GARD
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Abstracts

CAPSTONE SEMINAR: In recognition of the scientific contributions of Dr. Louise A. Rollins-Smith

June 8th, 1:30 pm

Immunity against chytridiomycosis: amphibian defenses and chytrid counter defenses

Rollins-Smith, Louise A[1]*
[1]Vanderbilt University School of Medicine
*louise.rollins-smith@vanderbilt.edu

The disease chytridiomycosis has been recognized for more than a quarter century, and new outbreaks and population declines in susceptible amphibian communities continue to occur. Because amphibians have complex and effective immune defenses against many pathogens, it remains a puzzle as to why many species are unable to control and clear this fungal infection. This presentation will summarize progress in our understanding of immune defenses and pathogen counter defenses for the pathogenic chytrids, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). Amphibian immune defenses in the skin include innate defenses such as antimicrobial peptides (AMPs) in the mucus, protective microbes in the skin microbiome, macrophage subsets, and other factors. In addition, the adaptive immune system is capable of producing anti-chytrid antibodies secreted into the mucus. In spite of these potential defenses, the chytrids appear to have evolved a set of counter defenses that enable them to persist in the skin. What we have learned and major questions that remain will be summarized to forge a path forward for further research. Support: USA National Science Foundation grants IOS-2147467 and BII 2120084.

KEYNOTE SEMINARS

June 8th, 8:25 am

The role of active museum collections in amphibian and reptile disease research: Historical baselines, geographic and taxonomic coverage, and opportunities for collaboration

Barrow, LN[1]*; Bradley HD[1]; Wiley DLF[1]
[1]University of New Mexico
*LNBarrow@unm.edu

Natural history collections have provided critical infrastructure for biodiversity research and discovery for centuries. Several retrospective studies have previously showcased the utility of museum specimens for pathogen surveillance, but their full integration with wildlife disease research has tremendous, untapped potential. This talk ponders how we can establish partnerships and implement common practices that maximize the utility of extended museum specimens for wildlife disease researchers. We discuss three on-going projects focused on anuran and snake pathogens in the United States to highlight the opportunities and challenges for collecting, interpreting, and sharing meaningful data. First, a large field sampling effort across 30 states, supplemented by museum tissue loans, enabled a study of infection dynamics across three amphibian pathogens and 12 host taxa, demonstrating infection differences among host species and environments. Second, examination of hundreds of snake specimens in the Southwestern U.S. has revealed a high prevalence of lesions of unclear origin, with additional histopathology and molecular testing underway. Third, we are investigating the influence of different tissues and preservation techniques on pathogen detection. We emphasize the importance of making disease data, including negative results, available via public databases and encourage deposition of whole specimens when possible to promote reproducible and extendable science.

June 8th, 3:55 pm

Unraveling the wild origins of common and significant pathogens of captive reptiles

Ossiboff, RJ[1]*
[1]University of Florida
*rossiboff@ufl.edu

While diseases of free-ranging reptiles are finally beginning to garner the necessary and appropriate attention to better understand the risks pathogens can play to wild populations, much of our knowledge of reptile pathogens comes from investigations of disease in captive reptiles. Parasites, such as *Cryptosporidium spp*., and viruses, such as ferlaviruses (paramyxoviruses) and reptarenaviruses (known primarily as boid inclusion body disease), are capable of causing significant morbidity and mortality in captive collections, and as such, they represent some of the best characterized reptile pathogens. Despite this, little is known about the natural wild origins of such important captive reptile pathogens. This talk will highlight recent studies that identify the occurrence of common captive reptile pathogens in free-ranging reptiles, with a focus on potential reservoir hosts, disease potential in free-ranging reptiles, and potential implications of the wild:captive interface on wild reptile health.

June 9th, 8:15 am

A conservationist's perspective on chytrid-related amphibian declines

Gratwicke, B[1]*
[1]Smithsonian's National Zoo and Conservation Biology Institute
*gratwickeb@si.edu

Since the 1989 World Herp Congress, amphibian biologists became conscious of disappearing amphibians, something they called enigmatic amphibian declines. Unravelling the mystery of these declines led to the discovery of the amphibian chytrid fungus, a global amphibian assessment and the first global amphibian conservation action plan. Part of that action plan involved building the global Amphibian Ark, to place some of the species at highest risk of extinction in human care while we work to mitigate the disease. I will discuss the efforts of the Panama Amphibian Rescue and Conservation Project and our efforts to work with zoos and aquariums to build captive assurance colonies for amphibians in Panama. We collaborated with academic partners to search for potential solutions to the amphibian chytrid fungus, and while no silver bullet has emerged, I will share some of the rationale for our recent rewilding efforts that explore potential pathways that might tip the balance in favor of the frogs.

June 9th, 1:30 pm

Measuring reptile health by establishing physiological profiles

Neuman-Lee, L[1]*
[1]Arkansas State University
*lneumanlee@astate.edu

Determining animal health requires consideration of the complicated interplay among many physiological processes. Determining the health of a reptile remains even more difficult as many baseline metrics of physiology are yet to be established. Therefore, we may be unable to understand the true effects of disease and infection on reptilian health. Two ways to address these gaps include: 1) measuring physiology of wild populations of reptiles to establish baseline physiological profiles and 2) performing simulated infections to create serialized profiles of immune and endocrine responses. Wild populations of reptiles have high variability in many physiological metrics. Thus, it is critical to measure many physiological parameters, including immune, reproductive, and endocrinological data. By then examining the connections and patterns among these multiple metrics, we can better establish normal physiological profiles for wild populations. In addition to gathering physiological data in the wild to create baseline physiological profiles, we also need to understand how reptiles respond to simulated infections to both predict and measure how they respond to actual infections. For example, in mammals, there are predictable, unique responses to bacteria, fungi, viruses, and parasites. By establishing similar predictable responses in reptiles, we are better able to identify animals potentially responding to pathogen types in the wild, particularly novel diseases. Using these two strategies, we can begin to understand the impact of disease on reptile individuals and populations.

