in comparison to more common species. To address this, we examined species-specific responses of less common herpetofauna within the Missouri Ozarks to even-aged and uneven-aged silvicultural systems at multiple scales, as well as their habitat associations. Using capture histories collected over 23 years (1992–2014) on the Missouri Ozark Forest Ecosystem Project (MOFEP) we examined the cumulative effects of two harvest entries (1996 and 2011) at both the local (stand-level) and landscape-scale (compartment-level) for eight uncommon amphibian and reptile species. We modeled capture probabilities with respect to multiple habitat- and harvest-related covariates. Three species showed compartment-level declines in the post-treatment period, however only two of the declines appeared to be related to forest management; the decline for the third species was observed in both treatment and control compartments suggesting that the cause was environmental. In contrast to compartment-level responses, we observed stand-level responses in five species, mostly positive. In general, our observed declines were minimal and currently we have no concerns that forest management will lead to the loss of any of the less common herpetofauna species considered here. Our models showed habitat associations for multiple species, which aids our understanding of species' life history strategies and can also guide future management efforts.

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## The relative contributions of multiarticular snake muscles to movement in different planes

*Jessica Tingle, Derek Jurestovsky, Henry Astley*

Muscles spanning multiple joints play important functional roles in a huge diversity of systems across tetrapod vertebrates, including human fingers, bird necks, and chameleon and primate tails. Despite the ubiquity and importance of multiarticular muscle systems, we still lack data on fundamental aspects of their mechanics, particularly the consequences of anatomical position on mechanical advantage. Snakes can serve as excellent study organisms for

advancing this topic. Their trunk muscles span from one or a few vertebrae to upwards of 30; moreover, muscle architecture varies among muscles and among species. Snakes rely on their axial musculoskeletal system for a huge range of activities, including striking, constriction, defensive displays, and locomotion. However, few studies have examined how snake muscle anatomy relates to function. We characterized the anatomy of major epaxial muscles in a size series of corn snakes, *Pantherophis guttatus*, including cross-sectional area and locations of attachments, using diceCT scans. We then used several approaches for calculating contributions of each muscle to force and motion generated during body bending, starting from a highly simplistic model and then moving on to increasingly complex and realistic models. Our results contribute to knowledge of snake muscles specifically and multiarticular muscle systems generally, providing a foundation for future comparisons across species and bioinspired multiarticular systems.

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## A Requirement Not a Band-Aid: The Role of Head-Starting in Recovering Mojave Desert Tortoise Populations

*Brian Todd, Mark Peaden, Kurt Buhlmann, Tracey Tuberville*

Tortoises have lengthy generation times that limit population growth. Thus, the removal of conservation threats alone may be insufficient to recover depleted populations over meaningful timeframes. Such concerns have led to increased studies of head-starting—the act of rearing animals through vulnerable life stages until they reach larger sizes when survival increases—as a recovery tool. Here, we constructed spatially-explicit population models of the Mojave Desert Tortoise (*Gopherus agassizii*) using published estimates and long-term data on survival, fecundity, movement, and behavior to better understand the context for head-starting as a recovery tool. We first examined the impact of roads on desert tortoise populations in isolation. We then examined potential population recovery from the installation of roadside fencing to mitigate desert tortoise road mortality. Finally, we investigated the potential for increased population recovery following the release of head-started desert tortoises into the population. Our models showed that roads caused substantial declines of desert tortoise populations in just 50 years. And, while mitigation fencing arrested declines caused by roads, recovery was slow, with <5% population increase over a subsequent 50 years. In contrast, populations recovered markedly when head-started tortoises were released for the first 3 or 5 years after fencing installation. Contrary to the outdated notion that head-starting may be a misguided conservation measure, our work here highlights the sound need for its inclusion alongside other conservation measures if viable populations of long-lived species are to be restored.

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## Phylogenetic Systematics and Species Diversity of South American Fossorial Frogs (*Microhylidae*: *Elachistocleis*)

*João Tonini, Rafael de Sá*

Phylogenetic systematics using molecular data have provided new insights into species diversity and phylogenetic relationships, particularly when phenotypic data does not present sufficient variation to tell species apart or does not contain a reasonable number of informative characters to define phylogenetic groups. South American fossorial frogs *Elachistocleis* comprise 18 described species distributed throughout forested biomes such as the Amazon and Atlantic Forest, floodplains of the Pantanal, and savannas and seasonally dry forests of the Cerrado, Caatinga, and Chaco. Despite experiencing a myriad of climatic and environmental conditions, these species have little differences in external phenotypic characters, varying mostly in body size, color pattern, and osteology. Genetic data have shown that newly sampled populations in Colombia represent new species and populations in Brazil, Bolivia, Paraguay, and Argentina have been hypothesized as cryptic species. We investigate *Elachistocleis* diversification by sequencing six genetic markers, three mitochondrial and three nuclear, and estimating phylogenetic trees using Maximum Likelihood. Then, we use the phylogenies to test species delimitation hypotheses. Our molecular matrix includes over 900 samples of *Elachistocleis* from populations across the geographic distribution of currently described species. The preliminary results show that all described species can be assigned to monophyletic groups. In addition, *Elachistocleis* might comprise twelve additional cryptic species. Future goals include visiting museum collections to acquire morphometric data and potentially advertisement calls to further test

the species hypotheses generated by the molecular data. Moreover, we will identify specimens for CT-scanning and 3D morphometrics analyses, given that *Elachistocleis* might present character variation in osteology.

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## Comparative Phylogenetics and Phylogenomics of the genus *Limia* (Cyprinodontiformes: Poeciliidae) using Next Generation Sequencing

*Patricia Torres-Pineda, Thomas D. Morgan, Hernán López-Fernández*

The family Poeciliidae constitutes an important component of Neotropical freshwater fish fauna, especially in Middle America (Central America and insular Caribbean regions). In the West Indies, the genus *Limia* is the largest freshwater clade of the region and is thought to have experienced explosive speciation, with Hispaniola (Haití and Dominican Republic) as the center of said radiation, where 19 out of the known 22 species occur. *Limia* has been proposed as a case of adaptive radiation, nevertheless this hypothesis has not been tested. To understand patterns and mechanisms of diversification in this group we conducted extensive fieldwork to sample all species of *Limia* present in the Dominican Republic, with collections spanning their known distribution throughout the country. We used a reduced-representation genomic approach (i.e., ddRAD) to sequence and compare thousands of loci from across the genome of *Limia* to build a species-level phylogeny for the genus. We also characterized intraspecific genetic diversity among contemporary populations. This high-resolution species-level phylogeny of *Limia* will serve as a comparative backbone to test hypotheses of both biogeographic and macroevolutionary processes underlying the diversification of *Limia* in the region.

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## Probing Conserved Regenerative Pathways in Frog Eye Regeneration

*Kelly Tseng*

Regeneration research has identified a myriad of genes and pathways in diverse models that regulate regrowth. However, this research has led to surprisingly few overarching principles that govern regeneration in general, even though an understanding of the evolution of regeneration could provide explanations for one of the field's most important yet unanswered questions: why regenerative ability varies greatly amongst species. Our approach is to look for shared molecular pathways in the different organs of the same model, which may help to identify mechanisms that can be used to stimulate regeneration regardless of tissue type. The African clawed frog, *Xenopus laevis*, has high regenerative ability and is a well-characterized model for animal development, especially for the eye. We found that tailbud embryos readily regrew eyes after ablation. The regrown eye reached a size similar to the uninjured contralateral eye and overall development was normal. The regrown eye contained the expected complement of tissues, connected to the brain, and was functional. Restoration of the eye largely recapitulated the developmental process. Using this model, we tested if mechanisms that are known to promote tadpole tail regeneration also drive eye regrowth. Cell death (apoptosis) and bioelectrical signaling via the H<sup>+</sup> pump V-ATPase are both required for initiating tail regeneration. Our studies showed that apoptosis and V-ATPase are separately required for embryonic eye regrowth. Together, these results suggest that bioelectrical signaling and apoptosis may represent conserved regenerative mechanism and as such help build a framework for future comparative studies.

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## Reverse Sexual Dichromatism in Golden Rocket Frogs

*James Tumulty*

Sexual dichromatism is common among animals, but examples of "reverse" sexual dichromatism, in which females are more brightly colored than males, are extremely rare. I report the discovery of reverse sexual dichromatism in golden rocket frogs (*Anomaloglossus beebei*). Only the females of this species are actually "golden," while the males are a drab tan color that becomes darker when they are calling. I use calibrated digital photography to measure these color differences, model color using different visual systems, explore the potential discriminability of the frogs from their natural backgrounds, and

catalog dichromatism among other Aromobatid frogs. While the function of bright coloration in females is still unclear, my results collectively implicate an important role of conspecific receivers in the evolution of sexual dichromatism in this species.

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## Movement of Western North Atlantic White Sharks through the Straits of Florida and Gulf of Mexico

*John Tyminski, Cade Roach, Bryan Franks, Alisa Newton, Fabián Pina Amargós, Brett McBride, Chris Fischer, Robert Hueter*

The white shark (*Carcharodon carcharias*) is a large, highly migratory, apex predator typically found in coastal and continental shelf environments of temperate waters across the globe. In the western North Atlantic (WNA), white sharks have been studied for decades through catch data and other observations along the U.S. Atlantic coast. Beginning in 2012, OCEARCH has coordinated a comprehensive long-term study of this species that includes tagging sharks with satellite-linked and acoustic tags to track their movements. Tagging occurred between Nova Scotia, Canada, and Jacksonville, Florida, USA, all on the Atlantic coast. Four life stages (young-of-the-year, juvenile, subadult, adult) were tagged, revealing the migratory cycles of this WNA population from age 0 through maturity. A combination of SPOTs, PSATs, and internal acoustic tags revealed all four life stages enter the Gulf of Mexico (GoM) through the Straits of Florida and utilize this habitat to a significant degree. Of 84 white sharks tagged, 42 (50%) spent time in the GoM and/or the Straits of Florida. In the Gulf, white sharks moved mainly from the Florida Keys north on the outer West Florida Shelf up to the Mississippi River, but some animals crossed into the western and southwestern GoM. In the Straits of Florida, sharks moved along the north coast of Cuba, where we have reports of other white sharks encountered in Cuban fisheries. These tagging and fisheries observations clearly demonstrate the high connectivity of the GoM with the U.S. and Canadian Atlantic coasts for the WNA white shark population.

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## Towards complete morphological descriptions of all reptile species

*Peter Uetz, Dustin Zeliiff, Sahil Sewani, Trang Ly, James Cahall, Yaa Adarkwa Darko, Alex Lupton, Olaf Voß*

About 14,000 species and subspecies of reptiles have been described. The Reptile Database now provides descriptions for about 8,000 species as well as photographs of more than 6,000 species. Links to GenBank for >8,300 species and to IUCN for 8,400 species provide DNA sequences and range maps for even more detailed descriptions.

In order to fully describe species, we have started to work on two further improvements.

First, a reference database of standardized images will allow users to directly compare species to each other. Our pilot project includes 16,000 high-resolution images of more than 1,000 species based on preserved specimens. Since these images do not show live colors, it complements the images of live images in the Reptile Database. However, we have also started to systematically collect color information on snakes to make this information searchable.

Second, we are working to convert textual descriptions of species into a structured database of traits, so that characters can be also compared and analyzed more systematically.

When integrated with geographic range maps, species identifications can be substantially simplified and made available also for non-experts. Finally, in combination with phylogenetic trees our dataset will allow detailed macroecological and other biological studies.

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## Big City Blues: The Effects of Urbanization on Western Fence Lizard (*Sceloporus occidentalis*) Body size and Ectoparasites

*Emily Urquidí, Breanna Putman*

As urbanization increases, animals are subjected to new stressors caused by human activity. If they adapt to these new conditions, animals are still left with limited land and resources. These harsh conditions along with novel stressors



may affect animal health. The health of animals can affect reproduction and survival (i.e., their fitness). Here, we compared body measurements from 145 lizards caught at two replicate urban and natural sites located in two different areas of Southern California: San Bernardino and Claremont. One sample was taken from an urban population, which consists of relatively high human population densities and high levels of impervious surface, and another from a natural population, which consists of mostly intact sage scrub habitat. We focused on measurements such as body size (SVL), body mass, body condition, and ectoparasites. Due to limited space and shifts in food availability caused by fragmentation, we predicted that urban lizards would be of smaller size, mass, and ultimately be in worse body condition. Some ectoparasites have complex life cycles, so we predicted that lizards from urban areas would have fewer parasites. We found that both the level of urbanization and study site affected SVL, as lizards in urban claremont had significantly lower SVLs compared to lizards in the natural claremont site. We also found there were fewer ectoparasites in urban areas. Lastly, we found that urbanization had no significant effect on body condition. In conclusion, our study shows that urbanization may affect certain aspects of health in western fence lizards.

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### **Parotoid Macrogland of *Phyllomedusa sauvagii* (Boulenger, 1882)**

*Paula Valchi, Maria Elena Ailin O'Donohoe, Eleonora Regueira, Lucila Nuñez Gorostidi, Alejandro Farias, Gladys Noemí Hermida*

Amphibian skin is a complex organ, rich in exocrine glands immersed in the dermis. According to morphological criteria, two types of glands are recognized: acinar glands (also known as mucous glands) and syncytial glands. The parotoid is a complex structure located in the postorbital- supratympanic (PoSt) region in several amphibians. It is involved in passive defense, an adaptation to avoid predators in frontal attacks. In particular, hylids are characterized by having macroglands in the mental and lateral regions, which are linked to a reproductive role. The aim of this study was to analyze the histomorphology of the PoSt region of *Phyllomedusa sauvagii*, a species of tree frog that is widely distributed in different regions of South America, and compare it with the dorsal skin. After histological analysis using light and scanning electron microscopy, it has been observed that the PoSt region is characterized by clustered large syncytial glands (LSG) disposed in close proximity to each other. The morphology and chemical nature of their secretion is similar to other glands distributed throughout the body. From this work emerges that the LSGs of the PoSt region of *P. sauvagii* have a secretion rich in lipids. This would be an indication that the parotoid would not only have a defensive role but could also contribute to prevent dehydration of the animal, being involved in the wiping behavior.

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### **Blunting a thousand cuts: integrated approaches to save Australian freshwater turtles**

*James Van Dyke, Donald McKnight, Deborah Bower, Ricky-John Spencer*

Australian freshwater turtles, like most turtles, have declined substantially for at least 50 years. One of the leading hypotheses of Australian turtle declines in the Murray-Darling Basin has been lost recruitment caused by high nest predation by invasive foxes. The fox hypothesis has been supported by both observations and experiments showing that nest predation rates exceed 95% in many regions, and with comparisons to other Australian systems where foxes are not present. Furthermore, intermittent population surveys have repeatedly found absences of juvenile turtles, and headstarting experiments have successfully replaced those juveniles. We are currently leading a nationwide citizen science program, 1 Million Turtles (1millionturtles.com), to engage local communities to protect turtle nests from foxes. Despite these efforts, we recently caught relatively large numbers of juvenile turtles at sites we study regularly that have not, to our knowledge, experienced nest protection. These observations coincide with three consecutive La Nina events, culminating in one of southeastern Australia's largest floods in 2022-23. Likewise, a study of turtle demographics in Australia and North America suggests that juveniles are difficult to detect, even in populations that have consistently recruitment. Modelling suggests that even small pulses of recruitment may be sufficient to maintain turtle populations in the face of fox impacts. Together, the preliminary evidence suggests that fox management is important, but that flood years

could also improve hatchling recruitment or detectability via other mechanisms, such as enhancing juvenile dispersal, improving food abundance, and providing hatchlings with access to shelter from aquatic predators.

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### **Is the Parallel Evolution of Large Body Size in *Anolis sagrei* the Result of Parallel Genomic Changes?**

*Alyssa Vanerelli, Jonathan Losos, Anthony Geneva*

*Anolis* lizards in the Greater Antilles are well-known for their remarkable ability to convergently adapt to similar environments. *Anolis sagrei* and its close relatives are in the trunk-ground ecomorph class, meaning that they possess adaptations that allow them to specialize in this specific microhabitat. While most species in this clade are medium-sized lizards with poorly-developed toepads, some populations have evolved a different phenotype. Populations from the Swan islands (*A. s. nelsoni*) and Cayman Brac (*A. s. luteosignifer*), where there are no other competing *Anolis* species, have independently evolved a significantly larger body size, more lamellae on their toepads, and an increased degree of sexual dimorphism. This is likely due to ecological release, as species without competitors are able to use different niche spaces than species with competitors. While the parallel evolution of these morphological changes has been well characterized, it is unclear if they have evolved via similar genetic mechanisms. In this study, we performed whole genome resequencing of these lineages and their close relatives to determine whether the same genetic changes underlie the parallel evolution of large body size. We use these genomic data and a recently published reference genome for *Anolis sagrei* to perform population genomic analyses to detect regions experiencing recent and recurrent natural selection in each population and then test whether the same regions have independently evolved in large-bodied populations.

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### **Long-term movements of silky sharks, *Carcharhinus falciformis*, in the Eastern Tropical Pacific Ocean**

*Jeremy Vaudo, Pelayo Salinas de León, Ryan Logan, Jenifer Suarez-Moncada, Bradley Wetherbee, Mahmood Shivji*

Despite the silky shark, *Carcharhinus falciformis*, being ubiquitous in tropical and sub-tropical pelagic waters and one of the most common species in the fish trade, little is known about its long-term horizontal movements. We tagged 40 adult and sub-adult silky sharks within the Galapagos Marine Reserve (GMR) with SPOT satellite tags during February and July 2021. Sharks were tracked for up to 602 days, with 12 sharks tracked for over 1 year. All but one shark left the GMR, however, sharks used the reserve all months of the year and individuals were often in the reserve for months at a time. The number of sharks present within the reserve per month tended to decrease over the course of the year, but increased during February and March 2022, suggesting the Galapagos Islands may serve as a seasonal aggregation site. During the cold season (Jun-Dec), female sharks leaving the reserve often displayed round-trip journeys to the west traveling up to ~4000 km from the reserve. These movements were latitudinally constrained, falling primarily between the equator and 5° N latitude, in an area with a deeper mixed layer; the westward and eastward components of these tracks aligned with prevailing currents. Male sharks did not make long directional movements. Overall, for a wide-ranging species, silky sharks spent a great deal of time within the protected waters of the GMR, but their movements extend far beyond the GMR and newly designated marine reserves, putting them at risk from commercial fisheries operating in the region.

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### **Evolution on the Forest Floor: Patterns of Diversification of Vipers (*Bitis*: *Macrocerastes*) in the African Rainforest**

*Eugene Vaughan, Eli Greenbaum, Wolfgang Wüster, Olivier Pauwels, Zoltán Nagy, Lotanna Micah Nneji, Abiodun Onadeko, Adeola Ayoola, Segun Oladipo, Vašek Gvoždík, Raffael Ernst, Laurent Chirio, Jean-François Trape, Chifundera Kusamba, Stefan Lötters, Aaron Bauer, Lauren McFarland, Vergie Musni, Philip Lavretsky*

The major biogeographical patterns characterizing the diversification of widely distributed species in the African rainforest remain poorly understood

because of a low number of well-sampled studies that are focused on this region. We present findings on the phylogeography and population genetics of two broadly sympatric sister lineages of large African forest vipers, the rhinoceros viper (*B. nasicornis*) and the Gaboon vipers (*Bitis gabonica* and *B. rhinoceros*). Despite their close relationship, these two lineages have notably different patterns of divergence in time and space. A time-calibrated phylogeny with sequence data from five genes, and population structure and PCA analyses of a SNP dataset show three major clades of *B. nasicornis*. Pleistocene refugia seem to play the primary role in their isolation, with the Congo and Ubangi rivers playing a potentially secondary role as a biogeographic barrier. Parallel datasets and analyses examining *B. gabonica* and *B. rhinoceros* demonstrate the Dahomey Gap as the barrier dividing the two species, with little population structuring within *B. rhinoceros*. The Niger River was shown to isolate a highly localized and very distinctive population of *B. gabonica* in southwestern Nigeria from all other populations of the species, which are otherwise very genetically similar over a vast span from south-eastern Nigeria to coastal Tanzania and South Africa. These results suggest that modern barriers (including rivers and the Dahomey Gap) and recent refugia likely play inconsistent roles in the population structuring of African rainforest species, likely depending on specific adaptations such as dispersal capability and degree of microhabitat specificity.

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### Natricine Snakes a non-model system in Macroevolutionary studies

*Deepak Veerappan, Natalie Cooper, David Gower*

Natricine snakes are morphologically diverse and currently distributed in Asia, Africa, Europe and north-central America (NCA). They are primarily semi-aquatic or ground-dwelling terrestrial snakes, but some are aquatic, burrowing, or aquatic and burrowing in habit and may be generalist or specialist in diet. Natricines are thus an interesting though understudied system to test biogeographic and diversification hypotheses, possibly using an ecomorphology framework. We included 80% of the 260 natricine species in a new phylogeny, and we collected natural history data on diet, habit and parity. We quantified morphological disparity in head shape among 191 species and applied phylogenetic comparative methods to test for convergence. Natricine snakes are of Asian origin, the lineage is roughly 40 million years (MYA) old. The monophyletic natricines in NCA dispersed there approximately 25 MYA and radiated, with higher diversification rates observed only after 11 MYA. Natricines in sub-Saharan Africa and Europe-Central Asia are depauperate compared to the Asian and NCA lineages. There are gaps in the data on natural history of natricine snakes especially for Asia. Natricine head shape is largely correlated with habit, but in some burrowers is better explained by dietary specialization. Convergence in head shape is especially strong for aquatic burrowing, semi-aquatic, and terrestrial ecomorphs and less strong for aquatic and burrowing ecomorphs. The ecomorph concept is useful for understanding natricine diversity and evolution, though would benefit from further refinement, especially for aquatic and burrowing taxa.

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### Algal Symbiosis in North American Ranids

*Zachary Vegso, Ryan Kerney, Sharyn Marks*

The symbiotic relationship between the chlamydomonad green alga *Oophila amblystomatis* and embryos of certain amphibian species has been documented for over 130 years. While the relationship is presumed to be mutualistic, the existence of a mutualism has only been experimentally tested and established in two closely related ambystomatid salamanders: *Ambystoma maculatum* and *Ambystoma gracile*. These experiments show a positive correlation between algal density and embryonic growth, survival, hatching synchrony, and hatchling body size. The embryos benefit from supplemental oxygen provided by the algae via photosynthesis, and the algae benefit from ammonia produced by the embryo, which is possibly used as a nitrogen source. *Oophila* has been documented within egg capsules of a growing number of amphibian species, including several frogs in the family Ranidae. However, the nature and extent of this relationship remains unclear. Ranid eggs are better oxygenated than ambystomatid eggs due to the presence of water channels flowing between individual eggs. Thus, the presence of mutualistic algae may be less important in ranid hosts compared

to ambystomatid hosts. We raised Northern red-legged frog (*Rana aurora*) and wood frog (*Rana sylvatica*) egg masses under three light treatments (24-hour light, 12:12 light:dark cycle, and 24-hour darkness) to test whether the symbiotic relationship between these ranid frog species and *Oophila amblystomatis* is a mutualistic one. Data analysis is ongoing, but preliminary analysis of *Rana sylvatica* data suggests that eggs raised in 24-hour darkness experienced decreased survival compared to lighted treatments, suggesting *R. sylvatica* embryos benefit from the presence of *Oophila*.

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### A Peep into Chondrichthyan Immunogenetics and its Relevance to Evolution, Management and Conservation

*Ana Veríssimo, Arnaud Gaigner, Tereza Almeida, Martin Flajnik, Yuko Ohta, Fabiana Neves, Antonio Munoz, Alessia Rota, Filipe Castro, André Machado, Pedro Esteves, Teresa Moura, Bárbara Serra-Pereira*

Chondrichthyans are the most basal living jawed vertebrates dating back ~400 Myr and occur in all aquatic environments, from fresh to salt waters and from shallow to deep waters. Their key phylogenetic position in vertebrate evolution and remarkable ecological diversity makes Chondrichthyans particularly important in studying the emergence and diversification of one of the most complex features of modern vertebrates – the adaptive immune system. This talk showcases the relevance of studying immune genes in Chondrichthyans to understand the evolution of this ancient group and to identify the ancestral and derived features of vertebrate adaptive immunity. At the species-level, immune genes may also be better at detecting biologically relevant population units compared to neutral markers since their genetic diversity is thought to be driven by pathogen-induced selection leading to locally adapted populations. Here, we focus on genes from Major Histocompatibility Complex (MHC), which is responsible for initiating adaptive immune responses against pathogen invasion and preventing infection and disease. Our results showed an unexpectedly high diversity of MHC class I gene lineages in Cartilaginous fish likely targeting different antigens and performing distinct functions, as well as ancient MHC class II lineages in sharks. Reconstructing the genomic architecture of MHC regions in Chondrichthyans also provided new information of the primordial adaptive immune complex of jawed vertebrate ancestors. Finally, we show that MHC genes exhibit increased intraspecific genetic diversity when compared to nuclear microsatellites in *Scyliorhinus canicula*, portraying a distinct pattern of genetic population structure with implications in fisheries management and conservation.

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### Xantusia revisited: Social network analysis of a kin-based social system in lizards

*Andressa Viol, Luis Zaman, Alison Davis Rabosky*

Social aggregations in reptiles represent convergent instances of sociality, reflecting adaptive benefits of grouping behavior across the tree of life. Extensive work on winter aggregations of desert night lizards (*Xantusia vigilis*) has uncovered a kin-based facultative social system, likely accompanied by thermal as well as social benefits. Here, we leverage network analysis to re-examine mark-recapture data collected over 5 years and 10 field seasons to further characterize social structure in this species and to test novel hypotheses. We use spatially- and temporally-informed null networks and node-based measures compared across individuals to better understand the consequences of sociality for this species.

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### Using Distance Sampling to Characterize Landscape-level Variation in Gopher Tortoise Densities on Avon Park Air Force Range, Florida

*Kameron Voves, Betsie Rothermel*

The Gopher Tortoise (*Gopherus polyphemus*) is a fossorial, burrowing species of the southeastern US Coastal Plain, where it occupies an array of upland habitats at widely varying densities. This natural variation in density has important implications for population dynamics and management of this threatened species, as well as the numerous commensals that use tortoise burrows. Line transect distance sampling (LTDS) surveys for Gopher Tortoises were initiated at Avon Park Air Force Range (APAFR) in 2009, the primary goal being

to obtain habitat-specific population densities. As in other parts of peninsular Florida, Gopher Tortoises at APAFR are primarily associated with fire-maintained mesic pine flatwoods and Florida scrub communities. Mesic flatwoods have abundant forage but high water tables that may hinder burrowing and nesting. In contrast, scrub has better-drained soils but relatively sparse forage. Our 2022 LTDS survey of scrub yielded an estimate of 0.80 adult tortoises/ha (95% CI 0.62 – 1.04) with a CV of 13%. In flatwoods, we implemented systematic transect placement and cluster-based analysis to improve model estimates given the patchy distribution of tortoises in this habitat and lower success of burrow scoping due to water in burrows. Gopher Tortoise density was lower in flatwoods than in scrub communities on APAFR, but overall abundance was higher because of the much greater spatial extent of flatwoods habitat. While spatial variation in Gopher Tortoise density within this large, naturally heterogeneous landscape provides insights into habitat quality and factors influencing ecological carrying capacity, demographic data are needed to clarify underlying population processes.

## Can scaling rattlesnake translocations to activity ranges improve mitigation outcomes?

*Jayne Waldron, Elizabeth Johnson, Kevin Oxenrider, Shane Welch*

Negative interactions between humans and venomous snakes are usually managed with translocation protocols that aim to mitigate risks from negative encounters. Venomous snake translocations are frequently scaled to public safety, such that release sites are located far from capture sites, regardless of habitat quality or the species' spatial ecology. However, translocation protocols that scale translocation distance to species' activity ranges have improved outcomes for rattlesnakes. To mitigate risks from human-rattlesnake encounters at popular recreation areas, we developed and implemented a translocation protocol scaled to Timber Rattlesnake activity ranges at two state forests in West Virginia. Using radio telemetry data collected from free-ranging rattlesnakes, we examined the effects of two translocation treatments (intra-range and extra-range) on movement, survival, and proximity to high-risk areas (e.g., trails, roads, and picnic areas). We placed snakes in translocation treatments (intra, extra, or control) one year into the study, allowing us to calculate baseline ranges for determining intra- and extra-range distances. We used mixed effects models to examine the effects of translocation on range area, daily movement, and proximity to high-risk habitats. We failed to detect an effect of translocation on activity range area and proximity to high-risk habitats. However, translocated snakes made longer, more variable daily movements than controls. Known fates survival models detected significant negative effects of body size and translocation on survival, with snakes in the extra-range treatment having lower survival. Our results highlight the importance of using biologically-relevant translocation scales based on the ecology of local populations when human-rattlesnake encounters warrant mitigation.

## Determining the Relationship Between Spectral Signals and Retinal Sensitivity in Dendrobatid Frogs

*Whitney Walkowski, Corinne Richards-Zawacki, William Gordon, Nicolas Bazan, Hamilton Farris*

Anuran visual behavior is perhaps best studied in the clade of Dendrobatoidea, as there is extensive variation in skin reflectance: perceived as a range from cryptic to bright coloration. These visual signals are important in intraspecific communication. Thus, we hypothesized that the visual systems of dendrobatids should also vary with, and perhaps be specialized for, reception of conspecific visual signals. Consistent with the matched filter hypothesis, we predicted that the retina of frogs will be tuned to portions of the visible light spectrum that match their body reflectance. Using full spectrum electroretinograms (ERGs), spectrometer measurements, and color calibrated photography, we compared the retinal sensitivity and reflectance of two cryptic species (*Allobates talamancae* and *Silverstoneia flator*), two intermediate species (*Colostethus panamensis* and *Phyllobates lugubris*), and two aposematic species (*Dendrobates tinctorius* and *Oophaga pumilio*). The retina of all species tested responded to light spanning the visible light spectrum and into the ultra-violet spectrum. The retinas of cryptic and intermediate species were more sensitive across the spectrum, but this effect was broadband and there was no evidence of spectral tuning to specific wavelengths. However,

spectral tuning was found to be different between morphologically distinct populations of *O. pumilio*, where Popa Island morphs exhibited retinal sensitivity that covaried with reflectance. This evidence for sensory specialization is particularly interesting given the rapid phenotypic divergence exhibited by this species and their behavioral preference for sympatric skin reflectances. Overall, this study suggests that retinal sensitivity and tuning is coevolving with reflective strategy and spectral reflectance in dendrobatids.

## Efficient Identification of Marbled Salamanders (*Ambystoma opacum*) Using Wild-ID

*Amadeus Waluga, Kira McEntire*

Being able to estimate population size and movement of individuals is essential for understanding if preserved areas are functioning as intended. Using pattern recognition is a common, non-invasive method to identify individual animals and estimate populations. Previous studies had great success using the program Wild-ID for small species of salamander with subtle pattern differences. Marbled salamanders, *Ambystoma opacum*, have very distinctive markings and can easily be evaluated for similarities in pattern, however previous studies suggested Wild-ID was less effective than other methods. We evaluated the potential to use Wild-ID for marbled salamanders by cropping the photos to the head, as has been done for other salamander species. Using 175 photos of adult marbled salamanders taken over three active seasons, at least 30 photos were detected as recaptures by Wild-ID in the first match. Compared to only 8 matches in the top 20 matches for the full body pictures. This method requires little photo manipulation and manual confirmation of matches suggest 100% detection of adults when cropped to the head. Further research is needed to determine the accuracy with juveniles and the youngest age when the markings become reliable identifying markers.

## The Restoration and Digitization of a Neglected Ichthyology Collection

*Rowan Ward, Emmy Delekta, Devya Hemraj, Autumn Magnuson, Morghan McCool, Matt Kolmann*

Natural history collections are crucial for understanding the world's biodiversity. These collections also hold important value in educating students about biodiversity, particularly phenotypic diversity. The University of Louisville (UofL) Department of Biology holds a natural history collection, a wet collection of preserved fishes, that appears to have been maintained up through the 1990s. However, much of the collection was relocated and what remains has been neglected for many years. Here, we describe our efforts to rehabilitate, revitalize, and digitize what is left of UofL's ichthyology collection - transforming it into a teaching collection for organismal biology courses. Ultimately, we hope this collection will be used as a teaching tool for ichthyology, vertebrate zoology, and aquatic biology courses. Our first priority was to triage damaged specimens, attempt to rehydrate desiccated specimens, and replace faulty jars. Next, we cataloged the surviving specimens, organized them, and rehoused them. We cataloged 228 species from 27 different orders and 36 different families. Specimens were collected from all over Kentucky and the southeastern US, as well as rare specimens (haplochromine cichlids) from Lake Malawi. Other specimens of interest include many troglodytic taxa and several specimens collected before they were classified as threatened, vulnerable, or endangered in Kentucky (lampreys, minnows, amblyopsids, and darters). Our next effort is to create a searchable database. In the future, we will dedicate sampling efforts to acquire representative specimens of all native Kentucky fish species.

## Testing a Connected Classroom Course-based Research Experience Using a Large-scale Salamander Research Network, SPARCnet

*Alexa Warwick, Louise Mead, Tracy Melvin, Kristine Grayson, Caitlin Fisher-Reid, Raisa Hernández Pacheco, Tanya Matlaga, Sean Sterrett, David Miller*

Understanding wildlife responses to climate change has never been more urgent. The Salamander Population and Adaptation Research Collaboration

Network (SPARCnet) was founded in 2013 to provide a consistent framework for understanding population trends of the Eastern Red-backed Salamander (*Plethodon cinereus*), while delivering education opportunities for students. It has grown to include collaborators at over 30 institutions. Researchers, educators, and citizen scientists apply the same cover-board plot study design and sampling methods across the geographic range of the salamander in eastern and north-central North America. Terrestrial salamanders in the genus *Plethodon* are often used in ecological studies as a key component of forest ecosystems, and indicators of forest health. Understanding larger scale spatiotemporal patterns in population dynamics in relation to climate requires expanding beyond studies at single institutions. We developed course-based undergraduate research experience (CURE) modules that engage students in SPARCnet through connected classrooms across different institutions. Here we describe the effects of CURE modules on students' knowledge of local ecosystems, climate change in general, and climate change effects on salamanders using pre-, post-learning correlative tests. Pre-, post-learning was assessed using Likert-scale questions on students' awareness of ecology, climate change, and species lists of local ecosystems. Overall trends from 229 students indicate learning increases in both recollection of number of species and phylogenetic/taxonomic diversity, and a deepening awareness of ecosystem-scale and study species climatic effects. Our results suggest that CURE modules can be an effective tool for both landscape scale climate research and training the next generation of scientists.

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### State of the “ZooMu Nation”: progress toward bridging gaps between zoos and museums.