June 10th, 8:15 am

Ten years of research on amphibian herpesviruses: All that we do not know

Origgi, FC[1,2,3]*
[1]University of applied sciences and arts of Southern Switzerland (SUPSI)
[2]University of Messina
[3]University of Bern
*francesco.origgi@supsi.ch

During the last ten years, we have characterized three previously unknown herpesviruses in distinct amphibian populations in Europe. The detection of three previously undescribed viral agents of the same group in distinct families (*Ranide, Bufonidae, Alytidae*) of amphibians, and within a relatively short time span, calls attention to their clinical and biological significance in the context of the ecology of the free ranging amphibian population and of the amphibian global decline. Thanks to a through and consistent investigative approach, including classic morphology (gross-, histo- and ultrastructural pathology) and molecular tools (genomic, transcriptomic, in situ hybridization), it has been possible to highlight common hallmarks of the diseases associated with each of the three viruses characterized to date (RaHV3, RaHV5 and BfHV1). These include an obvious proliferative skin disease with a local cellular immune suppression, likely virally driven, and a recurrent seasonal disease pattern, consistent with a likely viral latency. Infection may occur already in the tadpole, and viral prevalence is most likely higher than that assessed on gross lesions alone. The elusive behavior of the hosts and fast scavenging of their carcasses calls for readouts, other than mortality, to assess the actual impact of these viruses on the free-ranging amphibian populations.

June 10th, 4:00 pm

From infection to intervention: immune–microbiome interactions in amphibian health and disease

Ellison, A[1]*; Osborne, O[1]; Urrutia-Carter, J[2]; Madison, J[2]; Hartmann, A[2]; Grayfer, L[3]; Gratwicke, B[2]; Muletz-Wolz, C[2]
[1]Bangor University
[2]Smithsonian's National Zoo and Conservation Biology Institute
[3]George Washington University
*a.ellison@bangor.ac.uk

The drivers of variation in amphibian susceptibility to chytridiomycoses remains enigmatic. However, mounting evidence suggests a complex interplay between abiotic factors, host genetics, immune responses, skin secretions and their cutaneous microbial communities. In this talk, I will explore the utility of transcriptomic and microbial profiling methods to understand differential responses to *Batrachochytrium dendrobatidis* infections, factors shaping amphibian skin microbiomes, and the amphibian skin immune-microbiome interface in health and disease. Collectively, this work demonstrates intra- and inter-specific gene expression responses may be pivotal to chytrid disease outcomes and are associated with skin microbial community composition. In addition, by examining natural variation in skin microbiomes and host responses to microbiome manipulation, this work provides the foundations for improved strategies to augment animal microbiomes for improved disease resilience.

June 11th, 9:00am

Parasites in paradise: Evolutionary battles in Mediterranean and Macaronesian lizards

Megía-Palma, R[1]*
[1]Complutense University of Madrid
*rmegia01@ucm.es

The principles of the Red Queen hypothesis propose that parasites and their hosts evolve strategies that maximize their biological fitness, allowing them to offset the costs of their close interactions. One such strategy is sexual reproduction, which enhances genetic diversity and helps organisms stay ahead in the evolutionary arms race. A particular group of parasites, the apicomplexan protozoa, can undergo both sexual and asexual reproduction, combining the advantages of both strategies. Some species complete both cycles in vertebrates, while others generate most of their genetic diversity in invertebrates, where constraints on parasite genetic diversification are less restrictive. I will present examples of relationships involving these blood parasites, and mite vectors, in lizards from the Iberian Peninsula and the Canary Islands. I will discuss parasite diversity, as well as patterns of host specificity and the temporal and spatial dynamics of infection at both local and geographic scales. In particular, I will focus on Tenerife, where increasing aridification and climate change appear to be intensifying parasite pressure on endemic lizard populations. Notably, geographic variation in male color dimorphism across the island correlates with a climate-linked gradient of parasite pressure, supporting the idea that sexual selection operates in proportion to parasite intensity.

June 11th, 1:30 pm

Developing amphibian models to unravel defensive microbiome assembly and function

Bates, KA[1]*
[1]Queen Mary University of London
*k.bates@qmul.ac.uk

The amphibian skin microbiome plays a crucial role in host defence, particularly against pathogenic chytrid fungi. This has raised the tantalising possibility of developing microbiome-based interventions, including probiotics, to protect vulnerable species. However, despite their promise, *in vivo* probiotic trials have consistently shown poor microbial persistence and short-lived host protection. These limitations reflect a broader, fundamental challenge across vertebrate systems: how do protective microbiomes assemble and function in nature? For amphibians, answering these questions is further hindered by a sparsity of tractable experimental tools compared to mammalian systems. In this talk, I will present research from my lab that seeks to address these knowledge gaps by combing theory with wild and novel experimental amphibian models. By uncovering the mechanisms governing microbiome assembly and function, our work ultimately aims to inform the design of more effective, durable microbiome-based therapies to mitigate pathogen threats and conserve imperilled amphibians.

PARC DTT ETHOS

June 8th, 8:15 am

Guidance for ethical engagement and disease mitigation in amphibian and reptile conservation through PARC's ETHOS and Disease Task Teams

Garrido, JR[1,2]*; Palmisano, JN[2,3]*; O'Hanlon, B[1]; Christman, M[1]; Duffus, A[1]; Oxenrider, K[1]; Cozad, R[1,2]
1 *Partners in Amphibian and Reptile Conservation*
2 *Amphibian and Reptile Conservancy*
3 *University of Central Florida*
PARC_coordinators@PARCplace.org

Threats to amphibians and reptiles are multifaceted and can sometimes be exacerbated by the communities that value them most. Recreational herping and wildlife observation have expanded rapidly alongside social media and nature-based recreation, increasing human–herpetofauna interactions and associated risks including habitat disturbance, stress, and pathogen transmission. Partners in Amphibian and Reptile Conservation (PARC) has two complementary initiatives addressing these challenges: the Ethical Herping and Observation Standards (ETHOS) and the Disease Task Team (DTT). PARC recently developed ETHOS to establish best practices through Ethical Herping Tenets and Gold Standards, promoting in-situ observation, minimizing handling, reducing habitat disturbance, limiting location sharing, and preventing disease spread. Complimenting ETHOS, is the longstanding DTT, which provides resources for education, management, and control of herp pathogens and diseases through the Herpetofaunal Disease Alert System to advance disease surveillance, reporting, and mitigation through standardized decontamination protocols and response guidance. Together, these task teams provide an integrated framework linking human behavior with conservation outcomes including structure, guiding principles, and products from both teams that highlight their application across recreational, research, and management contexts. By aligning engagement with evidence-based practices, PARC aims to reduce anthropogenic impacts while maintaining opportunities for meaningful interaction, ultimately supporting long-term population health and resilience.