*Gregory Watkins-Colwell, Alexander Shepack, Steven Whitfield, Sinlan Poo*

Like museums, zoos and aquariums hold a wealth of biological resources in their collections that are unique to these institutions. As modern zoological institutions transition from exhibits to conservation organizations, zoos are starting to focus on ways to enhance their contributions to the advancement of biological sciences. Driven by this need, in 2021 iDigBio hosted a 3-day workshop bringing together zoo and museum professionals and researchers in the larger scientific community to discuss plans for improved access to zoo collections and increased collaboration across institutions. Thus began the ZooMu initiative. These efforts catalyzed a much-needed discourse on the underutilization and appreciation for living and preserved collections and the tremendous capacity they hold for scientific research and biodiversity conservation. The similarities between traditional museums of natural history and living collections are numerous; including databases, archiving, biological sample collection and care, as well as the more obvious shared interests in biodiversity conservation and education. The cultures may differ, but zoos and museums share many goals. This presentation will summarize where things are now and what the next steps are.

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### Home Range and Movement Patterns of Ornate Box Turtle (*Terrapene ornata*) in Eastern New Mexico

*Rachel Weaver, Thanchira Suriyamongkol, Sierra Shoemaker, Joshua Gonzalez, Ivana Mali*

The ornate box turtle (*Terrapene ornata*) is a small terrestrial Emydidae with limited mobility and slow walking speeds. *Terrapene ornata* are well adapted to semiarid conditions and do not rely on standing water to survive; however, the species can still face issues with water retention. Due to the species' small home range and travel distances, human-related disturbances (i.e., habitat fragmentation) and climate change (i.e., exceptionally prolonged droughts) can greatly impact sustainability of ornate box turtles. Understanding species home range and movement patterns in response to these stressors is important for long-term management. We used radiotelemetry to study movement and home range of 10 ornate box turtles in the Great Plains region of eastern New Mexico from 2019 to 2022. The annual home range varied from 0.6 to 30.1 ha based on Minimum Convex Polygon and the mean inter-annual home range overlap was 27%. Average distance traveled between two consecutive points during their active season varied from 33 to 96 m, by which the longest average monthly movement was observed in July (75 m). The greatest changes in movement and home range occurred after a drought-related

wildfire in March 2022. We found that 50% of turtles significantly increased their home range by moving to the unburned areas in May immediately after coming out of hibernation. However, these turtles ultimately exhibited homing behavior by returning to their original activity centers by June 2022. We encourage future studies to continue evaluating how this species responds to exceptional drought in the southwestern United States.

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### Cutaneous Evaporative Water Loss of Lizards Consistently Acclimates in Response to Vapor Pressure Deficit

*Savannah Weaver, Evan Odberg, Tess McIntyre, Taylor van Rossum, Emily Taylor*

Testing acclimation plasticity informs our understanding of functional biodiversity and applies to conservation management amidst our rapidly changing climate. While there is a wealth of research on the plasticity of thermal and hydric physiology in response to temperature acclimation, there is a comparative gap for research on acclimation to different hydric regimes, as well as the interaction between water and temperature. To fill this gap, we exposed Western Fence Lizards (*Sceloporus occidentalis*) to different temperature and humidity conditions, and measured cutaneous evaporative water loss (CEWL) under common conditions before and after the experiment to assess plastic changes in CEWL. Lizards acclimated to humid conditions always experienced increased CEWL while lizards acclimated to dry conditions usually experienced decreased CEWL. Each combination of acclimation temperature and humidity conditions resulted in a unique vapor pressure deficit to which lizards were exposed, and change in CEWL among individuals across experiments was negatively related to treatment vapor pressure deficit. Exposure to high vapor pressure deficits (dry air) related to a decrease (negative change) in CEWL. This negative relationship between vapor pressure deficit and the acclimation response of CEWL mirrors the negative relationship between habitat aridity and adapted CEWL rates among species. The physiological plasticity we measured will be essential for predictive models of activity and survival for animals under climate change.

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### Fasted, Fed, or Dead? Dorsum, Ventrums, or Head? Cutaneous Evaporative Water Loss Differs By Physiology, Body Region, and Measurement Method

*Savannah Weaver, Shane Siers, Rachel Jolley, Jeried Calaor, Emily Taylor*

With the growth of physiology-based mechanistic species distribution models, variation in those physiological traits within an organism must be considered. We assessed how skin permeability to water loss is impacted by body region, feeding, and toxic baiting by measuring cutaneous evaporative water loss (CEWL) of Brown Treesnakes (*Boiga irregularis*) using a handheld AquaFlux evaporimeter. We also compared CEWL to total evaporative water loss (TEWL), measured using respirometry-hygrometry. The biggest differences in CEWL were across body regions: CEWL of the top of the head was higher than CEWL of the dorsum. The differences we found among body regions align with other within-organism comparisons and suggest that CEWL rates may reflect dense vasculature in the head related to blood supply to sensory organs or to cooling needs of the brain, or alternatively, water conserving needs of the dorsum. Snakes digesting a mouse meal did not have altered CEWL at any body region, indicating that CEWL might not be impacted by feeding. Finally, deceased snakes that had been experimentally fed lethal dosages of acetaminophen had lower CEWL of the head, but no difference in CEWL of the dorsum, relative to live, fasted snakes. This suggests that the mechanisms underlying CEWL rates must at least partially be structural, but that higher CEWL in the head may be actively controlled. These results add to the growing literature around the adaptation of and variability in CEWL.

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### Distinguishing Pattern from “Chaos”: Assessing the Structural and Functional Organization of Superficial Neuromasts in Fishes

*Jacqueline Webb*

The mechanosensory lateral line system of fishes is comprised of two types of receptor organs (canal neuromasts and superficial neuromasts, SNs) that



mediate the detection of unidirectional and low frequency water flows of biotic and abiotic origin. Vital staining with fluorescent mitochondrial stains (e.g., 4-di-2-ASP) has revolutionized our ability to simultaneously image all neuro-masts on the head and trunk demonstrating how SN distributions, in particular, have been grossly underestimated among fishes. SEM allows other features of SNs to be assessed (size, shape, hair cell orientation [=directional sensitivity to flows]) that define their functional organization. Using data for two taxa with proliferations of SNs (characids, gobiids) and an integration of data from the literature on SNs in a wide range of teleost taxa, a system was established to describe the hierarchical organization of SNs found among fishes re: groupings, arrangements within groupings, and in functional groups based on innervation.

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### **Influence of Tissue Type and Biological Information on the Development and Accuracy of Epigenetic Clocks in a Deepwater Scorpionfish**

*Nick Weber, Andrew Fields, Derek Chamberlin, William Patterson, David Portnoy*

Age estimation in fishes has traditionally relied on the analysis of growth zones in a variety of ageing structures, including otoliths, vertebrae, scales, and fin rays. Recent studies, however, have demonstrated that DNA methylation levels at certain CpG sites (cytosines followed by guanines) exhibit strong correlations with chronological age, allowing for the development of DNA methylation-based, age-predictive models referred to as epigenetic clocks. While the utility of epigenetic age estimation has been demonstrated for a handful of fishes, the ability to develop a single epigenetic clock from different tissue types and the influence of incorporating biological information into age-predictive epigenetic clocks are poorly understood. Therefore, we developed epigenetic clocks for a long-lived, deepwater scorpionfish, blackbelly rosefish *Helicolenus dactylopterus* (n = 61; age 9 to 60 years) using two different tissue types (fin clips and muscle). Bisulfite-converted restriction site-associated DNA sequencing (bsRADseq) was used to identify CpG sites that exhibit age-correlated DNA methylation, and epigenetic clocks were developed from 100s of CpG sites found within and across tissue types. In addition, we assessed whether the inclusion of sex and/or length data improves the accuracy of the age estimates obtained. Overall, results have important implications for future epigenetic clock development and application.

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### **The Perks of Being a Eupercarian: Rapid Skull Shape Evolution in a Massive Radiation of Bony Fishes**

*JoJo West, Rose Faucher, Elizabeth Miller, Guillermo Orti, Lily Hughes, Sandra Alvarez-Carretero, Giorgio Carnevale, Aintzane Santaquiteria, Emily Troyer, Samantha Gartner, Mark Westneat, Carole Baldwin, Ricardo Betancur-R, Dahiana Arcila, Kory Evans*

With over 6,000 species of teleost fishes, Eupercaria reigns supreme as one of the most spectacularly diverse clades of vertebrates, extant or otherwise. They have colonized all seven continents, their range extends to almost every aquatic habitat from freshwater to the deep sea, and they exhibit numerous distinct ecologies and morphologies. Accordingly, many questions remain about the mode and tempo of the Eupercarian radiation. Here, we use 3D geometric morphometrics, phylogenetic comparative methods and a novel phylogenetic hypothesis based on exon capture genomic data to quantify the tempo and mode of skull shape evolution across 600 species of Eupercarian fishes. We find a rapid burst in the rate of skull shape evolution and an increase in morphological disparity that roughly coincides with the KPG extinction event 66 mya, suggesting that this mass extinction event opened niches which ancestral eupercarians swiftly filled. We also find high rates of morphological evolution and disparity in Acanthuriformes and Tetraodontiformes while other clades like Perciformes and Labriformes exhibit intermediate levels of disparity and rates of skull shape evolution.

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### **'A human values issue': Actors, Arguments, and Regulatory Change in the Florida Land-based Shark Fishery**

*Julia Wester, Dylann Turffs, David Shiffman, Catherine Macdonald*

Recreational shark fisheries have been the subject of recent public attention because of the possible impacts on shark populations and conflicts between stakeholders. Associated conflicts are rooted in both the growing visibility of recreational fishing through new media (e.g., social media platforms) and in broader cultural shifts in attitudes towards wildlife generally. I present a case study based on discussions that took place in summer 2018 about potential changes to policies regulating recreational land-based shark fishing in Florida. Comments from public meetings and workshops (totaling 15.8 hours) and online comments (1,050) submitted to the Florida Fish and Wildlife Conservation Commission (FWC) were analyzed using the lens of Actor-Network Theory. The case study explores the intersection of conflicting stakeholder interests and types of knowledge, including how stakeholders variously defined the problem of user conflict in a coastal public space, and how they aligned themselves in relation to the problem and to each other. It further illustrates how shifting values and norms can intersect with changing technologies and environmental realities to produce new or increasing conflicts between natural resource user groups. This study underlines the importance and potential benefits of regulatory agencies explicitly and transparently engaging with value trade-offs when managing conflicts among users of natural resources.

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### **Differential contribution of historical processes to structuring of Central California populations of the desert night lizard, *Xantusia vigilis*.**

*Michael Westphal, Iris Holmes, Ivan Monagan, Paul Johnson, Alison Davis Rabosky*

We compared population structure and connectivity inferred from microsatellites and RAD loci among 17 populations of the desert night lizard, *Xantusia vigilis*, in Central California to test among historical processes structuring population-level genetic diversity. Both marker types enabled us to reconstruct population genetic structure in the region, including a major phylogenetic break maintained between two populations separated by less than 10 kilometers in the Cuyama Valley. Although either marker type could be used to understand generalized demographic patterns, populations exhibited varying levels of marker discordance in heterozygosity and allelic privacy. Rather than treating this discordance as a drawback, we instead leveraged it to resolve the effects of differential population histories across the landscape and test among otherwise indistinguishable biogeographic hypotheses. Our method thus provides powerful opportunities for leveraging marker discordance for analytical benefit, particularly for understanding the creation and maintenance of contact zones among clades.

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### **Comparative tank study of two species of Amphistichinid surfperches: the redbelt surfperch, *Amphistichus argenteus*, and the calico surfperch, *A. koelzi*.**

*Michael Westphal, Kristine Lesyna, Benson Chow, Mark Okihiro, Jesús Jimenez, Ken Oda*

Although the large, sandy-beach-dwelling surfperch of the family Embiotocidae / subfamily Amphistichinae compose an important commercial and recreational fishery, little has been published on their life histories or ecology. We collected a mixed gender sample of 15 adult fish of two species: redbelt surfperch, *Amphistichus argenteus*, and calico surfperch, *Amphistichus koelzi*, and kept them live in tanks for one year. Upon capture they were injected with a standard dye to facilitate the estimation of annual growth via otolith inspection. The redbelt surfperch showed aversive behaviors and experienced high mortality early on, while the calico surfperch seemed to adapt better to the tanks and experienced less, and later, mortality. We found parasites to be a potential cause of mortality, and successfully treated fish with a freshwater bath. We recommend immediate freshwater treatments upon capture for both species for further tank studies, and present preliminary data from otolith analyses.

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## Historical connections between river systems revealed through phylogeographic analysis of Western Blacknose Dace

*Courtney Weyand, Jonathan Armbruster*

The Western Blacknose Dace, *Rhinichthys obtusus*, is a wide-ranging species found throughout eastern North America. In 2004, the species was hypothesized as extirpated from the southernmost portion of its range, Black Warrior River (Mobile Basin) based on infrequent and singleton collections. Recently, multiple populations have been observed within the Sipsey and Locust Fork Rivers of the Black Warrior, both of which are close to the Tennessee and Coosa River systems. Several Tennessee aquatic endemics have been observed within the Black Warrior River, outside of their presumed ranges and it is suggested that transfer between the systems could be the result of stream capture, subterranean transfer, or human bait bucket introduction. Given the many recent finding of *R. obtusus* within the Black Warrior River, it is evident that the species is still occupying the system, but to what extent and the source of entry are unknowns. In this study, we used a RADseq dataset to identify the source population(s) of species within the Black Warrior, assess the timing of transfer, and determine if gene flow is still occurring. Preliminary analyses recovered three separate and historic transfer events, with the Sipsey Fork populations sister to the Tennessee River and the Locust Fork populations sister to the Coosa River. Additionally, two specimens from the upper Coosa (Conasauga River) were recovered as sister to neighboring Tennessee River populations. Overall, results from this study indicate that *R. obtusus* was likely never extirpated from the Black Warrior and populations may represent unique evolutionary lineages.

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## The Influence of Marine Protected Areas on Decadal Changes in Fish Diversity in the Center of Marine Diversity

*John Whalen, Rebecca Ruiz, Abner Bucol, Chris Bird, Kent Carpenter*

The Visayas Region (VR) of the Philippines is a global epicenter of marine fish biodiversity. Hypotheses attribute this peak of species richness within the Coral Triangle to processes that occur at geological time scales. However, changes in biodiversity may occur on ecological time scales, allowing for the assessment of the potential impact of anthropogenic activities. Low species richness in the VR has previously been associated with stressors such as habitat degradation and overfishing. Many marine protected areas (MPAs) have been established throughout the Philippines to mitigate the cumulative negative effect of these stressors. While most of the country's MPAs lie within the VR, this region has been termed the "epicenter of conservation adversity" within the Philippines. This study examines the effect of MPAs on changes in fish diversity in the VR between surveys conducted in the 1970s, 2010s, and 2020s. Individual- and sample-based species accumulation and rarefaction curves were used to create snapshots of species diversity from these distinct periods. Site-specific species richness and MPA indices (distance, size, age) were compared to determine the effect of MPAs on fish diversity. Results indicate that species richness decreased between the 1970s and 2010s then increased in the 2020s. Furthermore, there's a relationship between MPAs and species richness suggesting that species richness has increased over time with proximity to MPAs, potentially indicative of the spillover effect. In conclusion, this study suggests an effect of era and MPA on species richness over four decades in the center of marine fish diversity.

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## Evidence for Ecological Tuning of Novel Anuran Biofluorescent Signals

*Courtney Whitcher*

Our study aimed to assess the variability of amphibian biofluorescence to provide insight into its potential functions and role in anuran evolution. To do so, we discovered and documented patterns of biofluorescence in tropical amphibians. We nearly quadrupled the number of species to have been tested for this trait and our dataset added representatives from additional Anura families. We found evidence for ecological tuning (i.e., the specific adaptation of a signal to the environment in which it is received) of the novel anuran biofluorescent signals. Across groups, the fluorescence excitation peak matches the wavelengths most available at twilight, the light environment in which most

frog species are active. Additionally, biofluorescence emission spans both wavelengths of low availability in twilight and the peak sensitivity of green-sensitive rods in the anuran eye, likely increasing contrast of this signal for a conspecific receiver. With evidence of tuning to the ecology and sensory systems of frogs, our results suggest frog biofluorescence is likely functioning in anuran communication.

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## Sharks and Recreation: Characterising the Soundscape of an Urban Shark Nursery

*Clemency White, Catherine Hobbs, Ben Williams, Andy Radford, Matthew Witt, Catherine Macdonald, Steve Simpson*

Sound is an important cue in the marine environment, mediating behaviour across taxa. Human activity is increasingly modifying underwater soundscapes, reducing reproduction, modifying behaviour and changing movement in marine mammals, teleost fish and invertebrates. While our understanding of elasmobranch hearing is limited, it is suggested that they are primarily sensitive to low-frequency sound (10-1500Hz) in the particle motion domain. Many anthropogenic noise sources, including motorboats, are also low-frequency dominant, however the role of sound in ecology and the effects of anthropogenic noise in elasmobranchs are not well studied. Considering that shark-boat interactions occur in a variety of circumstances, including fishing, provisioning and depredation, there are valuable applications for developing this understanding. We begin to address this by describing the soundscape of the Biscayne Bay (Miami, FL), a described shark nursery with very high levels of boating activity. Through the deployment of hydrophones and particle motion sensors, we collected twelve weeks of acoustic data across the Biscayne Bay. We then applied machine learning algorithms to acoustic outputs to quantify the relative contributions and temporal trends of biological and anthropogenic noise. These sound recordings were consequently integrated into playback studies, played through underwater speakers alongside baited remote underwater video systems (BRUVS) to expose sharks in-situ to multiple boat noise treatments. Our results provide insight into if and how sharks detect and respond to boat noise and are used to develop discussions on why deepening our understanding of shark sensory ecology should be considered in their conservation.

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## Perceptions of scientific value of zoo-housed specimens by zoo and museum collections staff

*Steven Whitfield*

The living collections of zoos and aquariums hold millions of individual specimens across thousands of species. Zoos collect long-term records on individuals in their collections over their lives - including information on major life events (birth, reproduction, death), animal health and behavior data, pedigrees, etc. In some cases, zoos also collect biological samples from animals in their collection over their lives (blood, tissue, gametes, etc). Upon death, zoo-housed animals undergo a thorough necropsy and are typically then destroyed by incineration. An alternative to post-mortem destruction of specimens for some zoo-housed specimens is permanent deposition in natural history collections - ideally with any associated records and biological materials collected through the life of the individual. However, resource constraints will limit the number of zoo-housed specimens that can be transferred to natural history collections, and there have been no formal efforts to assess perceptions of scientific value of zoo-housed specimens. Here, we report the results of surveys of collection staff from zoos and museums to evaluate perceptions of scientific value of zoo-housed specimens. We use a survey instrument and focal interviews with collection staff at both zoos and museums to identify attributes of high-value zoo-housed specimens across vertebrate taxa, but with particular focus on fishes, amphibians, and reptiles. Based on survey results, we identify a set of best practices to facilitate transfer of specimens from living collections to traditional natural history collections. This research is foundational to the development of prioritization schemes for specimen transfer from living collections to traditional preserved natural history collections.

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## ZooMu Network: Integrating Zoos and Natural History Museums to Enhance Capacity for Biological Collections Research

Steven Whitfield, Sheila Poo, Gregory Watkins-Colwell, Alex Shepack

Zoos and natural history museums are both collections-based institutions with important missions in biodiversity research and education. Animals in zoos are a repository and living record of the world's biodiversity, whereas natural history museums are a permanent historical record of snapshots of biodiversity in time. Surprisingly, despite significant overlap in institutional missions, formal partnerships between these institution types are infrequent. Life history information, pedigrees, and medical records maintained at zoos should be seen as complementary to historical records of morphology, genetics, and distribution kept at museums. Through examining both institution types, we synthesize the benefits and challenges of cross-institutional exchanges and propose actions to increase the dialog between zoos and museums. With a growing recognition of the importance of collections to the advancement of scientific research and discovery, a transformational impact could be made with long-term investments in connecting the institutions that are caretakers of living and preserved animals.

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## Phylogenetic Analyses Reveal Potential New Species Within *Pseudopaludicola* (Anura: Leptodactylidae)

Molly Wierman, Grace Davis, João Tonini, Rafael de Sá

*Pseudopaludicola* consists of 25 described species distributed throughout South America. In this study, our goal is to investigate whether newly discovered populations of *Pseudopaludicola* from Colombia cluster with described species known to occur in the country, if they could be potential new species, or if they represent geographic extensions of species not previously reported in the country. Colombia is an incredibly biodiverse country with several phenotypic similar but genetic distinct species – cryptic species. There are four *Pseudopaludicola* species found in Colombia: *P. boliviana*, *P. ceratophyses*, *P. llanera*, and *P. pusilla*. We utilized DNA extracted from muscle and liver samples originating from different locations in South America and sequences four genetic markers, three mitochondrial and one nuclear. Then, we created phylogenetic trees using Maximum Likelihood and used species delimitation methods to identify potential new species. Our results confirmed that *P. boliviana* does not occur in Colombia. In addition, *P. pusilla* and *P. llanera* form monophyletic groups, with *P. llanera* exhibiting wide geographic range and high phylogeographic structure. Our results revealed one clade in the tree that is a potential undescribed species identified in the field as *P. boliviana*, *P. llanera*, and *P. pusilla*. Future work includes acquiring phenotypic data from the new clade to test whether it may potentially represent *P. ceratophyses*, which is unlikely due to geographical distance of this population to the type locality of the species, or if it represents a new species. In addition, we will add two more nuclear markers to the data set.

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## Selling Out Sawfish: Closing Loopholes in U.S. State-Level Protections that Leave Endangered Species at Risk

Tonya Wiley, Matthew McDavitt, Lukas Heath, Sonja Fordham

Wildlife trade is a major threat to marine species particularly the exceptionally threatened sawfishes. The long, toothed snouts (rostra) of sawfish have been valued around the world for centuries as decorations, weapons, and medicines. Their tendency to become entangled in fishing nets has been the primary factor in sawfish depletion. All five species (*Anoxypristis cuspidata*, *Pristis clavata*, *P. pectinata*, *P. pristis*, and *P. zijsron*) are classified as Endangered or Critically Endangered on the IUCN Red List and as Endangered under the United States (U.S.) Endangered Species Act (ESA). Despite ESA protections, the sale of sawfish rostra continues in the U.S. because of incomplete coverage and legal loopholes. The ESA only restricts interstate trade, leaving intrastate commerce regulation up to the states. Only 23 states currently prohibit the sale and trade of sawfish rostra. We documented 453 sawfish sale listings from around the world posted between March 2016 and August 2022; the U.S. is the top-ranked nation with 195 offerings. Rostra listings were found in 38 states; 45.1% of this activity was legal. To strengthen the country's network of sawfish safeguards, we outline specific improvements to inadequate state

laws. Other recommendations cover the need to raise the priority for publicizing and enforcing both state and federal sawfish protections. Minimizing the incentive to kill sawfish (or remove their saws) through stricter U.S. state bans on rostra trade and sale is most relevant for *P. pectinata*, the only sawfish species with a U.S. population.

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## Food fight? Assessing the trophic overlap of resident bottlenose dolphins (*Tursiops truncatus*) and coastal shark species off Sarasota, Florida

Krystan A. Wilkinson, Randall S. Wells, Robert E. Hueter, John Morris, Lisa A. Hoopes, Judith Bakker, Maurits P.M. van Zinnicq Bergmann, Sam Rossman, Theresa-Anne Tatom-Naecker, Valerie N. Hagan

The identification of shared prey resources among top predators can help disentangle complex trophic interactions which may have profound implications for ecosystem structure and function. Sharks and dolphins play critical roles in coastal marine environments as top predators. Sarasota Bay, Florida, is home to a long-term resident community of common bottlenose dolphins in addition to various shark species. Bull sharks and tiger sharks are thought to be the most frequent predators of Sarasota Bay dolphin residents, while other shark species – such as blacktip sharks and sandbar sharks – may be possible competitors for prey fish resources. However, shark-dolphin dietary overlap and the potential for sharks to engage in intraguild predation (where sharks can be both a predator and a competitor) with dolphins are unexplored in Florida Gulf waters. We used stable isotope, fatty acid and DNA metabarcoding analyses to investigate the trophic position, as well as isotopic and dietary niche overlaps, among bull, tiger, sandbar and blacktip sharks and compared them to information available for Sarasota Bay dolphins. Results indicated that resident dolphins primarily fed in seagrass environments, while the sharks fed in more open-water habitats. DNA metabarcoding results identified mullet, pinfish, and herring species – among others – in the diets of the coastal sharks. These prey species are also relatively frequent diet components of the local dolphin community. Overlap in prey could indicate some dietary competition between sharks and dolphins which may have consequences when prey resources are scarce, such as during harmful algal blooms.

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## Caecilian Vision

Mark Wilkinson

Caecilians are an ancient radiation of mainly fossorial amphibians with reduced visual systems (their name being a reference to their being blind). Adult caecilian eyes are small, covered by skin and sometimes by bone, with retinal cells that may only contain rod opsins, associated with scotopic (dim light) vision. I present an overview of what is known of the structure, function and evolution of the caecilian visual system. Morphological comparisons suggest different degrees of visual system reduction probably associated with different degrees of dedicated subterranean burrowing lifestyles, with independent losses of many components of the visual system or their modification to serve other functions. Spectral sensitivities of some caecilian photoreceptors have been determined with microspectrophotometry and visual pigment gene sequenced. Rod opsins regenerated in vitro with 11-cis retinal give pigments with spectral sensitivity peaks at 493nm or below, short-wavelength shifted in terms of the maximum absorption of light when compared with other amphibian lineages. The significance of this shift and its molecular mechanism remain to be determined. Because of the obvious potential for regressive evolution to lead to convergent losses that might confound morphological phylogenetics, comparative morphological data on caecilian eyes has been a focus for studies of methods of character coding and of detecting character independence. Additionally, these data have been used to address the utility of discrete character data in analyses of disparity. However, there are many uncertainties and potential inaccuracies in the underlying observations some due in part to histological artefacts. Some of these problems are illustrated.

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## **The Recovery Potential of Juvenile Nurse Sharks (*Ginglymostoma cirratum*) to Catch-and-Release Recreational Angling**

*Christopher Will, Jake Jerome, Julia Wester, Evan D'Alessandro, Danielle McDonald, Catherine Macdonald*

Overfishing has altered marine ecosystems, led to global population declines in coastal shark species, and increased concern about shark conservation. The nurse shark (*Ginglymostoma cirratum*) is a globally-distributed coastal species of elasmobranch assessed as "Vulnerable" by the IUCN. Although nurse sharks are considered resilient to fishing pressure, traits such as low standard metabolic rates, low fecundity, late-age of maturity, and relatively slow growth rates suggest they may be more vulnerable to pressures from fisheries, including recreational angling, than has been assumed. The stress response following capture in recreational fisheries can lead to a cascade of physiological changes and potential decreases in overall fitness. The speed of recovery from these stressors has rarely been examined in sharks but can inform recreational fisheries management. This study examines the recovery potential of juvenile nurse sharks to recreational angling by using indicators such as lactate and hematocrit to assess recovery from capture stress over time. Better understanding the length and intensity of physiological effects of recreational fisheries capture can help influence development of best practices and management recommendations around shore-based recreational angling of juvenile nurse sharks.

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## **Revision of Atlantic Shark Management in the United States**

*Ann Williamson, Guy DuBeck, Karyl Brewster-Geisz, Carrie Soltanoff*

The Highly Migratory Species Management Division of the National Marine Fisheries Service (NOAA Fisheries) is responsible for the management of the U.S. federal shark fisheries in the Atlantic Ocean, including the Gulf of Mexico and Caribbean Sea. Federal management of Atlantic sharks began in 1993, and the management measures established at that time are the basis for those in place today, including permitting and reporting requirements, management complexes, commercial quotas, and recreational bag limits. Over the years, NOAA Fisheries has implemented various management measures for Atlantic shark fisheries; however, the breadth of new science and information available for shark species today supports revision of domestic shark management. The goals of these changes are to increase management flexibility to react to additional factors impacting the Atlantic shark fisheries and optimize the ability for the commercial and recreational shark fisheries to harvest available, science-based shark quotas, to the extent practicable. The changes that are underway will continue to need strong scientific research and support.

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## **Assessing Diversity and Abundance of Seven Taxonomic Freshwater Fish Orders in The Guayas River Basin using New Genetic Reference Library**

*Hannah Willis, Olivia Schweikart, Caleb McMahan, Windsor Aguirre*

This study examined the use of the 16s locus from a new metabarcoding library to successfully amplify neotropical freshwater fishes native to Western Ecuador in metabarcoding applications. To demonstrate this, residual metabarcoding data from 2022 river-water samples (Chimbo, Milagro, and Taura) were analyzed for detection rate of seven taxonomic orders in the Guayas River Basin (Characiformes, Siluriformes, Cichliformes, Gobiiformes, Cyprinodontiformes, Gymnotiformes, and Perciformes). Data was adjusted for bias in primer binding to allow for estimations of taxonomic abundance at different sampling localities. The results of this study demonstrate the 16s locus to be effective for freshwater fish detection in Western Ecuador. The Oxford MinION nanopore sequencer was used in obtaining the environmental DNA sequences used in this study because of low read-cost and its accessibility for use in the field. Pairing the benefits of the nanopore with the scope of the biodiversity data obtained from this survey emphasizes the importance to increase the frequency at which metabarcoding surveys are performed on freshwater fish populations in Western Ecuador, particularly the Guayas River Basin.

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## **Evaluation of Physical Abnormalities in Freshwater Turtles of the Lower Pecos River, New Mexico**

*Emma Wilson, Laramie Mahan, Ivana Mali*

Due to their longevity and durability, turtles often function as indicators of environmental quality for the ecosystems they occupy. Existing research has seldom explored the prevalence of physical abnormalities in freshwater turtles, yet sublethal effects can provide insight into the extent of environmental stressors. The lower Pecos River has suffered anthropogenic disturbance through dam construction, channelization, and oil and gas production with the recent megadrought further affecting water quality, availability, and flow. These stressors can be manifested in physical abnormalities and cumulatively affect the fitness of freshwater turtles in this fragile ecosystem. We examined photographs of 884 turtles captured on the lower Pecos River, New Mexico in 2020 and 2021. We looked for signs of poor body condition such as missing limbs, abnormal shell shape, and shell lesions. We also recorded cases of melanism in males and abnormal scutellation. Of these, the most prevalent conditions were melanism (27% of males), abnormal scutellation (10%), and shell lesions (26%). 4% of turtles also exhibited dovetail syndrome. The most severe cases (i.e., abnormal shape and missing limbs) could be attributed to predation attempts or human-caused injuries including heavy agricultural machinery and boat propellers. Despite the relatively high prevalence of milder abnormalities, including dovetail syndrome, little information exists on their causes. We emphasize the importance of assessing body conditions while conducting freshwater turtle surveys and encourage future research to explore potential genetic and environmental factors that affect embryonic and post-hatching development of turtles.

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## **Cryptic species add to diversity of the acanthomorph fish genus *Polymixia* in the Atlantic, Caribbean, and Gulf of Mexico**

*Mark Wilson, Terry Grande, Carole Baldwin, Ross Robertson, Jesus Leal*

The primitive acanthomorph fish genus *Polymixia* (beardfishes) has long been studied anatomically because of its relevance to teleost phylogeny. Known species are demersal in moderately deep water over mostly soft substrates, which they probe with a pair of barbels. They inhabit continental slopes and flanks of seamounts and oceanic islands. Until recently, about 10 species of *Polymixia* were recognized, based on meristics and some morphological traits. Only two (*P. nobilis* and *P. lowei*) were known from the Atlantic, Gulf of Mexico, and Caribbean. However, multi-locus molecular sequences and DNA barcodes recently suggested at least two cryptic species exist in the region. The Bermuda Beardfish was named in 2021 as *P. hollisterae*, based on two specimens from Bermuda and one small juvenile from the Gulf of Mexico. A second cryptic species, also suggested by multi-locus sequences and barcodes, was revealed as a distinct group of *Polymixia* in the Caribbean off Panama. We report here further evidence for the existence of a southern Caribbean species, using meristics, external and internal morphology, radiographs,  $\mu$ -CT scans, and landmark-based multivariate morphometrics. Specimens of *Polymixia* spp. from museum collections allow us to distinguish individuals of *P. lowei* in the Gulf of Mexico and northwestern Atlantic from those of its formerly cryptic, sibling species in the southern Caribbean. Examination of numerous specimens has also revealed evidence for additional potential species. The Gulf of Mexico and the Caribbean are now known to be home to species representing at least three of the four main lineages within *Polymixia*.

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## **Functional Implications Reflected by Shape Variation in Neurocranial Morphology of Lamniform Sharks (Elasmobranchii: Lamniformes)**

*Jake Wood, Kenshu Shimada*

Lamniform sharks (Elasmobranchii: Lamniformes) display diverse habitat and diet preferences. Based on museum specimens and published illustrations, we examined the variation of neurocranial (skull) shapes across all extant lamniform sharks. We ran landmark-based geometric morphometric analyses to infer the importance and patterns of sensory organs to habitats and diets in lamniform sharks, where variation in skull shape influenced by the rostral,



olfactory, optic, and otic regions serves as a proxy of the relative importance of each sensory region (electroreception, olfaction, vision, and 'coordination') to its habitat and diet. Our study suggests that there are three basic skull shapes in lamniform sharks, an 'elongated-rostral' and 'reduced-optic' shape (Group 1; *Mitsukurina owstoni*), an 'intermediated-rostral-optic' shape (Group 2; *Carcharodon carcharias*, *Carcharias taurus*, *Cetorhinus maximus*, *Isurus oxyrinchus*, *I. paucus*, *Lamna ditropis*, *L. nasus*, *Megachasma pelagios*, *Odontaspis ferox*, and *O. noronhai*), and a 'shortened-rostral' and 'enlarged-optic' shape (Group 3; *Alopias pelagicus*, *A. superciliosus*, *A. vulpinus*, and *Pseudocarcharias kamoharui*), where all lamniform sharks have essentially one basic olfactory and otic region shape. Besides phylogenetic influences, we found that habitat and diet preferences in lamniform sharks are also broadly correlated with skull shapes where Group 1 is a sluggish species that feeds on midwater teleosts, cephalopods, and crustaceans, Group 2 sharks are largely represented by migratory species that feed on epipelagic food sources (e.g., zooplankton, teleosts, marine mammals), and Group 3 sharks are active species that feed on smaller teleosts and crustaceans. Our study provides new insights into the evolutionary history of lamniform sharks.

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### The Effects of Pesticides and Salinization on Brain Shape of Larval Northern Leopard Frogs

*Sarah Woodley, Gabriela Agostini, Devin Jones, Rick Relyea*

Wetland communities are increasingly threatened by contaminants such as pesticides and salinization. This is a concern because prior research has found that exposure to sublethal concentrations of an organophosphate insecticide (chlorpyrifos) altered the brain shape and behavior of larval and metamorphic amphibians. Using wetland mesocosms, we tested whether additional insecticides (two organophosphates, two pyrethroids, and two neonicotinoids) impacted tadpole (*Lithobates pipiens*) brain shape in the presence and absence of added salt (NaCl). We found that brain development in tadpoles exposed to chlorpyrifos altered larval brain shape, consistent with previous studies, but the other organophosphate pesticide (malathion) did not. Of the pyrethroid pesticides, permethrin, but not cypermethrin, altered brain shape. There was no effect of the neonicotinoids (imidacloprid and thiamethoxam) on brain shape. Thus, the impact of pesticides on brain shape was not dictated by the type of pesticide. Salt also altered brain shape, although the effect was strongest in the absence of pesticides. Together, our results indicate that environmentally realistic levels of pesticides and salinization have interactive effects on larval development. The changes in brain shape are likely associated with changes in behavior and potentially performance and fitness. Our study highlights the importance of studying the impacts of naturally-occurring levels of pesticides and other anthropogenic stressors on vertebrate neural development.