ORAL PRESENTATIONS

June 8th, 8:55 am

Are we ready for a novel disease outbreak? Lessons learned from a complex captive disease outbreak

Bletz, MC[1]*; Grant, E[2]; Gray, M[3]; Grear, D[4]; McDonnell, N[5]; Warwick, A[6]; Woodhams, D[5], Disease Response Consortia
[1]Pennsylvania State University
[2]USGS Eastern Ecological Science Center
[3]University of Tennessee
[4]USGS National Wildlife Health Center
[5]University of Massachusetts
[6]Michigan State University
* mcb6342@psu.edu

Are we ready for a novel disease outbreak? This is a question the Bsal Task Force recently asked, 10 years after its creation. In Fall 2025, we were put to the test. Sick animals in a captive collection, with signs reminiscent of *Batrachochytrium salamandrivorans*, were brought to the attention of disease researchers. Rapid collaboration, coordination, and complexity ensued in the weeks following as cross-sector teams of researchers, state and federal agencies, and amphibian hobbyists came together to determine the disease, coordinate response efforts on the ground, trace potential trade related movement and communicate across stakeholders. When testing showed that Bsal was not the culprit, we were met with a potentially even greater challenge: when an outbreak of unknown cause emerges – how do we act? How do we evaluate risk? How do we work together to take action to something we know little about? This talk will provide an overview of the interdisciplinary puzzle pieces at play when a disease emergency occurs. I will highlight synergistic collaborations that facilitated rapid progress, the complexities that challenged us, and the dimensions we never saw coming. The lessons learned can shape future response efforts and generate strategies that minimize the chance of disease outbreaks in the first place.

June 8th, 9:10 am

Interception and investigation of an elusive and novel salamander disease

McDonnell, NB[1]*; Bletz, MC[2]; Richards, A[1]; Gray, MJ[3]; Grear, DA[4]; Grant, EHC[4]; Miller, DL[3]; Shearn-Bochsler, VI[4]; Lorch, JM[4]; Tawidian, P[4]; Robert, J[5]; De Jesus, F[5]; Buttimer, SM[2]; Inman, B[1]; Doolin, ML[1]; Haifley, P[1]; Williams, K[1]; Whetstone, RD[1]; Barr, T[1]; Petit Frere, W[1]; Woodhams, DC[1]
[1]University of Massachusetts Boston
[2]Pennsylvania State University
[3]University of Tennessee Knoxville
[4]U.S. Geological Survey, National Wildlife Health Center
[5]University of Rochester
*nina.mcdonnell@umb.edu

In November of 2025, the discovery of an unknown disease in a large private collection of salamanders raised concerns for spread though the pet trade and into native amphibian populations. *Salamandra spp*. in the affected collection exhibited epidermal necrosis, ulcers, and significant mortality. We initiated a rapid series of preliminary investigations to assess transmissibility, native species susceptibility, and captive treatment strategies. Diagnostic evaluations found multiple features associated with lesions, including fungi, nematodes, and at least one virus. These candidate agents were isolated and experimentally evaluated for their ability to reproduce the disease. We expect trials to be completed by the time of this presentation. We hope these results will clarify disease etiology, thereby informing response.

June 8th, 9:25 am

Navigating a morbidity event in the pet trade

Miller, DL[1]*; Shearn-Bochsler, V[2]
[1]University of Tennessee
[2]USGS National Wildlife Health Center
*dmille42@utk.edu

In late 2025, a private amphibian hobbyist and breeder reported concern about sick salamanders. Although grossly the lesions presented similar to *Batrachochytrium salamandrivorans* (Bsal) chytridiomycosis, diagnostic testing ruled out Bsal. Trade of animals to other private hobbyists resulted in similar morbidity occurring at these sites, causing concern for a transmissible infectious agent. Animals were submitted to multiple laboratories for concurrent testing. Chilled, frozen, and formalin-fixed animals from both the initial site and at least one of the secondary sites were examined by microscopy. Gross findings were not consistent in all animals but variably included sloughing skin, varying numbers of randomly distributed pinpoint to 1 mm diameter flat dark red to black foci throughout the body, and occasional up to 5 mm diameter ulcerations. General microscopic findings included multifocal epidermal necrosis with occasionally one or more of fungal hyphae, bacteria, or nematode larvae. Culture results from frozen tissues revealed multiple fungal and bacterial colonies. Ongoing investigations include metagenomics and virus isolation. This case highlights the importance of working as teams to tackle morbidity events.