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### Effects of Invasive American Bullfrogs on Western Pond Turtles in Yosemite National Park

*Sidney Woodruff, Robert Grasso, Brian Todd*

Success of many conservation efforts for California's declining Western pond turtle (WPT) may hinge on the management of invasive American bullfrogs. Anecdotal evidence and some studies suggest that WPT populations are declining in part due to this voracious predator of hatchling turtles. Recent research has also uncovered another phenomenon—greater average carapace lengths for WPTs co-occurring with bullfrogs compared to populations where bullfrogs are absent. Given the call for research on WPT conservation strategies, coupled with concerns over the invasiveness of the bullfrog, we studied how the native turtle's population structure and body size are affected by the co-occurrence of bullfrogs and how bullfrog eradication may aid WPT recovery efforts. From 2016–2022, we monitored WPT populations with varying degrees of bullfrog presence using mark-recapture. In 2018, we began removing bullfrogs, recording data on bullfrog size, population structure, and stomach contents. Preliminary findings at sites without bullfrogs showed all WPT life stages (hatchling, juvenile, and adult), whereas sites with bullfrogs yielded little to no observations of hatchling and juvenile WPTs, except for two WPT hatchlings found in bullfrog stomachs during the first year of bullfrog removal. While analyses are ongoing, findings from this study will inform conservation management where increased recruitment is needed

to sustain WPT populations. Further, this study highlights how the removal of introduced species, though cumbersome, can be promising to aid restoration of a declining native species.

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### The four million year heist: The evolutionary history of unisexual salamanders and their relatives (genus *Ambystoma*)

*Parker Woodward, Rob Denton*

Unisexual *Ambystoma* are the oldest unisexual vertebrates known to exist, with multiple studies showing the emergence of the unisexuals to be between 4–6 million years ago. This single all-female lineage reproduces through a process called kleptogenesis, in which sperm of primarily two sexual species (*A. laterale* and *A. jeffersonianum*) is used to fertilize eggs and often introgresses into the unisexual lineage. We present new analyses of over 300 samples from all three groups across their entire range. We sequenced thousands of UCE loci to build comprehensive phylogenetic trees and conducted analyses to reveal how sexual *Ambystoma* have exchanged genetic information with their unisexual counterparts. We show that an in-depth examination of the deep evolutionary history of the unisexual *Ambystoma* provides strong evidence that kleptogenesis has varied widely over time and across geographic space. This work will be helpful in understanding the evolution of this unique reproductive mode and frames future hypotheses about the drivers of introgression into the unisexual lineage.

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### Using Baited Remote Underwater Video (BRUV) to Characterize Shark Nursery Habitat Use in a Low-Visibility Estuarine System

*Sarah Worthington, Jim Gelsleichter*

Recently, there has been increasing use of baited remote underwater video (BRUV) surveys for assessing the species composition and relative abundance of shark populations in marine ecosystems. This approach can be useful because it may provide a non-destructive, non-extractive, and cost-effective alternative to traditional fishing-based methods. However, to date, most studies using BRUVs have focused on their use in high-visibility ecosystems, such as tropical reefs. Furthermore, few studies have compared results from BRUV surveys with those obtained using traditional methods to evaluate the efficacy of this approach. Therefore, the goal of this study is to evaluate the effectiveness of using BRUV surveys to assess shark populations in low-visibility ecosystems. We will address this goal by comparing shark presence and abundance surveys conducted using BRUVs and bottom longline fishing in the Tolomato River, a northeast Florida estuary that has been shown to serve as communal nursery habitat for juveniles from multiple coastal shark species including the sandbar (*Carcharhinus plumbeus*) and the scalloped hammerhead (*Sphyrna lewini*). This presentation will showcase preliminary results from initial deployments of BRUVs in this estuary.

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### Unprecedented rates of parthenogenesis in ex situ zebra sharks

*Jennifer Wyffels, Kady Lyons, David Portnoy, Christine Dudgeon, Kevin Feldheim*

Parthenogenesis is known to occur across elasmobranchs in both viviparous and oviparous species. Cases of parthenogenesis have been primarily documented by happenstance when husbandry staff discover neonates in exhibits where adult females are housed in single-sex populations; therefore, the true rate of captive parthenogenetic births is unknown. This study focused on the zebra shark (*Stegostoma tigrinum*), an oviparous species commonly housed in aquariums where this mode of reproduction has been documented to occur, to quantify baseline rates of parthenogenesis. Over the course of one year, all yolked egg from 13 females across 7 institutions were incubated for several weeks to allow for visual identification of embryos in early development at the time of dissection. Tissue samples were taken for microscopy and genetic confirmation of parthenogenesis through microsatellite genotyping. Parthenogenesis was detected in all females and across institutions, regardless of male presence or absence. There was one exception, a

maturing female who laid only five eggs during her first year of laying where no embryos were identified either visually or through genetic testing of egg fluid. For institutions where all eggs were monitored for an entire year, the baseline rate of parthenogenesis was unexpectedly high, suggesting that this phenomenon occurs more often than previously recognized. The prevalence of this phenomenon may complicate conservation efforts aiming to release genetically-diverse offspring back into the wild.

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### Collecting DNA from Sharks using Non-Invasive Skin Sampling

*Lila Xenakis, Megan Winton, Greg Skomal, Toby Daly-Engel*

Elasmobranchs are ecologically important keystone predators, and many species are currently under threat. Genetic diversity is a known buffer against extinction risk, and understanding patterns in diversity can improve conservation measures. But sampling DNA from elasmobranchs remains both logistically difficult and invasive, especially large shark species that are among the most vulnerable. To address this issue, we developed and tested a new, non-invasive method of collecting DNA from elasmobranchs to enable both individual and population-level genetic testing. Using an extendable pole-mounted scrubby sponge, four white sharks (*Carcharodon carcharias*) found feeding on a floating dead whale were swabbed to collect DNA. Tissue samples were also taken using a conventional biopsy-tipped pole spear to compare sequencing quality between collection types. We extracted and amplified 800 bp of the D-loop portion of the mitochondrial control region, and we found that the resulting sequences were consistently clean, and the readability did not differ between collection methods. Similar results were obtained for nuclear microsatellites. With this new technique, researchers will be able to collect DNA from white sharks and other threatened species non-invasively via pole or indirectly via spear or arrow, ensuring that the animal is less impacted without sacrificing DNA quality.

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### Effects of urbanization on limb length and locomotion of Western Fence Lizards (*Sceloporus occidentalis*)

*Daisy Xiong, Angela Horner, Breanna Putman*

As the human population continues to increase, the need for urbanization also increases and challenges organisms to adapt at a rapid rate. An ongoing concern is to what degree urbanization negatively impacts organisms or whether these populations have the potential to adapt. In this study we measured Western Fence Lizard (*Sceloporus occidentalis*) morphology from relatively urbanized and natural locations in Claremont and San Bernardino, CA. We chose *Sceloporus occidentalis* because their geographical range extends throughout the entire Western United States, and this lizard species is known to adjust well to changing habitats and environments. Limb length in lizards has been shown to be highly correlated with locomotion and performance, while toe and claw length is correlated with clinging ability. Urban and natural habitats have different substrates; for example, urban habitats have a higher degree of smooth surfaces than natural habitats. In this study, we predict that urban lizards will show a decrease in limb length and toe lengths because living in urbanized habitats requires less use of climbing abilities and more time on flat, smooth surfaces. We also explored the relationships between different limb measurements and will be analyzing their correlation. This study is ongoing, but preliminary results suggest that the urban populations have longer limb lengths compared to their natural counterparts. We do not know of the long term effects that urbanization can have on the physiological abilities of these lizards, but we hope this study helps us to determine the degree in which urbanization is impacting organisms.

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### Evolutionary History and Population Genetic Structure of the Commercially Important Thorny Skate (*Amblyraja radiata*)

*Lei Yang, Pierre Lesturgie, John Denton, Shannon Corrigan, Jeff Kneebone, Stefano Mona, Gavin Naylor*

The thorny skate population continues to decline in US waters despite nearly twenty years of protected management. We have explored the use of genetic data to guide the management of this commercially important fish. Previous

studies have shown some evidence of regional genetic differentiation, but no samples have been examined from US waters. Additionally, two size morphs (large and small) co-exist in the Gulf of Maine, but no genetic explanations for this difference in size have been found to date. Here we analyzed complete mitogenome sequences from 527 specimens across the geographic range of the species. We also generated a high-quality reference genome for the thorny skate and sequenced whole nuclear genome for an additional 50 individuals sampled throughout the species range. Both the mitogenome data and nuclear data support the idea that the populations from the west (Gulf of Maine and Newfoundland) and the east (Greenland, Iceland, North Sea, and Norway) are well differentiated, while the Gulf of Maine and Newfoundland populations are only weakly differentiated. Ancestral range reconstruction results based on mitochondrial data suggest that there have been 28 historical East-West colonization events and two historical West-East colonization events. These events occurred within the last ~1 million years and have contributed greatly to the formation of the current population genetic structure of thorny skates. The mitogenome data was found to be inadequate to explain the evolution of the two size morphs. However, results from nuclear genome scanning have revealed the possible genetic basis for the different size morphs.

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### Impoundments be dammed: Assessing a threatened watersnake within different habitats of the Brazos River watershed, Texas.

*Jessica Yates, Stephen Harding, Dustin McBride, David Rodriguez, Matthew Kwiatkowski, Stephen Mullin*

Species with restricted ranges are among the most imperiled groups of organisms. Semiaquatic species are disproportionately affected by habitat alterations because they rely on both terrestrial and aquatic habitats. Brazos River Watersnakes (*Nerodia harteri*), endemic to north-central Texas, have one of most restricted geographic ranges of any North American snake. Since their description in 1941, there has been relatively little research on this species in comparison to its congeners. River impoundments likely threaten *N. harteri*, and a proposed reservoir in an upper portion of the watershed will further deprive this species of its preferred habitat. We quantified demographic parameters of *N. harteri* occurring in river and reservoir habitat from 2018-2021. Survivorship of *N. harteri* was poor, with individuals having a 2.14% chance of surviving year-to-year. Within riverine sites, adult females and neonates of both sexes were more likely to be encountered, contributing to a more even age-class distribution than that of reservoir populations. Combining our data with that of prior surveys, estimates of body condition improved over time, but the condition of individuals from reservoirs did not respond in similar fashion. Our results indicate that river impoundment has long-term effects on *N. harteri* and that, within intact riverine habitat, the species can be reliably detected. To promote the conservation of this threatened snake, long-term monitoring and habitat protection are needed, especially along the site of the proposed reservoir.

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### Diet of Deep-reef Lionfish in the Caribbean using DNA Metabarcoding

*Sarah Yerrace, Carole Baldwin, Ross Robertson, Katherine Maslenikov, Luke Tornabene*

Indo-Pacific Lionfish (*Pterois volitans*) are a well-established invasive species in the western Atlantic that have been observed on deep reefs down to ~300m. Lionfish presence on deep reefs is concerning given their negative impacts on biodiversity and recruitment seen on shallow reefs. Deep reef fish communities are poorly known, but preliminary studies indicate that deep reefs are home to many rare, undescribed, or highly endemic species, making them highly vulnerable to lionfish predation. Understanding what lionfish are eating and how they are moving on the reef is a necessary first step to understand what impact these foreign predators will have on rare photic communities in the Atlantic. Using spearfishing and a manned submersible, 137 lionfish were collected off the southern coast of Curacao down to 200m. Gut contents were identified using a combination of visual identification and DNA metabarcoding of the mitochondrial cytochrome c oxidase I gene. DNA sequences were matched to a custom database of 16,000+ sequences from fishes collected in the Western Atlantic. Preliminary results suggest that lionfish collected

on deep reefs are feeding on both deep- and shallow-reef prey species, suggesting some degree of movement of lionfish across the reef slope. Our results provide key insight as to the threats lionfish may pose to vulnerable deep-reef ecosystems, as well as the potential for deep-reef lionfish to be controlled by shallow-water culling efforts.

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## Are Two Heads Better Than One? Brain and Sensory Organization in a Dicephalic Blue Shark Embryo, *Prionace glauca*

*Kara Yopak, Omar Santana Morales, Dan Cartamil, Gary Cowin, Shaun Collin*

Dicephaly is a developmental anomaly, whereby the embryo is axially bifurcated and presents as conjoined twins, with two heads on a single body. Although this abnormality is rare, there are more descriptions in blue sharks (*Prionace glauca*) than any other shark species, likely given their global distribution, importance in several fisheries, and relatively high fecundity. However, no study to date has assessed patterns of brain organization in any shark species with this malformation. In this study, a dicephalic *P. glauca* embryo was incidentally collected from a pregnant female off Baja California, Mexico and imaged on a 9.4T Bruker Biospec small animal scanner, using contrast-enhanced, T1-weighted MR imaging. The brain, cranial nerves, and key sensory systems (olfactory rosette, eyes, semicircular canals) were segmented from the 3D data. Externally, the embryo has shared gill slits, trunk, umbilical opening, and a single coiled caudal fin. Anterior to the gills, there are two rostra, two mouths, four distinct nares, and four eyes, two of which that are fused just at the edge of the ocular cavity. Correspondingly, the brain shows four distinct olfactory rosettes and olfactory bulbs, two distinct telencephala, which fuses just beyond the mesencephalic midbrain. Posteriorly, the specimen has a partially bifurcated cerebellum, a single medulla, and a single set of semicircular canals. Three additional blue shark embryos of a similar developmental stage without malformations were scanned and assessed for comparison. Data on the dicephalic specimen will be contextualized within normal embryonic and ontogenetic brain and sensory development in *P. glauca*.

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## How Colourful is the Nocturnal World for Different Frogs?

*Carola Yovanovich*

Anuran amphibians are mostly nocturnal and have long been known to possess quite sensitive visual systems that allow them to navigate, feed and mate in dimly lit environments. The remarkable visual performance of nocturnal anurans relies partly on their possession of two different kinds of retinal rod photoreceptor cells, unequalled among vertebrates, which enables colour discrimination in extremely dim light in specific behavioural contexts, at least in some species. These unique ability and visual system configuration are equally relevant for the fields of visual neuroscience and amphibian's visual ecology. For the first, they provide a unique opportunity to study the involvement of rods in colour vision, and this phenomenon in itself can offer valuable insights into the driving forces shaping the evolution of signal processing circuitry involved in colour discrimination in general. For the latter, the suitability of the eyes of classical model species for nocturnal vision opens the question as to whether those of diurnal species share their features or have specific adaptations to enhance vision in bright light. In this presentation I will discuss the recent progress made on both fronts, from the use of model species to unveil the reach and limitations of colour vision abilities in anurans, to the range of retinal configurations and associated ocular features potentially enabling -or precluding- colour vision in anurans with different diel patterns.

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## A community genomics approach to natural hybridization

*Zachery Zbinden, Marlis Douglas, Tyler Chafin, Michael Douglas*

Hybridization is a complicated, oft-misunderstood process. Once deemed unnatural and uncommon, hybridization is now recognized as ubiquitous among species. But hybridization rates within and among communities are poorly understood despite the relevance to ecology, evolution, and conservation. To clarify, we examined hybridization across 75 freshwater fish communities within the Ozarks of the North American Interior Highlands

(USA) by SNP genotyping 33 species (N=2,865 individuals; ddRAD). We found evidence of hybridization (70 putative hybrids; 2.4% of individuals) among 18 species-pairs involving 73% (24/33) of study species, with the majority being concentrated within one family (Leuciscidae/minnows; 15 species; 66 hybrids). Interspecific genetic exchange—or introgression—was evident from 24 backcrossed individuals (10/18 species-pairs). Hybrids occurred within 42 of 75 communities (56%). Four selected environmental variables (species richness, protected area extent, precipitation [May and annually]) exhibited 73–78% accuracy in predicting hybrid occurrence via random forest classification. Our community-level assessment identified hybridization as spatially widespread and environmentally dependent (albeit predominantly within one large, diverse family). Our approach provides a more holistic survey of natural hybridization by testing a wide range of species-pairs, thus contrasting with more conventional evaluations.

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## Palatal Muscle Diversity in Reptiles and their Significance for Cranial Kinesis

*Christopher Zobel, Conner Verhulst, Anmol Sethi, Alec Wilken, Casey Holliday*

Cranial kinesis is highly diverse across vertebrates, from the completely akinetic skulls of crocodilians and turtles to the extremely kinetic skulls of snakes. This diversity is formed and powered by the assemblage of jaw muscles that control the jaws and palate. The mPPT (m. protractor pterygoideus) which attaches the pterygoid to the braincase, is thought to play a large role in cranial kinesis, along with the mLPT (m. levator pterygoideus) and mRPT (m. retractor pterygoideus) when they are present. Here we utilize DiceCT (iodine-contrast microCT) and Avizo Xfiber muscle fascicle tracking to reconstruct the architecture of the mPPT, mLPT, and mRPT in a broad sample of Reptilia to understand how they impact cranial function. We imaged, segmented, and modeled the muscles of species that employ a wide range of cranial kinesis, including snakes (*Agkistrodon contortrix*, *Morelia spilota*, *Pantherophis obsoletus*), lizards (*Anolis sagrei*, *Gekko gecko*, *Varanus exanthematicus*), and birds (*Anas carolinensis*, *Megasceryle alcyon*, *Psittacus erithacus*). We segmented each muscle and collected data on volume, attachments, resultants, fiber architecture and pennation. We found that these muscles greatly differ in pennation, resultants and moments, supporting our expectations that the muscles responsible for cranial kinesis are quite variable across Reptilia. Snakes have rostro-caudally oriented, parallel fibered mPPT, while lizards and birds have a greater variety of pennation and fiber orientations. In all these groups, the musculature powering cranial kinesis require additional study to understand their functional, ecological, and evolutionary significance.

# Abstract Book

## A

Abbott, Kevin.....	102
Abel, Dan C.....	35
Abel, Daniel.....	48, 66
Abercrombie, Debra.....	81
Abesamis, Rene.....	19
Able, Kenneth.....	3
Adams, Amy Yackel.....	47
Adams, Carissa.....	3, 45
Adams, Cory.....	3, 95
Adams, Dean.....	42, 60
Adamski, Jonathan.....	20
Adams, Lance.....	66
Afonso, Gabriel Vinicius Felix.....	11
Agostini, Gabriela.....	117
Agudo, Kandice.....	41
Agugliaro, Joseph.....	3, 64
Aguirre, Windsor.....	6, 99, 116
Ahmad, Norhayati.....	38
Ajei, Anjela.....	55
Ajemian, Matthew.....	8, 9, 18, 31, 70
Akin, Daniel.....	3
Akinrinade, Ayomikun.....	4
Albert, James.....	62
Alcala, Angel.....	19
Alda, Fernando.....	32
Alemdag, Melike.....	84
Alesker, Ron.....	4
Alexander, Mitchell.....	102
Alfonso, Noel.....	54
Allan, Beth.....	34
Allen, Larry.....	17
Alloway, Hannah.....	4
Almeida, Tereza.....	110
álvarez, Alicia.....	96
Alvarez-Carretero, Sanda.....	113
Alvarez-Carretero, Sandra.....	73
Amargós, Fabián Pina.....	108
Anderson, Alena.....	31, 89
Anderson, Brooke.....	4
Anderson, Carlie.....	5
Anderson, James.....	5, 65, 102
Anderson, Thomas.....	5, 91, 96
Andres, Alyssa.....	6
Anthony, Carl.....	6, 40
Apodaca, Joseph.....	3
Appel, Giulliana.....	87
Araújo, Malu.....	96
Araya, Kunio.....	38
Arcila, Dahiana.....	73, 113
Arguedas, Triana.....	18
Arifin, Umilaela.....	13
Armbruster, Jonathan.....	3, 6, 28, 105, 114
Arnett, John.....	6

Arnett, John Michael.....	105
Arnold, Kaleigh.....	6
Aronis, Ariana.....	81
Assad, Victoria.....	6
Assaoui, Meryem.....	38
Astley, Henry.....	107
Atkinson, April.....	53
Atkinson, Matthew.....	7
Atkinson, Matthew S.....	85
Aubert, Joseph.....	32
Aubrey, Benjamin.....	54
Augustine, Lauren.....	7
Austin, Adam.....	7
Austin, Christopher.....	14
Avrit, Amanda.....	59
Aydin, Ilhan.....	84
Ayoola, Adeola.....	109

## B

Babcock, Elizabeth.....	77
Bach, Elizabeth.....	42
Bahm, Tanner.....	7
Baiser, Benjamin.....	47
Baker, Patrick.....	8
Baker, Sarah.....	30
Bakkegard, Kristin A.....	8
Bakker, Judith.....	115
Baldesimo, Jemelyn.....	19, 39, 91
Baldesimo, Jemelyn Grace P.....	8
Baldwin, Carole.....	42, 73, 113, 116, 118
Ballard, Kirsty.....	9
Bangley, Charles.....	8
Banker, Sarah.....	5
Barber, Diane.....	57
Barbosa, Julia Laterza.....	62
Barcia, Laura Garcia.....	8
Baremore, Ivy.....	10
Barnes, Matthew.....	73
Barney, Joseph.....	29
Barry, Shannon.....	8
Barthelemy, Jacob.....	9
Bart Jr., Henry.....	18
Bartoszek, Ian.....	57
Bartram, Brian.....	88, 92
Bassos-Hull, Kim.....	9, 18, 39
Bataineh, Ola.....	101
Bauder, Javan.....	43
Bauer, Aaron.....	9, 20, 34, 63, 85, 86, 87, 109
Bauer, Aaron M.....	33
Bauer, Shelby.....	68
Baxley, Danna.....	51
Bazan, Nicolas.....	111
Beachy, Christopher.....	15
Bean, Megan.....	16, 31

Beard, Allyson.....	9
Beaupre, Steven.....	19, 64, 83
Beauvais, Tom.....	31
Becker, C. Guilherme.....	43
Bedard, Robin.....	9, 59
Bednarik, Nicole.....	10
Bedore, Christine.....	25
Beehler, Makenna.....	10
Beerli, Peter.....	5
Beeson, Mikayla.....	10
Behere, Ashmika.....	10
Belabut, Daicus.....	38
Belen, Janniry Cabrera.....	71
Bellquist, Lyall.....	80, 105
Bell, Rayna.....	4, 10, 37, 55, 60, 74, 78, 92, 98, 103
Bell, Rayna C.....	10, 14, 43, 87
Bemis, Katherine.....	42, 79, 99
Bemis, Katherine E.....	11, 75
Bemis, William E.....	11
Ben-Aderet, Noah.....	80
Bergmann, Maurits P.M. van Zinnicq.....	115
Bernard, Andrea.....	104
Bernardi, Giacomo.....	66
Bernstein, Justin M.....	11
Bernt, Maxwell.....	12
Berzins, Rebecca.....	103
Betancur-R, Ricardo.....	73, 113
Bevier, Catherine.....	12
Bicknell, Jake.....	28
Bielmyer-Fraser, Gretchen.....	27, 82
Bieri, Eli.....	12
Biesack, Ellen.....	12, 22, 95
Bird, Chris.....	39, 69, 91, 114
Bird, Christopher.....	19
Bird, Christopher E.....	8, 92
Bishop, Lacey.....	12
Bittencourt, Gabriela B.....	12
Blackburn, David.....	60, 100
Blackburn, Jason.....	47
Black, Corinthia.....	13
Blanco-Ortiz, Paula.....	42
Blanton, Rebecca.....	4, 26
Blinco, Kayla.....	80
Blouin-Demers, Gabriel.....	55
Blumsetin, Daniel.....	90
Boback, Scott.....	101
Bock, Samantha.....	56
Bodensteiner, Brooke.....	65
Bogan, Sophia.....	99
Boggio-Pasqua, Atlantine.....	9
Bogisich, Allison.....	13, 51
Bolaños, Federico.....	58
Bonilla-Johnson, Annais.....	13
Borstein, Samuel.....	26
Boswell, Kevin.....	104



## Author Index

Bourdeau, Paul.....	47	Burrows, Lee.....	17	Chakrabarty, Prosanta.....	14, 32, 66, 93, 102
Bowden, John.....	40	Butler, Brett.....	20	Chamberlin, Derek.....	113
Bower, Deborah.....	109	Byington, Russ.....	93	Chandler, Houston.....	20
Bowers, Beth.....	14	Byrd, Greg.....	9	Chang, Ann.....	60
Bowlby, Heather.....	4	Byrne, Allison.....	76	Chang, Belinda.....	68, 90
Boyce, Frederick.....	14, 33	Byrne, Michael.....	98	Chang, Belinda SW.....	55
Boyd, Charlotte.....	105	Byrum, Steven.....	18	Chang, Chi-Wei.....	20
Boyd, David.....	14			Chao, Ning Labbish.....	20
Boyette, John L.....	14			Chapman, Demian.....	75, 77
Boyle, Kelly.....	90			Charney, Noah.....	42
Bracken-Grissom, Heather.....	104			Chatfield, Matthew.....	12
Bracken, Jason.....	107			Chaves-Chaves, Noé.....	103
Bradke, Danielle.....	14, 15, 68			Chen, Meng-Hsien.....	20
Bradley, Jarius.....	15			Cherry, Kristen.....	29
Brady, Damian.....	83			Chilel-Lopez, Francis.....	21
Braker, Emily.....	59			Ching, Christian.....	21
Brandl, Simon.....	79			Chirio, Laurent.....	109
Brandt, Stephanie.....	26			Chow, Benson.....	113
Branham, Lauren.....	38			Church, Gabriella.....	88
Braun, Camrin.....	37			Ciccotto, Patrick.....	21
Braun, Raquel.....	15			Cicero, Carla.....	59
Brennan, Patricia.....	15, 57			Cinnamon, Haim Flores.....	53
Bressman, Noah.....	21, 26			Cisz, Kaitlyn.....	22, 75
Bretzing, Robert.....	16			Clark, Brett.....	74
Brewster-Geisz, Karyl.....	16, 116			Clark, Elizabeth.....	88
Brewster, Lauran.....	70			Clark, Rene.....	39, 91
Bright, Dani.....	38			Clark, René.....	19
Brill, Richard W.....	52			Clark, Scott.....	46
Brittany, Maldonado.....	15			Claunch, Natalie.....	18, 22
Britto, Marcelo.....	96			Clause, Justin.....	22
Brock, Tyler.....	15			Cleland, Timothy.....	62
Brodbeck, Bethany.....	16			Clerkin, Paul.....	22
Brodman, Robert.....	16			Cleveland, Danielle.....	54
Bronson, Allison.....	16			Close, Matthew.....	22
Brooks, Elizabeth.....	105			Cocks, Karina.....	81
Brooks, George.....	16, 20, 32			Coffey, Daniel.....	23
Brosnan, Erin.....	7, 85			Cohen, Jonathan.....	7, 63
Brothers, Ed.....	56			Collatos, Caroline.....	23, 59
Brouwer, Myra.....	17			Collazo, Jaime.....	92
Brown, Donald.....	9			Collette, Bruce.....	23
Brown, Rafe M.....	11			Collins, Angela.....	31
Brownscombe, Jacob.....	19			Collins, Cassandra.....	13
Bryant, Dylan.....	17			Collin, Shaun.....	119
Bryn, Samantha.....	67			Collins, Richard.....	56
Bubley, Walter.....	17			Colón-Piñeiro, Zuania.....	22, 48
Buckmaster, Nicholas.....	22			Comşa, L. Caden.....	23
Bucol, Abner.....	19, 39, 91, 114			Condez, Thais.....	23
Buehler, Matt.....	3			Condray, Cass.....	38
Buhlmann, Kurt.....	107			Connally, Neeta.....	6
Burbrink, Frank.....	47, 61, 81			Conrad, Hailey.....	24
Burge, Erin J.....	35			Converse, Paul.....	93
Burger, Isabella.....	57			Conway, Kevin.....	16, 31, 61, 88
Burger, Joanna.....	34			Conway, Kevin W.....	53, 83
Burgess, Tristan.....	12			Cook, April.....	24, 104
Burkhart, Jacob.....	5			Cook, Dave.....	82
Burns, Elizabeth.....	17			Cooke, Steven.....	19
Burriel-Carranza, Bernat.....	60			Cook, Ryan.....	24

## Author Index

Cooper, Natalie.....	110	Deary, Alison.....	78	Dye, Mysia.....	5
Cormier, Emily.....	24	Dedman, Simon.....	49	Dyke, James Van.....	109
Correa, Sandra.....	96	Dedrickson, Angie.....	51		
Correa, Vanessa.....	96	Deeds, Jonathan R.....	11	<b>E</b>	
Corrigan, Shannon.....	118	Degroot, Breanna.....	44	Earl, Julia E.....	32
Cortes, Enric.....	60	DeGroot, Breanna.....	9, 18	Easterling, Ian.....	57
Coster, Stephanie.....	58	Dehn, Jessica.....	29	Ebersole, Sandy.....	58
Cotton, Charles.....	74	Deitloff, Jennifer.....	29, 74, 102	Ebert, Sarah.....	3, 45
Cotton, Charles F.....	24, 51	Delekta, Emmy.....	111	Eckert, Ryan.....	18
Covill, Toby.....	25	DeLoache, Allison.....	29	Edelson, Charles.....	25, 32
Cowin, Gary.....	119	DeLoof, Bethany.....	29	Edwards, Daniel J.....	32
Cox, Christian L.....	43	Denommé, Melanie.....	29	Einig, Amanda.....	4
Crabtree, Delanie.....	78	Denton, John.....	16, 118	Elbassiouny, Ahmed.....	68
Craig, Chestina.....	25	Denton, Rob.....	117	Elías, Diego.....	37, 72
Craige, Lauren A.....	25	Denton, Robert.....	54	Elias, Diego J.....	93
Crampton, William.....	68	DeShazo, Jamie.....	53	Elias, Diego J.....	32
Crane, Derek.....	66	Devred, Emmanuel.....	24	Ellefsen, Hans-Frédéric.....	54
Crawford, Brian.....	14	Dheilly, Nolwenn.....	25	Ellison, Amy.....	62, 76
Crawford, John.....	30, 31	Diago-Toro, Maria.....	49	Emerson, Kyle.....	32
Crawford, Lisa.....	25, 32	Diaz-Lameiro, Alondra.....	30	Engler, Tori.....	32
Crear, Dan.....	25, 70	Diaz, M. Isabel.....	20	English-Clarke, Traci.....	18
Criscitiello, Michael.....	20	Dillman, Will.....	53	Erdmann, Mark.....	43
Crookston, Claire.....	15	Discher, Brooke.....	27	Eric, Hilton.....	11
Crovo, Jenna.....	44	Distel, Chris.....	49	Erkaya, Mustafa.....	33
Crowell, Hayley.....	26	Dodd, Jon.....	76	Ernst, Raffael.....	109
Crowe-Riddell, Jenna.....	26	Dodson, Thomas.....	30	Eroğlu, Oğuzhan.....	84
Crowther, Claudia.....	26	Doll, Andrew.....	59	Espindoola, Vinicius.....	33
Crum, Zach.....	26	Domínguez-Domínguez, Omar.....	55	Espinoza, Mario.....	68
Culley, Alexis.....	26	Donaldson, Linda.....	4	Esteves, Pedro.....	110
Cunningham, Connor.....	93	Donatelli, Cassandra.....	104	Ethridge, Abigail.....	91
Cunningham, Curry.....	81	Dorr, Laurence.....	80	Eto, Koshiro.....	38
Currylow, Andrea.....	47	Dougherty, Dean.....	9	Evans, Kory.....	73, 79, 113
Curtis, Tobey.....	25, 26, 59, 70	Doughton, Caitlin.....	30	Evans, Kristine.....	77
		Douglas, Marlis.....	119	Evers, Teah.....	6
		Douglas, Michael.....	119		
<b>D</b>		Douglas, Ron H.....	43	<b>F</b>	
Dacostagomez, Tatyana.....	27	Douppnik, Craig.....	66	Falk, Bryan.....	47
Dales, Jack.....	27	Drazen, Jeffrey.....	6	Falterman, Brett.....	104
D'Alessandro, Evan.....	116	Drennan, Julia.....	30	Falvey, Cleo.....	33
Daly-Engel, Toby.....	8, 10, 20, 100, 118	Dreslik, Michael.....	30, 31, 51	Farallo, Vincent.....	30
D'Antonio, Ashley.....	52	Driggers III, William B.....	34	Faria, Guilherme.....	72
Danylchuk, Andy.....	19	Driskell, Amy C.....	11	Farias, Alejandro.....	109
Darko, Yaa Adarkwa.....	108	Drymon, J Marcus.....	70	Farina, Stacy.....	33
Datovo, Alessio.....	90	Drymon, Marcus.....	8, 31, 77, 89, 105	Farrell, Terence.....	3, 14, 33, 64, 84, 87
Davenport, Jon.....	5	Duarte, Paula Sánchez.....	96	Farris, Hamilton.....	111
David, Dr. Solomon.....	102	DuBeck, Guy.....	116	Faucher, Rose.....	73, 113
Davis, Cameron.....	27	Duclos, Kevin.....	31	Faulkner, Lauren.....	33
Davis, Drew R.....	27, 41	Dudaitis, Vilma.....	97	Fawcett, Michael.....	82
Davis, Grace.....	27, 115	Dudgeon, Christine.....	117	Feinberg, Jeremy.....	34
Davis, Matthew.....	27, 41, 102	Dudley, Katie.....	65	Feinstein, Divia.....	34
Davis, Samara.....	42	Dunlap, Faith.....	31, 71	Feldheim, Kevin.....	105, 117
Davis, Samuel.....	27	Durkee, Steve.....	25	Fellenstein, Anna.....	88
Dawdy, Ashley.....	28, 87	Durso, Andrew.....	53	Fenner, Kelsey.....	34
Daye, Daniel.....	28, 40	Duryea, Katie.....	48	Fenwick, Allyson.....	34, 38
Dayer, Ashley.....	82	Dutton, Jessica.....	88, 92	Ferry, Lara.....	100
Day, Julia.....	100	Dutton, Peter.....	52	Fields, Andrew.....	16, 105, 113
Daza, Juan.....	49	Dye, Kayla.....	31		
DeArmon, Emily.....	27				