June 8th, 9:40 am

From rare to widespread: the rise of Batrachochytrium dendrobatidis hybrids in a tropical forest

Carvalho, T[1,2]*; Pontes, MR[3]; Gonçalves-Souza, T[1]; Jenkinson, T[4]; James, TY[1]
[1]University of Michigan
[2]University of Michigan
[3]Universidade Estadual de Campinas
[4]California State University
*tamilie@umich.edu

The amphibian chytridiomycosis panzootic, driven by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) and the Bd-GPL lineage, reshaped amphibian communities worldwide. Hybrid Bd genotypes produced by recombination between divergent lineages may pose renewed epizootic risk, yet their frequency, distribution, and spatiotemporal dynamics remain largely unknown. Here we present genomic surveillance in Atlantic Forest amphibian communities, combining (i) whole-genome sequencing (WGS) of Bd isolates and (ii) Bd genotyping from field tadpoles using a sequence-capture target-enrichment approach, followed by high-throughput sequencing. Across a multi-year time series, we observe a shift toward hybrids: hybrid prevalence increases through time across multiple localities and, in several sites, hybrids rise to dominate the detected Bd genetic composition. We also detect Bd-GPL and Bd-Brazil, with Bd-Brazil less represented. Patterns of hybrid increase and dominance are consistent with the hypothesis that

hybrids may be competitively superior under local ecological conditions. WGS further shows that many hybrid isolates exhibit high genome-wide heterozygosity, consistent with early-generation (F1-like) ancestry. Initial phylogenomic patterns suggest multiple, potentially independent hybrid origins, with ongoing analyses to refine ancestry inference, evaluate directionality, and assess the contribution of loss of heterozygosity. Together, these data help address a major knowledge gap by providing time-resolved genomic evidence for the emergence and rise of Bd hybrids.

June 8th, 9:55 am

Impacts of Forest Restoration on Snake Fungal Disease (*Ophidiomyces ophiodiicola*) Prevalence in a Southeastern Snake Community

Robinson, SM[1]; Walker, DM[2]; Cochran, A[3]; **Sutton, WB[1]***
[1]Tennessee State University
[2]Middle Tennessee State University
[3]National Forests of Alabama
*wsutton@tnstate.edu

In the southeastern United States, forest restoration is important for restoring forest health of the imperiled longleaf pine ecosystem. However, changes in habitat structure can alter wildlife communities, along with the pathogens that impact these species. Therefore, we quantified the prevalence of Snake Fungal Disease (*Ophidiomyces ophiodiicola*, Oo hereafter) and evaluated host- and habitat-level predictors of infection prevalence in a southeastern snake community across a restoration chronosequence. In 2023–2024, we monitored the snake community in 16 forest restoration stands across 3,718 trap nights, which yielded 512 unique snake captures. We detected Oo in snakes from all four restoration treatments (Control, Early planting, Late planting, and Desired Forest Condition), indicating forest-wide distribution. Prevalence varied significantly among species and between years, and we found a significant positive association between body size (snout–vent length) and Oo prevalence. Infection prevalence was also greater in forest stands with greater average maximum relative humidity. Restoration status alone was not a strong predictor of infection status. Clinical signs of ophidiomycosis were observed in some individuals, but fungal load did not correspond with infection severity, suggesting frequent subclinical infections. Overall, Oo occurrence was more strongly structured by host traits and microclimate than by forest restoration status.

June 8th, 10:10 am

Selection and survival in great crested newt populations at the Bsal frontline

Westerlinck, L[1]*; Mulder, KP[1]; Pasmans, F[1]; Beukema, W[2]; Gilbert, M[2]; Duchatel, C[1,2]; Wielstra, B[3,4]; Martel, A[1]
[1]Ghent University
[2]Reptile, Amphibian and Fish Conservation Netherlands
[3]Leiden University
[4]Naturalis Biodiversity Centre
*Lars.westerlinck@Ugent.be

Amphibians are disappearing at disproportionately high rates compared to other vertebrate groups, placing them among the most threatened taxa in the current biodiversity crisis. Infectious diseases, like chytridiomycosis, are a major driver. The chytrid pathogen *Batrachochytrium salamandrivorans* (Bsal) is particularly problematic in Europe, where it poses an ongoing threat to the urodelan diversity by its rapid spread and high mortality rate. Since its discovery in 2013, Bsal has established itself in multiple salamander and newt populations, yet the genetic and evolutionary responses of these affected populations remain poorly understood. This study investigates the population genetics of the great crested newt (*Triturus cristatus*) in The Netherlands by comparing populations exposed to Bsal with nearby uninfected populations. Using targeted sequence-capture on non-invasive skin swabs, the variation across the coding regions of the *Triturus* genome will be assessed to detect population bottlenecks and signals of selection. We aim to show how pathogen introduction affects population-level genetic diversity and to identify genetic markers linked to resistance or susceptibility. Understanding these dynamics is crucial, as strong selective pressures can reduce genetic variation, compromise population health, and increase vulnerability.

June 8th, 10:55 am

Global strategies to minimize pathogens in amphibian trade

Gray, MJ[1]*; Pasmans, F[2]; Bogaerts, S[3]; Boratto, R[4]; Borzée, A[5]; Chen, KP[6]; Collins, J[7]; Galvin, DJ[1]; Hartman, K[8]; Jewell, S[9], Lesbarrères, D[10]; Marschang, RE[11]; Marston, A[8], Martel, A[2]; McDemott, CT[12]; Miller, DL[1]; Myers, G[13]; Oefner, S[14]; Penner. J[15]; Singheiser, M[16]; Tseng, YH[6]; Warwick, A[17]; Wenninger, M[18]; Willard, J[19]
[1]University of Tennessee
[2]Wildlife Health Ghent
[3]Strijperstraat 55a
[4]GuArdean Centre for Conservation Research
[5]College of Life Sciences
[6]Full of Frogs
[7]Reptile and Exotic Pet Trade Association
[8]Veterinary Services
[9]U.S. Fish and Wildlife Service (Retired)
[10]Environment and Climate Change Canada and Université de Sudbury
[11]Laboklin GmbH & Co. KG
[12]Department of Veterinary Clinical Sciences
[13]Zoo Miami
[14]Auffangstation für Reptilien
[15]Museum für Naturkunde
[16]Bundesverband fuer fachgerechten Natur-, Tier und Artenschutz e.V.
[17]Michigan State University
[18]Cincinnati Zoo and Botanical Garden
[19]Josh's Frogs
*mgray11@utk.edu

Global amphibian trade is implicated in the spread and emergence of chytrid fungus and ranavirus. We evaluated the regulations, policies and programs that exist in the United States, Canada, United Kingdom (UK), European Union (EU), China, and Taiwan. We found that despite membership of these nations in the World Organization for Animal Health (WOAH) and the notifiable listing of chytrid fungus and ranavirus, only the EU requires animal health certificates for imported amphibians; however, enforcement and testing requirements vary among EU nations. Due to the threat of *Batrachochytrium salamandrivorans* (*Bsal*) to native biodiversity, the United States and Canada issued import trade bans on salamanders. The UK requires that salamanders are imported to government-approved facilities and verified negative for *Bsal* prior to sale. Taiwan is the only nation that provides free diagnostic testing for amphibians being exported. China has laws forbidding the sale of amphibians infected with chytrid fungus; however, there is no enforcement. Non-profit organizations in Europe and North America produce educational materials on best biosecurity practices for businesses and pet owners. One non-profit organization, Healthy Trade Institute, certifies businesses in practices that reduce pathogen transmission and disease outbreaks. Our review provides evidence that governments allocate limited resources to reduce pathogens in amphibian trade and emphasize the need for industry-led standards that are self-imposed by businesses.