## Author Index

Fields, Andrew T. ....	34	Fuller, Keith .....	38	Goldspiel, Harrison.....	42
Findley, Kearstin .....	35	Fuller, Nicole .....	47	Gomes, Amanda .....	72
Fink, Allison .....	91			Gomez-Buckley, Marta.....	43
Finnegan, Thomas .....	69			Gonzalez, Flor.....	41
Finn, John.....	19			Gonzalez, Joshua.....	112
Finucci, Brittany.....	35			Goode, Matt .....	43
Firidin, Şirin .....	84			Gordon, William .....	111
Fischer, Chris .....	8, 53, 108			Gorostidi, Lucila Nuñez.....	109
Fischer, Eva.....	76			Gower, David .....	4, 10, 37, 74, 98, 100, 103, 110
Fischer, George C .....	37			Gower, David J .....	10, 87
Fisher-Reid, Caitlin.....	111			Gower, David J .....	14, 21, 43
Fitak, Robert .....	84			Gragson, Daniel.....	44
Fitzgerald, Lee .....	95			Graham, Jasmin.....	18, 44, 65
Fitz, Kyra.....	19			Graham, Rachel.....	10
Flajnik, Martin.....	110			Grande, Terry.....	31, 116
Flamio Jr., Richard .....	49			Grant, Evan.....	35
Flanigan, Kristina.....	35, 66			Graser, Bill.....	42
Fleming, Jill.....	35			Grasso, Robert.....	46, 117
Fleming, Jillian.....	74			Gratwicke, Brian .....	44, 62, 76
Flemming, Adania .....	35, 36			Graves, John E. ....	71
Flemming, Joanna Mills .....	8			Gray, Chelsea.....	44
Flores, Nicko Amor L .....	11			Gray, Emily.....	3
Floyd, Michael.....	26			Gray, Emily R.....	45
Fogg, Alexander.....	9			Gray, Jaimi.....	100
Folfas, Edita.....	36, 77			Grayson, Kristine .....	58, 80, 111
Fong, Vicky .....	86			Greenbaum, Eli.....	9, 109
Fontenelle, João Pedro .....	68			Green, David .....	36, 45
Fontenot, Dr. Clifford .....	9			Green, David M.....	56
Fordham, Sonja.....	36, 115			Grey, Erin.....	42
Ford, Jessica.....	36			Grient, Jesse van der.....	6
Foust, Isabella.....	36			Griffin, Lucas.....	19
Fox, Dewayne.....	29, 69, 99			Gros, Gaelyn.....	45
Fox, Gerad.....	70			Gross, Joyce.....	60
Frable, Benjamin.....	41			Grubbs, Dean .....	8, 28, 29, 74, 82, 87, 100, 105
França, Rayane .....	72			Grubbs, Faye.....	88, 92
France, Ethan.....	11			Grubbs, R. Dean.....	24, 27, 40, 45, 51, 81, 89
Franco, Ana Clara Sampaio .....	96			Grullon, Madelene .....	45
Franklin, Michael .....	17			Guertin, Jeffery .....	9
Franks, Bryan.....	8, 27, 37, 53, 82, 108			Gull, Henderson .....	33
Frank, Tamara .....	6, 104			Gumm, Jennifer .....	98
Fraser, Gareth.....	18			Gutiérrez, Ángela.....	96
Frazier, Bryan.....	8, 14, 27, 37, 40, 72			Gutierrez, Elmer.....	46
Frazier, Bryan S. ....	34			Guttridge, Annie E.....	49
Frederick, Cheryl.....	12			Guttridge, Tristan L. ....	49, 89
Frederick, Lindsey.....	59			Gvoždík, Vašek.....	109
French, Martin.....	19				
Frey, Amy .....	52				
Friedman, Matt .....	59				
Friedman, Sarah.....	4				
Friess, Claudia .....	17				
Frishkoff, Luke .....	36, 37, 77				
Fuentes-Montejo, César .....	37				
Fujita, Matthew .....	4, 10, 37, 74, 77, 92, 98, 103				
Fujita, Matthew K .....	10, 87				
Fujita, Matthew K. ....	14, 43				
Fujiwara, Kyoji .....	38				
Fukuyama, Ibuki.....	38				

## G

## H

## Author Index

Halstead, Brian .....	46	Hermida, Gladys Noemí .....	109	Hussain, Lameace .....	54
Halvorsen, Mark .....	106	Herod, Tristan .....	50	Hussey, Nigel .....	15, 37, 53
Hamadou, Radhouane.....	33	Herrera, Juliana.....	96	Hutt, Cliff .....	70
Hamidy, Amir.....	38	Hess, Marta.....	51	Hyatt, Michael .....	76
Hamilton, Blake.....	87	Hickerson, Cari-Ann .....	6, 40		
Hamilton, Marlena.....	46	Higgs, Jeremy M. ....	34		
Hammerschlag, Neil.....	8, 89	Higham, Rebecca.....	12		
Hampton, Cecilia .....	39	Hileman, Eric .....	9		
Hanafi, Norhafiz.....	47	Hillard, Cory .....	51		
Hancock, Gabriella.....	65	Hillard, Scott .....	80		
Hanifin, Charles.....	86	Hilton, Eric .....	22, 71, 84		
Hanscom, Ryan .....	43	Hilton, Eric J. ....	51, 75, 76		
Harding, Stephen.....	118	Hinck, Jo Ellen .....	54		
Harman, Madison.....	40, 47	Hiner, Blaine .....	51		
Harman, Rose .....	47	Hitchens, Casey.....	45		
Harms, Craig A. ....	95	Hobbs, Catherine.....	114		
Harper, Shawn .....	10	Hobbs, Jean-Paul .....	66		
Harrington, Richard.....	47	Hobel, Gerlinde .....	18		
Harrington, Sean.....	25, 47	Hoeflich, Jennifer.....	51		
Harris, Rachael.....	48	Hoekstra, Luke .....	26		
Hartmann, Arik .....	22, 31, 48, 71	Hoffmayer, Eric R. ....	98		
Hart, Pamela.....	73	Holden, Courtney .....	4		
Hartzheim, Alyssa.....	48	Holding, Matthew .....	51		
Harvey, Guy.....	28	Holguin, Charlie.....	52		
Harvey, Jessica .....	28	Hollenbeck, Christopher.....	16, 31, 62		
Haskell, Craig.....	93	Hollenbeck, Christopher M.....	89		
Hasselman, Daniel .....	8	Holliday, Casey .....	119		
Hassinger, Alyssa.....	5	Holloway, John.....	3, 45		
Hastings, Philip.....	80	Holmes, Iris .....	113		
Hata, Harutaka.....	48	Hoopes, Lisa A. ....	115		
Haulsee, Danielle .....	29, 69	Hopkins, Gareth .....	52		
Hauser, Frances.....	68	Hopkins, William .....	16, 82		
Hauzman, Einat.....	43	Horne, John.....	52		
Haverkamp, Silke.....	43	Horner, Angela .....	118		
Hawkins, Noah .....	49	Horodysky, Andrij Z. ....	52		
Hawk, Rileigh.....	48	Horswill, Cat.....	58		
Haxton, Tim .....	54	Horton, Mackenzie.....	53		
Hayden, Elise.....	49	Horvath, Shannon .....	53		
Haydt, Natalie .....	49	Hossman, Yazid .....	38		
Hayes, William .....	70	Hostetter, Nathan.....	92		
Haynes, Laura .....	51	Howard, Owen.....	6		
Head, Alyssa.....	65	Howey, Christopher .....	53		
Heath, Lukas.....	115	Hrabik, Robert.....	40		
Hedrick, Brandon .....	57, 65	Huang, Junman .....	49		
Heim, Vital .....	49	Hueter, Robert.....	37, 53, 108		
Heinicke, Matthew.....	12, 49	Hueter, Robert E. ....	115		
Heist, Edward.....	49	Hughes, James.....	8		
Heithaus, Michael .....	8, 105	Hughes, Lily.....	73, 113		
Hemraj, Devya.....	111	Hughes, Rachel.....	8		
Hendon, Jill M. ....	34	Hugi, April.....	79		
Hendricks, Kimberly .....	17	Hull, Kim Bassos.....	44		
Hendrickson, Dean.....	55	Hull, Peter .....	9		
Henen, Brian.....	80	Humphrey, Anna.....	53		
Hengstebeck, Kodiak .....	50	Hunt, Elizabeth .....	16, 88		
Henn, Dakotah.....	50	Hunt, Elizabeth P. ....	53		
Heptinstall, Tucker.....	50	Hunter, Briar .....	53		
Hereford, Scott.....	51	Hunter, Elizabeth S. ....	11		



## Author Index

Julian, Anja.....	22	Kouete, Marcel.....	100	Litmer, Allison.....	64
Julien, Allison.....	57	Kresge, Colby.....	60	Lituma, Christopher.....	9
Julkowski, Katherine.....	76	Krochmal, Aaron R.....	41	Liu, Juan.....	51, 63, 65
Jurestovsky, Derek.....	107	Krueger, Caleb.....	60, 61	Liu, Kathy.....	65
<b>K</b>		Kruizenga, Gretchen.....	61	Liu, Kwang-Ming.....	79
Kacev, Dovi.....	105	Kubel, Jacob.....	42	Liu, Linlin.....	79, 80
Kaefer, Igor.....	87	Kubicek, Kole.....	61	Liu, Min.....	64
Kaganer, Alyssa W.....	42	Kuchta, Shawn.....	61	Liu, Ming.....	20
Kajiura, Stephen.....	14	Kudson, Haley.....	46	Liu, Xinchun.....	103
Kaloczi, Juliana.....	4	Kuhn, Arianna.....	61	Lively, Curt.....	74
Kamouyiaros, Maria.....	12	Kunz, Bethany K.....	54	Livingston, Elizabeth.....	81
Karns, Daryl R.....	11	Kupferberg, Sarah.....	61	Livingston, Ethan.....	65
Kasana, Devanshi.....	77	Kusamba, Chifundera.....	109	Lizaso, Christian.....	38
Katz, Rachel.....	103	Kutos, Steve.....	62	Ljustina, Oliver.....	65
Keall, Susan.....	79, 80	Kuznar, Shannon.....	62	Loader, Simon P.....	12
Keck, Benjamin.....	4	Kwiatkowski, Matthew.....	98, 118	Lochetto, Stephen.....	65
Keeffe, Rachel.....	57	<b>L</b>		Loew, Ellis.....	10, 37, 103
Keller, Bryan.....	57	Labare, Michaela.....	62	Logan, Ryan.....	109
Kellner, Kenneth.....	107	Laclaire, Linda.....	51	Lo, Javon.....	8
Kelso, Sarah.....	57	LaDuc, Travis J.....	41	Long, Dede.....	65
Kemp, Ashlynn.....	57	LaFond, Jacob.....	40	Longo, Ana.....	22, 31, 48
Kern, Claudia.....	58	Lameiro, Alondra Díaz.....	4	Longo, Ana V.....	71
Kerney, Ryan.....	110	Landa, Mario.....	99	Looney, Blake.....	52
Kesling, Joshua.....	52	Lane-Harvard, Liz.....	34	López-Fernández, Hernán.....	79, 108
Kessler, Ethan.....	30	Langer, Emily.....	62	Lopez, Ivan.....	19, 39
Kilfoil, James.....	37	Lange, Zach.....	77	Losos, Jonathan.....	109
Kim, Daemin.....	58, 88	Lanoue, Katherine.....	62	Lötters, Stefan.....	109
Kim, Sora.....	65	Latour, Robert.....	82	Lotze, Heike.....	24
Kindsvater, Holly.....	16, 24, 52, 58	Lattanzio, Matthew.....	17, 46, 63, 98, 102	Lovejoy, Nathan.....	68, 90
King, Richard.....	42	Lavoie, Camille.....	87	Lowe, Christopher.....	33, 68, 73, 102
Kinney, Ian.....	69	Lavoué, Sébastien.....	48	Lowe, Christopher G.....	55, 65, 91, 96
Kinney, Michael.....	105	Lavretsky, Philip.....	109	Lowerre-Barbieri, Susan.....	39
Kiviat, Erik.....	34	Lawrence, J.P.....	97	Lowndes, Ryan.....	35, 66
Klank, Jeremy.....	58, 103	Leaché, Adam.....	92	Ludt, William.....	66
Kleeman, Patrick.....	46	Leal, Jesus.....	116	Luer, Carl.....	66
Klinard, Natalie.....	80	Lee, James.....	51	Luo, Xiao-Zhu.....	60
Klocke, Blake.....	44	Lee, James R.....	13	Lupton, Alex.....	108
Kneebone, Jeff.....	23, 29, 59, 118	Lee, Justin.....	63	Lyakurwa, John.....	12
Knight, Marissa.....	58	Leek, Grady.....	46	Lyons, Kady.....	25, 66, 117
Knotek, Ryan.....	59, 89	Lemmon, Alan.....	5	Ly, Trang.....	108
Koeller, Krista.....	59	Lemmon, Emily Moriarty.....	5	Lytton, Adam.....	17
Kohilakis, Melina.....	88	Leong, Derrick.....	63	<b>M</b>	
Kolmann, Matt.....	104, 111	León, Pelayo Salinas de.....	109	Macdonald, Catherine.....	15, 65, 66, 113, 114, 116
Kolmann, Matthew.....	59, 68	Leontiou, Andrea.....	63	Macdonald III, Kenneth S.....	11
Komoroske, Lisa.....	105	Leow, Chi Jing.....	56	MacFarlane, Zira.....	67
Kong, Brandon.....	59, 101	Lesbarrères, David.....	53	Machado, André.....	110
Koo, Michelle.....	60	Lesturgie, Pierre.....	118	Machado, Sheila Rodríguez.....	93
Koo, Michelle S.....	59	Lesyna, Kristine.....	113	Mack, Mariah.....	5, 67
Koppetsch, Thore.....	60	Lewis, Johanne.....	25	MacKnight, Maisie.....	67
Kornfeind, Scott.....	45, 60	Lewis, Levi.....	17	MacKnight, Maisie G.....	67
Kortyna, Michelle.....	5	Liedtke, H. Christophe.....	43	MacLeod, Leo.....	67
Kotlinski, Nicholas.....	28	Lim, Burton.....	28	Macneil, M. Aaron.....	77
Kotsakiozi, Panayiota.....	88	Li, Mi-Ling.....	7	Macpherson, Esme.....	68
Kouba, Andrew.....	57	Linardich, Christi.....	27, 64, 77	Madave, George.....	29
Kouba, Andy.....	98	Lin, Baian.....	64	Maddin, Hillary.....	23
Kouba, Carrie.....	57, 98	Lind, Craig.....	3, 64	Maddock, Simon.....	100

## Author Index

Maddox, Dylan.....	72	McCarthy, Elizabeth.....	68	Moniz, Haley.....	59, 74
Madrigal-Mora, Sergio.....	68	McCauley, Shannon.....	67	Monroe, Alison.....	62
Maerz, John.....	14, 15, 68	McClanahan, Ara.....	10	Montalvo, Miguel.....	75, 84
Magnuson, Autumn.....	68, 111	McClelland, Sara.....	45, 60, 71, 78	Montgomery, Chad.....	75
Magnuson, Sharon.....	19, 69	McCool, Morghan.....	111	Mooney, T. Aran.....	95
Magnuson, Sharon F.....	92	McDavitt, Matthew.....	115	Moore, Jennifer.....	75
Magoulick, Daniel.....	35	McDonald, Danielle.....	116	Moore, John.....	75
Mahan, Laramie.....	116	McDowell, Jan.....	12, 22, 95	Moore, Jon.....	104
Mahler, Luke.....	36	McDowell, Jan R.....	71	Morales, Cristian Hernandez.....	49
Maier, Paul.....	80	McElroy, Anne.....	25	Morales, Omar Santana.....	119
Maisey, John.....	16	McEntee, Kela.....	66	Morgan, Thomas D.....	108
Malabag, Marial.....	19, 91	McEntire, Kira.....	111	Morris, John.....	75, 115
Mali, Ivana.....	97, 112, 116	McFarland, Lauren.....	109	Moss, Jeanette.....	76
Malin, Pinsky.....	19	McGowen, Michael.....	10	Motomura, Hiroyuki.....	38
Malone, Hunter.....	4	Mcgrath-Blaser, Sarah.....	48	Mott, Cody.....	9
Mamoozadeh, Nadya R.....	71	McGrath, Patrick.....	71	Motz, Noah.....	69
Mangel, Marc.....	58	McGuire, Jimmy.....	60	Moura, Teresa.....	110
Mansfield, Kate.....	69, 70	McGuire, Jimmy A.....	11	Mowle, Kat.....	76
Marcy-Quay, Benjamin.....	105	McIntyre, Tess.....	71, 112	Moyer, Joshua.....	76
Mareska, John.....	77	McKinstry, Cory.....	31, 71	Mruzek, Joe.....	77
Marks, Sharyn.....	110	McKnight, Donald.....	109	Muallil, Richard.....	19
Marques, Mariana.....	20	McMahan, Caleb.....	37, 72, 87, 93, 99, 116	Muffelman, Sarah C.....	76
Marsaly, Benjamin.....	69	McMahan, Caleb D.....	32	Muletz-Wolz, Carly.....	62, 76
Marsh, David.....	69	McNamara, Dennis.....	72	Mull, Christopher.....	77, 80
Martel, André.....	54	McPhatter, Elliot.....	72	Muller, Tyler.....	77
Martens, Haley.....	26	Mead, Louise.....	111	Müller, Zoë.....	77
Martin, Amanda.....	69	Medd, Hannah.....	61	Mullins, Lindsay.....	77
Martin, Chelsea.....	70	Meese, Emily N.....	72	Mullin, Stephen.....	80, 118
Martinelli, Stacy.....	82	Melo, Bruno.....	72, 103	Munir, Misbahul.....	38
Martinez, Christopher.....	4	Melo, Marcelo.....	28, 72, 93	Munoz, Antonio.....	110
Martinez, Jackson L.....	71	Melvin, Tracy.....	111	Muñoz, Mariela Osorno.....	36, 106
Martin, James.....	68	Ménard, Frédéric.....	9	Munroe, Thomas.....	77
Martin, Katherine.....	70	Menezes, Rafael.....	94	Murphy, Kyle.....	17
Martin, Katie.....	85	Menna, Deborah.....	61	Murray, Alexander.....	77
Martin, Rene.....	70	Merson, Zachariah.....	73	Murray, Chris.....	29
Martins, Ana.....	77	Merson, Zachary.....	65	Murray, Christopher.....	9, 50
Martins, Renato A.....	43	Messerman, Arianne.....	5	Muscher-Hodges, Bekky.....	81
Martin, Summer.....	52	Meyer, Carl.....	23	Musick, John A.....	52
Maryati, Mohamed.....	38	Meyer, Michael.....	73	Musni, Vergie.....	109
Maslenikov, Katherine.....	118	Miguez, Joseph.....	73	Muth, Allyson B.....	67
Mastromonaco, Gabriela.....	53	Miller, David.....	35, 67, 111	Mutyal, Santoshi.....	78
Masumoto, Flávia.....	72	Miller, Elizabeth.....	73, 113	Myers, Edward.....	78, 85
Matich, Philip.....	49	Miller, Mary Cate.....	45	Myers, Lynn.....	106
Matlaga, Tanya.....	111	Miller, Melissa.....	47		
Matschiner, Michael.....	60	Miller, Ryan.....	74		
Matsui, Masafumi.....	38	Milligan, Rosanna.....	104		
Matthiopoulos, Jason.....	58	Million, Kara.....	74		
Mayer, Ryan.....	6	Mir, Wali.....	55		
Mayfield-Meyer, Teresa.....	59	Miskiewicz, Tony.....	74		
McAree, Danielle.....	31, 89	Mitra, Amartya.....	74		
McBride, Brett.....	37, 53, 108	Miyashita, Tetsuto.....	23		
McBride, Dustin.....	118	Moe, Brian.....	74		
McCabe, Kathryn.....	42	Moe, Brian J.....	24		
McCallister, Michael.....	70	Mohan, John.....	94		
McCallum, Anna.....	84	Molino, Eryka.....	91		
McCandless, Cami.....	60, 70	Monagan, Ivan.....	113		
McCandless, Camilla.....	99	Mona, Stefano.....	118		

## Author Index

[illegible]

## Author Index

Rajkumar, Anthony.....	90	Rosenbaum, David.....	3	Schalk, Christopher.....	3
Rallo, Trevor.....	91	Rose, Ned.....	11	Schlesinger, Matthew.....	34
Ralph, Gina.....	77	Rosenthal, William.....	25	Schloesser, Ryan.....	44
Ramos, Telton.....	97	Rossman, Sam.....	115	Schott, Ryan.....	4, 10, 37, 55, 74, 98, 103
Rangel, Joseph.....	92	Rossum, Taylor van.....	112	Schott, Ryan K.....	10, 87
Rao, RJ.....	91	Rota, Alessia.....	110	Schott, Ryan K.....	14, 21, 43
Rautsaw, Rhett.....	39, 50, 85	Rothermel, Betsie.....	110	Schreckengost, Shae.....	35, 66
Ravesi, Michael.....	6	Roth II, Timothy C.....	41	Schriever, Tiffany.....	7
Record, Nicholas.....	83	Rothman, Dale.....	44	Schultz, Andreanna.....	98
Regueira, Eleonora.....	109	Rowland, Hannah.....	103	Schultz, Mischa.....	98
Reichling, Steven B.....	13	Rowland, Hannah M.....	58	Schumm, Matthew.....	99
Reid, Brendan.....	19, 39, 91	Rowley, Jodi.....	60	Schwanz, Lisa.....	26
Reider, Kelsey.....	95	Royer, Anne.....	53	Schweikart, Olivia.....	99, 116
Reis, Marcos.....	72	Ruane, Sara.....	11, 61	Scott, Cassandra.....	61
Relyea, Rick.....	117	Rubin, Alexander.....	94	Scott, Dare.....	15
Rex, Patrick.....	5, 65, 91, 102	Rubio, Andrew.....	12	Scott, Devon.....	99
Richard, Kamryn.....	81	Ruck, Stephanie.....	94	Scyphers, Steven.....	31, 89
Richards-Zawacki, Corinne.....	111	Ruiz, Rebecca.....	114	Seah, Ying Giat.....	20
Rich, Caitlyn.....	43	Russell, Khalil.....	94	Searcy, Christopher.....	82
Ricks, Carter.....	92	Russo, Joseph Dello.....	19	Sedberry, George.....	17
Rieucan, Guillaume.....	90	Rypel, Andrew.....	17	Seitz, Andrew.....	81
Rigby, Cassandra.....	101			Selig, Gina.....	6
Rigby, Cassandra L.....	35			Selman, Will.....	46
Riggin, Kurt.....	41			Semmens, Brice.....	80, 105
Rincon-Sandoval, Melissa.....	73			Serra-Pereira, Bárbara.....	110
Rivera, Danielle.....	92			Serra-Silva, Ana.....	100
Rivera, Joshua.....	92			Serr, Megan.....	99
Roach, Cade.....	108			Sesler, Ryan L.....	99
Robertson, Jeanne.....	10			Sethi, Anmol.....	119
Robertson, Ross.....	116, 118			Sévêque, Anthony.....	105
Robertson, Sarah.....	31			Sewani, Sahil.....	108
Roberts, Roy.....	19, 39, 91, 92			Shah-Walter, Suni.....	99
Robinson, Kelly.....	17			Sharp, Steven.....	106
Robins, Robert.....	100			Shavit, Jordan.....	51
Robson, Olivia.....	92			Sheehy III, Coleman.....	100
Rocha, Cacilda.....	96			Shepack, Alex.....	115
Rodríguez, Daniela Nunez.....	96			Shepack, Alexander.....	112
Rodríguez, David.....	71, 118			Shepherd, Robert.....	11
Rodríguez, Jasmine.....	92			Sheridan, Jennifer.....	69
Rodríguez, Jordan.....	19			Sherlock, Miranda.....	100
Rodríguez, Noemi.....	43			Shevchik, Anya.....	65
Rodríguez-Velázquez, Adolfo.....	93			Shiffman, David.....	100, 113
Ro, Hyejoo.....	65			Shimada, Kenshu.....	116
Rolland, Virginie.....	106			Shimada, Tomohiko.....	38
Rolón, Alberto Puente.....	4			Shinohara, Gento.....	38
Romagosa, Christina.....	40, 47, 50			Shipley, Oliver.....	100
Romero, Cesar.....	72			Shivji, Mahmood.....	28, 40, 41, 60, 104, 109
Romine, Jason.....	93			Shoemaker, Sierra.....	97, 112
Ron, Santiago.....	12			Short, Graham.....	74
Rooker, Jay.....	104			Shortridge, Ayley.....	101
Rooker, Jay R.....	72			Shrimpton, Samuel.....	37
Roosenburg, Willem.....	93			Siciliani, Daphne.....	101
Root, Kyle.....	29			Siegfried, Tabitha.....	9
Roque, Pollyana.....	72, 93			Siers, Shane.....	112
Rosales-Garcia, Ramses.....	50			Siles, Jose Sandoval.....	43
Rosa, Ricardo.....	94, 97			Silva, Barbara da.....	72
Rose, Jonathan.....	46			Silva, Corina.....	65



## Author Index

Silver-Peavey, Courtney.....	101	Straube, Nicolas .....	79	Thompson, Michelle .....	28
Simões, Bruno F.....	43	Streicher, Jeffery W.....	87	Thorrold, Simon.....	37
Simons, Andrew.....	77	Streicher, Jeffery W.....	21	Thulander, Julia .....	78, 107
Simpfendorfer, Colin.....	101	Streicher, Jeffrey.....	4, 10, 37, 74, 98, 100, 103	Tillman, Brittany.....	16
Simpson, Steve.....	114	Streicher, Jeffrey W.....	10	Timm, Shelby.....	107
Sims, Michele A.....	95	Streicher, Jeffrey W.....	14, 43	Tingle, Jessica.....	107
Singer, Gabe.....	17	Streicher, Jeff W.....	12	Tittensor, Derek .....	24
Singer, Randy .....	26	Streich, Matthew .....	31	Tiutenko, Arthur .....	20
Singer, Ryan.....	59, 101	Strickland, Bradley .....	8	Toda, Mamoru.....	98
Sinopoli, Daniel.....	102	Struthers, Carl D. ....	85	Todd, Brian.....	80, 107, 117
Sipos, Michael.....	31	Stuart, Bryan L.....	11	Tokash, Alayna.....	93
Sison, Ke'alani.....	102	Stunz, Gregory W.....	72	Tonini, João.....	27, 106, 107, 115
Sisson, Michael.....	51	Stynoski, Jennifer L.....	58, 103	Tonini, João F. R.....	36
Skipwith, Philip.....	9	Suarez-Moncada, Jenifer.....	109	Tornabene, Luke.....	43, 73, 78, 118
Skomal, Greg.....	23, 29, 118	Subramaniam, Kuttichantran .....	31	Torquato, Felipe .....	33
Skomal, Gregory.....	8, 19	Sudin, Ahmad .....	38	Torres, Karen A. Paniagua .....	85
S., Luz Fernanda Jiménez.....	96	Sulikowski, James.....	4	Torres-Pineda, Patricia.....	108
Smaga, Christopher.....	56	Sulikowski, James A.....	98	Torres-Sánchez, María.....	22
Smeenk, Nicholas.....	93	Summers, Adam.....	104	Towey, Brian.....	42
Smith, Cynthia.....	44	Summers, Kyle.....	12, 60	Trape, Jean-François .....	109
Smith, Jaime.....	51	Suriyamongkol, Thanchira.....	97, 112	Travis, Joseph.....	74
Smith, Kate.....	37	Sutton, Tracey.....	24, 104	Tribuzio, Cindy.....	38
Smith, Leo.....	102	Swanson, Brad.....	90	Troyer, Emily.....	73, 113
Smith, Ryan.....	16	Sweet, Lynn .....	80	Tseng, Jack .....	38
Smith, Sellers.....	69	Sweezey, Brett.....	104	Tseng, Kelly.....	108
Smith, W. Leo.....	27, 41	Swenson, John.....	105	Tuberville, Tracey.....	107
Smukall, Matthew.....	8, 24, 27, 49, 81	Swift, Dominic.....	105	Tu, Eddie.....	101
Smukall, Matthew J.....	49	Swift, Dominic G.....	89	Tumulty, James.....	30, 108
Snyder, Andrew .....	28	Swinea, Savannah.....	89	Tupy, John .....	51
Solomon, Kara.....	99	Swinehart, Alyssa.....	75	Turffs, Dylann .....	113
Soltanoff, Carrie.....	116	<b>T</b>		Tyabji, Zoya.....	77
Sorensen, Johan.....	33	Talwar, Brendan .....	105	Tyler, James C.....	11
Soto-Centeno, Angelo.....	11	Tambascio, Michael.....	105	Tyminski, John .....	37, 53, 108
Sousa, Nivia .....	96	Tang, Kevin.....	105	<b>U</b>	
Souza, Lesley de.....	28, 87	Tan, Milton.....	13, 105	Uetz, Peter.....	108
Sparks, Eric.....	77	Taphorn, Donald.....	28	Umbers, Kate D.L.....	97
Sparks, John.....	19	Tarvin, Rebecca .....	60	Unmack, Peter.....	66
Spencer, Carol.....	60	Tassel, Hannah Van.....	47	Urquidi, Emily.....	108
Spencer, Ricky-John .....	109	Tatom-Naecker, Theresa-Anne.....	115	<b>V</b>	
Spurgeon, Emily.....	65, 102	Tattersall, Glenn.....	41	Valchi, Paula.....	109
Stahl, Sydney.....	102	Taylor, Aimee.....	93	Valek, Jessica .....	9
Stalker, Jeremy.....	27	Taylor, Emily.....	9, 59, 71, 74, 80, 101, 112	Valenti, Cassie .....	53
Stallsmith, Bruce.....	102	Telemeco, Rory.....	106	Valentine, Jordan.....	12
Steele, Zachary.....	18	Terrell, Vanessa.....	68	Vallejos, Johana Goyes.....	43
Steigerwald, Emma.....	60	Terry, Jennifer.....	48, 106	Vandenberg, Meg.....	104
Steinberg, Deborah K. ....	76	Terzi, Yahya.....	84, 102	Vanerelli, Alyssa .....	109
Stemp, Kenzi .....	5	Thammachoti, Panupong.....	38	Vanoven, Alexia.....	106
Stepanova, Natasha.....	103	Thomas, Kate.....	4, 10, 74, 98, 103	Vaudo, Jeremy.....	28, 40, 41, 60, 109
Stern, Cecily .....	69	Thomas, Kate N.....	10, 87	Vaughan, Eugene.....	109
Sterrett, Sean.....	103, 111	Thomas, Kate N.....	14, 21, 43	Veerappan, Deepak.....	110
Stewart, Tyler.....	31	Thomas, Matthew.....	26	Vegso, Zachary.....	110
Stiassny, Melanie.....	12, 72, 103	Thomason, Brandon.....	106	Verhulst, Conner.....	119
Stirling, Brian .....	102	Thomas, Rysa.....	106	Veríssimo, Ana.....	110
Stites, Andrew .....	30	Thompson, Daniel.....	42	Vinyard, Elizabeth A.....	34
Stoockle, Mark Y.....	42	Thompson, Kenneth .....	106		
Stokes, Maya.....	58	Thompson, Marten .....	102		
Stotz, Doug.....	28				

## Author Index

Viol, Andressa.....110  
 Violette, Veronica .....22  
 Voris, Harold K.....11  
 Voss, Joshua.....18  
 Voß, Olaf.....108  
 Voves, Kameron .....110  
 Vratil, Tristan .....71  
 Vredenburg, Vance .....60

### W

Waddle, Hardin.....85  
 Wagner, Catherine .....25  
 Wainwright, Peter .....51, 94  
 Waldron, Jayme .....3, 53, 62, 84, 111  
 Waldron, Jayme L.....45  
 Waldrop, Cassandra.....68  
 Walkowski, Whitney.....111  
 Walsh, Cathy .....66  
 Walter, Sunita Shah.....94  
 Waluga, Amadeus.....111  
 Ward, Rowan .....111  
 Warren, Tom .....25  
 Warwick, Alexa .....111  
 Warwick, Luke.....77  
 Watanabe, Yuuki.....23  
 Watkins-Colwell, Gregory.....112, 115  
 Watson, River.....26  
 Weaver, Rachel .....112  
 Weaver, Savannah.....9, 59, 71, 112  
 Webber-Schultz, Amani.....100  
 Webb, Jacqueline.....112  
 Webb, Jacqueline F.....82  
 Weber, Nick.....113  
 Weigt, Lee A.....11  
 Weinell, Jeffrey .....20  
 Welch, Shane .....3, 53, 62, 111  
 Welch, Shane M.....45  
 Wells, Randall S. ....115  
 Wells, R.J. David.....72, 104  
 Wen, Florence .....65  
 Wester, Julia.....15, 66, 113, 116  
 West, JoJo.....73, 113  
 Westneat, Mark.....73, 113  
 Westphal, Michael .....71, 80, 106, 113  
 Wetherbee, Brad .....29, 41  
 Wetherbee, Bradley .....28, 40, 60, 69, 109  
 Weyand, Courtney.....3, 114  
 Whalen, John.....39, 91, 114  
 Whitcher, Courtney .....114  
 White, Byron .....17  
 White, Clemency .....114  
 White, Connor.....59  
 Whiteman, John .....18  
 Whitenack, Lisa B.....99  
 Whitfield, Steven .....112, 114, 115  
 Whitney, Nick .....23, 59, 89  
 Whoriskey, Frederick.....8

Wierman, Molly.....27, 115  
 Wiley, Tonya.....8, 39, 65, 115  
 Wilken, Alec.....119  
 Wilkinson, Krystan .....9, 39  
 Wilkinson, Krystan A.....44, 115  
 Wilkinson, Mark.....100, 115  
 Wilkinson, Stacey Leonatti.....45  
 Wilkinson, Will.....45  
 Will, Christopher .....116  
 Williams, Ben.....114  
 Williams, Jason.....72  
 Williams, Jeffrey .....19  
 Williams, Jeffrey T. ....11  
 Williams, Kai .....38  
 Williamson, Ann .....116  
 Willis, Hannah .....99, 116  
 Willmes, Malte.....17  
 Wilson, Emma.....116  
 Wilson, Kristen .....24  
 Wilson, Mark .....116  
 Wingert, Juliana M.....96  
 Winton, Megan.....8, 83, 118  
 Wipfler, Benjamin .....60  
 Wisely, Samantha.....40  
 Wit, Kirsty de .....22  
 Witt, Matthew .....114  
 Wolf, Alexander.....107  
 Womack, Molly.....60, 74  
 Wommack, Elizabeth.....59  
 Wood, Jake .....116  
 Woodley, Sarah .....32, 117  
 Woodruff, Sidney.....117  
 Woodward, Parker.....117  
 Worthington, Sarah.....117  
 Wüster, Wolfgang.....109  
 Wyanski, David.....17  
 Wyffels, Jennifer.....66, 117

### X

Xenakis, Lila .....118  
 Xiong, Daisy.....118

### Y

Yamamoto, Takumi.....98  
 Yambun, Paul .....38  
 Yando, Erik.....56  
 Yang, Lei .....118  
 Yates, Jessica .....118  
 Yeckley, Sean.....17  
 Yerrace, Sarah.....118  
 Yong, Hoi-Sen.....38  
 Yopak, Kara .....119  
 Young, Matthew.....22  
 Yovanovich, Carola.....119

### Z

Zaman, Luis.....110  
 Zbinden, Zachery.....119  
 Zelif, Dustin .....108  
 Zhang, Jiangyong.....65  
 Zhou, Yiqun.....95  
 Zhou, Zehua .....63  
 Zobek, Christopher .....119  
 Zoungrana, Magalie .....100  
 Zuliani, Mario.....80