June 8th, 11:10 am

Amphibian and reptile pathogens detected in the pet trade: a collaboration with the Healthy Trade Institute

Galvin, DJ[1,2]*; Gray, MJ[1,2]; Brinkman, A[2,3]; Miller, DL[1,2]; Moherman, N[2,4]
[1]University of Tennessee
[2]Healthy Trade Institute
[3]Pet Advocacy Network
[5]Reptiles By Mack
*dgalvin2@utk.edu

The amphibian and reptile pet industry is a fast-growing industry with a market value of over 1.5 billion USD in the United States, and an estimated 3 billion USD globally. Ball pythons (*Python regius*), bearded dragons (*Pogona*

vitticeps), and leopard geckos (*Eublepharis macularius*) dominate the industry, although the proportion of amphibians is increasing, with species such as White's treefrogs (*Litoria caerulea*), red-eyed treefrogs (*Agalychnis callidryas*), and dart frogs (*Oophaga spp., Dendrobates spp.*, and *Ranitomeya spp.*) growing in popularity. While other pet trade industries (i.e. dogs and cats) in the US require pathogen testing and/or vaccinations to ensure healthy animals, no such testing is required for the amphibian and reptile pet trade. A recently launched certification program led by the Healthy Trade Institute trains businesses in biosecurity practices, quarantining, and pathogen surveillance to monitor the movement of pathogens including *Batrachochytrium dendrobatidis, B. salamandrivorans*, *Ranavirus*, *Agamid adenovirus I*, *Cryoptosporidium serpentis*, and *Ophidiomyces ophiodiicola*. Pathogen surveillance conducted in collaboration with the HTI and member businesses show that these pathogens vary in prevalence based on host species and location of origin (captive bred vs. wild caught). This surveillance provides important information for characterizing pathogen dynamics in the amphibian and reptile pet trade network.

June 8th, 11:25 am

Illegal exotic amphibian trade as a dual threat to India's amphibian biodiversity and biosecurity: A one-health risk assessment using Monte Carlo simulations

Manohara, A[1]*
[1]Azim Premji University
*amoghavarsha0103@gmail.com

Illegal trade in exotic amphibians represents a significant yet underappreciated biosecurity and conservation threat to India's amphibian fauna. India is home to over 450 amphibian species, approximately 40% of which are classified as threatened or Data Deficient, with high endemism concentrated in geographically restricted habitats. Many of these lineages lack documented historical exposure to globally emergent amphibian pathogens, increasing susceptibility to disease-mediated population declines. This study presents the first country-level dataset documenting illegal exotic amphibian trade in India, compiled through systematic online trade monitoring and marketplace documentation. The dataset records approximately 13 exotic amphibian species —including both anurans and urodelans—sold across eight major urban centers, with distinct city-specific market structuring and restricted species availability (e.g., Chinese giant salamander (*Andrias davidianus*) and Laos warty newt (*Laotriton laoensis*)), indicating spatially concentrated trade hubs and enforcement gaps. A semi-quantitative risk matrix integrating pathogen carriage potential, ecological overlap with native taxa, climatic suitability, escape probability, and market frequency was used to rank spillover risk. Stochastic Monte Carlo simulations (10,000–50,000 iterations), parameterized with realistic trade-volume estimates, quantified annual and cumulative probabilities of pathogen introduction, focusing on chytrid fungi (*Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*) and ranaviruses. Results indicate that even low propagule pressure from illegal trade can generate non-trivial annual probabilities of pathogen entry, with cumulative risk increasing rapidly over short timeframes. Given the logistical and economic infeasibility of species-wide susceptibility testing, prevention-focused biosecurity and enforcement represent the most effective risk-mitigation strategies.

June 8th, 11:40 am

Systematic surveillance to screen immerging infectious diseases and use of multifaceted collaborative approaches to mitigate the disease risks in Asia

Herath, J[1]*; Dan, S[2]; Ellepola, G[3]; Subramaniam, K[4]; Meegaskumbura, M[2]
[1]University of Peradeniya
[2]Guangxi University
[3]University of Peradeniya
[4]University of Florida
*Jayampathi_herath@yahoo.com

Asia is a major epicenter of emerging infectious diseases, yet surveillance of ranavirus, a globally emerging pathogen causing mass mortality in ectothermic vertebrates, remains limited. In southern China, particularly the Guangxi Zhuang Autonomous Region (GAR), the true disease burden and circulating ranavirus lineages are poorly understood. Based on the assessed prevalence, host range, and transmission potential of ranavirus in the Guangxi Zhuang Autonomous Region (GAR), this study aims to generate evidence to better inform and propose effective regional and global disease management strategies. A comprehensive, systematic surveillance program was conducted across the Guangxi Zhuang Autonomous Region (GAR), targeting fish, amphibians, and reptiles from both wild and farmed populations, including protected nature reserves. Based on the findings, multifaceted and collaborative approaches were suggested to mitigate ranavirus disease risks in Asia. Ranavirus infection was detected in 92 individuals from 18 ectothermic vertebrate species, indicating interspecies and interclass transmission. Infection rates in some wild frog populations reached 100%, even within protected areas. Introduced species used in food and pet trades pose

heightened risks for pathogen spread. These findings underscore the need for comprehensive surveillance, structured import risk analysis aligned with WTO and WOAH standards, and reporting to global systems such as GRRS. In the absence of treatments or vaccines, a coordinated One Health approach, strengthened biosecurity, and grassroots-level awareness are critical for mitigation.

June 8th, 11:45 am

Clean animals or clean operations? Assessing and comparing the consumer preferences in amphibian trade

Poudyal, NC[1]*; Adhikari, RK[2]; Warwick, AR[3]; Haddock, G[3]; Cavasos, K[1]; Gray, MJ[1]
[1]University of Tennessee
[2]Mississippi State University
[3]Michigan State University
*npoudyal@utk.edu

Disease transmission within the pet trade network and the potential for spillover into natural ecosystems poses significant threats to biodiversity. Market-based clean trade mechanisms, such as certification, are often proposed as solutions to environmental issues. However, little is known about consumer attitudes or willingness to pay for, certification related to animals or businesses in the pet trade. Using a case study of chytrid fungi and ranavirus in amphibians, this study compared the value U.S. pet amphibian consumers place on two certification schemes – that is, pet- vs business-focused. Pet owners demonstrated a strong willingness to pay for disease prevention, with a mean willingness to pay of 54% additional for amphibians certified as disease-free compared to not certified. Moreover, they were willing to pay even more (89% premium) for animals from a business certified as low risk. Trust in business certification and broader perceived benefits influenced willingness to pay, as did factors such as frequency of veterinary care, ownership size, age, and gender. Younger, female owners demonstrated the highest likelihood of paying a premium. These findings highlight the economic and ecological benefits of certification for healthy trade practices, providing insight into consumer preferences for both product- and business-level certifications.

June 8th, 11:50 am

The impact of trade relationships and business characteristics on biosecurity behaviors: A case study of the amphibian pet trade

Haddock, GA[1]*; Poudyal, N[2]; Gray, MJ[2]; Warwick, AR[1]
[1]Michigan State University
[2]University of Tennessee
*Haddock4@msu.edu

The global amphibian trade has been identified as a primary pathway of disease spread to naive amphibian populations in captivity and the wild. Three OIE reportable diseases, Bd, Bsal, and *Ranavirus* are of particular concern because of their virulence and pathogenesis. Improper or a lack of biosecurity actions performed by amphibian pet trade stakeholders are at least partially responsible for introducing these diseases and subsequent population declines. Understanding what motivates an amphibian pet trade business to partake in or prohibit biosecurity practices is critical to developing behavior change interventions. Here, we explore how a business' trade network centrality and position affect how many biosecurity practices they perform. We found that the percentage of animals sold to different places, mode of selling, importer status, and concern for public image had significant relationships with how many biosecurity practices a business performs. Additionally, we identify three distinct kinds of amphibian pet trade businesses. This information is useful in identifying what strategies to use to encourage more bio secure behavior and which subpopulations to target for outreach.

June 8th, 11:55 am

Amphibian population status across an altitudinal gradient in Guatemala: Insights from the San Marcos transect related to *Batrachochytrium dendrobatidis*

Ruano Fajardo, G[1,2]*; Galdino, JY[2]; Chang, MJ[2]; Catenazzi, A[1]
[1]Florida International University
[2]Universidad de San Carlos de Guatemala
*gruanofajardo1@gmail.com

Neotropical mountains are a global epicenter for amphibian declines. The primary causes of these declines include habitat loss, climate change, and wildlife diseases. Across climatic gradients, amphibian communities face different threats, and their species possess varying adaptive capacities. One of the most studied altitudinal gradients in the Neotropics is the San Marcos, Guatemala transect. Amphibian communities and chytrid fungus (*Batrachochytrium*

dendrobatidis – Bd) presence were studied from 1970 to 2009, which shed light on the enigmatic decline of salamanders. The main cause seems to be related to the synergy between habitat loss and the arrival of the chytrid fungus in the 1980s. Four decades after the arrival of chytrid fungus, we used a multi-species occupancy model approach to evaluate the status of amphibian populations, chytrid infection, and its association with forest canopy, anthropogenic disturbance, slope, altitude, and water presence along this gradient. Low amphibian detection probabilities were associated with higher altitudes in pristine and secondary pine-cypress forests. In general, high prevalence and low infection of the chytrid fungus were detected in cloud forest amphibian species breeding aquatically, showing similar trends to reported amphibians' Bd prevalences. On the other hand, direct-developing amphibians had lower detection and chytrid prevalence.

June 8th, 2:00 pm

Investigating amphibian host resistance and immunization against the fungal pathogen *Batrachochytrium salamandrivorans*

Roseman, S[1]*; Brunner, J[1]
[1]Washington State University
*Sarah.Roseman@wsu.edu

The chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), threaten amphibian biodiversity. Although not yet detected here, Bsal is of particular concern in North America, a hotspot of salamander diversity. It has caused declines in Fire Salamanders (*Salamandra salamandra*) in Europe after spreading from Asia via the pet trade, demonstrating the need for effective intervention strategies. In a study designed to understand how the magnitude and duration of heat treatments affect Bsal infections and recrudescence, Rough-skinned Newts (*Taricha granulosa*) were experimentally exposed to Bsal. Across three exposure attempts—from an initial exposure to accidentally inactivated zoospores to high doses of active zoospores—newts developed only rare, low-level, transient infections. These results suggest this species is more resistant to Bsal than predicted, or that they were inadvertently immunized against Bsal. After verifying that the Bsal culture is infectious (by exposing highly susceptible Eastern Newts; *Notophthalmus viridescens*), we are exposing both previously exposed and unexposed T. granulosa to compare infection outcomes. Similar levels of infection would suggest resistance, while more severe infections in naïve compared to previously exposed newts would suggest immunization. Results will be ready for presentation by the time of this conference.

June 8th, 2:15 pm

Selective pressure from endemic *Batrachochytrium dendrobatidis* shapes genomic variation in *Litoria verreauxii alpina*

Wendt, AS[1]*; Berger, L[1]; Skerratt, LF[1]; Brannelly, LA[1]
[1]University of Melbourne
*aswendt91@gmail.com

Endemic pathogens can drive rapid evolution, but genome-scale evidence from wild populations remains scarce, particularly in declining species. Amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd), has reduced many frog populations, yet some persist under long-term Bd exposure. We tested whether Bd imposes detectable selection on the alpine tree frog (*Litoria verreauxii alpina*) by identifying genomic variants associated with disease history and temporal change. We generated genome-wide single nucleotide polymorphism (SNP) data using Diversity Arrays Technology sequencing (DArTseq) from nine sites, including Bd-naïve and Bd-endemic populations, plus two populations resampled ~11 years apart. We ran Bayesian selection scans with BayPass and used the XtX (locus differentiation) statistic to detect unusually differentiated SNPs while accounting for shared demographic history. Candidate loci were annotated with SnpEff (variant effect prediction) and assigned putative functions using protein similarity searches. All contrasts recovered high-XtX outliers, with most candidates in intergenic regions and few in protein-coding sequences. Inferred functions were dominated by signalling, transcriptional regulation, and cell-cycle processes, with some canonical immune genes. Results point to early, polygenic, regulation-heavy responses to persistent Bd pressure. Linking these variants to gene expression and infection phenotypes is a key next step for conservation.

June 8th, 2:30 pm

Antifungal defenses against *Batrachochytrium dendrobatidis* from the skin secretions of Mexican Ambystoma species

Jacinto-Castillo, DF[1]*; Aguilar-Peralta, JS[2]; Auvynet, C[1]; Hernández-Orihuela, L[1]; Figueroa, M[1]; Hissa, DC[3]; Rebollar, EA[1]
[1]Universidad Nacional Autónoma de México
[2]Red de Manejo Biorracional de Plagas y Vectores, Instituto de Ecología, A.C. INECOL
[3]Universidade Federal do Ceará
*jacinto@ccg.unam.mx

Chytridiomycosis, a skin disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), has severely impacted amphibian populations worldwide. Bd has been detected in the skin of several Mexican Ambystoma species (axolotls), but they rarely develop chytridiomycosis, suggesting tolerance to the infection. However, knowledge about their innate immune system is still very limited. This study evaluated the antifungal capacity of axolotl skin secretions against Bd and characterized their antimicrobial peptides. Skin secretion samples were collected from the only four obligate paedomorphic species endemic to Mexico: *A. andersoni, A. taylori, A. dumerilii*, and *A. mexicanum*. The antifungal activity of skin secretions was evaluated through in vitro Bd growth inhibition assays, and peptide identification was done using HPLC and mass spectrometry. Inhibition assays showed that all samples inhibited Bd growth, providing the first evidence of antifungal activity in axolotl skin secretions against this pathogen. Peptide sequence analyses revealed molecules similar to previously reported antimicrobial peptides in other organisms, including cofilin, ubiquitin, ubiquicidin, and histones such as H2A, all of which have potential antifungal roles that require functional validation. This study represents the first comprehensive analysis of peptide composition of Ambystoma skin secretions and contributes to the understanding of innate immunity in axolotls against Bd.

June 8th, 2:45 pm

Linking infection mode, sporangial growth, and host damage to explain strain-specific virulence in *Batrachochytrium dendrobatidis*

Akram, N[1]*; Webb, R[1]; Berger, L[1]; Skerratt, L[1]
[1]University of Melbourne
*akramn@student.unimelb.edu.au

Batrachochytrium dendrobatidis (Bd), the causative agent of chytridiomycosis, remains a major driver of global amphibian declines. However, Bd strain-specific differences in infection strategy and virulence are poorly understood. Here, we compared seven Australian Bd isolates to determine how growth mode influences host-cell damage. Using a Xenopus laevis A6 epithelial cell infection model, isolates originating from multiple host species and geographic regions were assessed for epibiotic (surface-associated) and endobiotic (intracellular) growth, sporangial development, and cytopathic effects. Host-cell damage was quantified using 4′,6-diamidino-2-phenylindole (DAPI) staining. All isolates displayed both epibiotic and endobiotic growth, although the extent of intracellular development varied markedly. Zoospores primarily established infection via an endobiotic mode; however, isolates 103, 109 and 3 showed a strong bias towards intracellular growth compared with isolates 46 and 79, which exhibited a more balanced growth profile. Increased endobiotic growth correlated with larger, faster-growing sporangia and significantly greater epithelial cell damage, whereas isolates with limited intracellular proliferation caused minimal cytopathic effects. These findings demonstrate that Bd virulence is driven by the combined effects of infection mode and fungal burden, with endobiotic growth emerging as a key determinant of host-cell damage. Ongoing work will apply this model to evaluate frog-skin-derived antifungal compounds and proteases.

June 8th, 3:00 pm

Multi-tissue transcriptomic analysis of amphibian immune response to an emerging bacterial pathogen

Floreste, FR[1]*; Garcia-Neto, PG[1]; Muxel, SM[1]; Titon, SCM[1]; Gomes, FR[1]; Assis, VR[1,2]
[1]University of São Paulo
[2]University of West Alabama
*felipe.floreste@gmail.com

Susceptibility to infection is an urgent issue in global wildlife health, making the characterization of immune responses essential for developing effective conservation strategies. The opportunistic bacterium *Aeromonas hydrophila* is commonly present in the amphibian microbiota, but can become pathogenic in immunocompromised hosts, often leading to death. While host–pathogen interactions involving fungal and viral agents have been extensively studied, much less is known about the amphibian immune responses to lethal bacteria. We generated a *de*

novo transcriptome of *Rhinella diptycha* toads (Anura: Bufonidae) to characterize early immune responses to *A. hydrophila* across major immune organs and identify tissue-specific differential gene expression following bacterial stimuli. Toads were injected with heat-killed *A. hydrophila*, and RNA-seq was performed on spleen and liver tissues six hours post-injection. Preliminary results reveal higher tissue-specific differential gene expression in the spleen compared to the liver, indicating organ-specific immune activation during systemic bacterial infection. Ongoing functional enrichment analyses will identify tissue-specific signaling cascades and gene families involved in this response. Our results provide a multi-tissue transcriptomic framework for understanding amphibian immune responses to *A. hydrophila*. Because these pathogens are present in healthy hosts, immune activation may depend on host condition, offering insight into mechanisms underlying susceptibility and survival.

June 8th, 3:15 pm

In vitro characterization of the *Ranavirus ambystoma1* viral eukaryotic translation initiation factor 2-alpha homologue protein's functional domains

Steel, R[1,2]; **Jancovich, JK**[1]*
[1]California State University San Marcos
[2]University of Rochester
*jjancovich@csusm.edu

Ranavirus ambystoma1, also known as Ambystoma tigrinum virus (ATV), encodes a full-length homologue of the cellular eukaryotic translation initiation factor 2α protein (vIF2αH) in open reading frame 57R. Previous studies demonstrated that the ATV vIF2αH gene is non-essential in cells in culture but is required for full pathogenesis in an animal model. In addition, the vIF2αH protein promotes the degradation of protein kinase R (PKR), a key modulator of the innate immune response. Characterization of a ranavirus vIF2αH protein using a yeast-two-hybrid system suggests that the amino- (N-) terminal S1 and central helical domains (HD) are sufficient for PKR inhibition. However, characterization of the vIF2αH functional domains has never been performed using a ranavirus model system. Therefore, we investigated the functional domains of the ATV vIF2αH protein by generating revertant and truncated S1, HD and C-terminal domain (CTD) mutants in ATV by homologous recombination. Our results suggest all three domains of the vIF2αH protein are necessary for full function of the vIF2αH protein. Therefore, this study has established approaches to express revertant and truncation mutant ranavirus proteins while providing insight into ATV vIF2αH protein's role in viral replication, modulation of PKR and host immune evasion during a native infection.

June 8th, 4:25 pm

The path to verifying amphibian Perkinsea as a lethal frog pathogen: Optimizing culturing methods from samples collected during a Florida tadpole mortality event

Brosnan, EB[1]*; Ossiboff, RJ[2]; Savage, AE[1]
[1]University of Central Florida
[2]University of Florida
*erin.brosnan@ucf.edu

The informally described protist termed "amphibian Perkinsea" (Pr) lacks cultured isolates and experimental confirmation of lethality, despite broad surveys suggesting it is the second-leading cause of amphibian mortalities in North America. Our overarching goal is to validate that Pr is a lethal pathogen, verifying its ecological significance and promoting further research on its biodiversity impacts. In order to fulfill Koch's postulates and demonstrate lethality, the first step is to isolate and culture Pr, which has not yet been achieved. We collected tadpoles with signs of perkinsiosis from a Florida mortality event in February 2025. Pr presence was verified via qPCR and formal histopathological assessment. Using a fully factorial design, we identified optimal culturing conditions for Pr by evaluating the effects of media, pH, antibiotics and temperature on Pr replication. Colony development was quantified daily by counting replicating cells via inverted microscope and hemocytometer. Pr growth across treatments was analyzed using generalized mixed-effect models, and demonstrates successful conditions for optimal Pr isolation and replication. Continuing work will generate the first Pr reference genome and demonstrate mortality and morbidity in tadpole hosts using our cultured isolates.

June 8th, 4:30 pm

Microsporidia and Cryptosporidium in headstarted Jamaican iguanas (*Cyclura collei*)

Rainwater, KL[1]*; Khan, S[2]; Armien, AG[3]; Tillis SB[4]; Wellehan JFX[4]; Conley, KJ[5]
[1]Fort Worth Zoo
[2]Hope Zoo Preservation Foundation

[3]University of California Davis
[4]University of Florida
[5]Disney's Animals, Science, and Environment
*krainwater@fortworthzoo.org

The critically endangered Jamaican iguana (*Cyclura collei*) endemic to the southeastern coast of Jamaica encountered rapid population decline in the late 1800s and was thought to be extinct. The Jamaican Iguana Recovery Group formed and mobilized conservation efforts in the 1990s after the species was rediscovered. A headstart program was created in which hatchlings are brought to the Hope Zoo in Kingston and cared for until reaching a size sufficient to evade predators. Annual health assessments of headstarted iguanas have been conducted since the early 2000s, typically in late winter. In 2024, histologic examination revealed cause of death in two iguanas to be microsporidiosis – one with intestinal and hepatic microsporidiosis and the other with a multisystemic microsporidial infection. Electron microscopy on the latter case revealed Microsporidia morphologically consistent with *Encephalitozoon sp*. In 2025, molecular diagnostics on a subset of feces from apparently healthy headstarted iguanas revealed one iguana was positive for *Cryptosporidium varanii* and three were positive for Microsporidia, one with 90% similarity to *Encephalitozoon cuniculi* and the other two with 99.6% similarity to *Vairimorpha ceranae*, a microsporidian of honeybees. Continued surveillance in both Jamaican iguana populations is needed to assess risks posed by these pathogens to the recovery effort.

June 8th, 4:45 pm

From FROG to FASTQ: Isolating an African amphibian trypanosome genome directly from natural infections in the peripheral blood with single cell sequencing

Jordaan, BJ[1]*; Castelli, M[2]; Gammuto, L[2]; Netherlands EC[1]
[1]University of the Free State
[2]University of Pavia
*berniejordaan01@gmail.com

Trypanosoma infecting African amphibian hosts are among the least understood taxa within the genus. However, they show considerable potential to elucidate the largest evolutionary split within the genus. Availability of new techniques, namely Next-Generation Sequencing and single cell isolation, allow for improved diagnoses, characterisation, and resolution of mixed infections. Recent studies highlight *Trypanosoma grandicolor* as evolutionarily significant, occupying a basal position within the phylogeny, at the primary divergence between aquatic groups. Building upon recent work, this study aimed to utilise single cell isolation and advanced molecular techniques to characterise this species' genome and shed light on the evolutionary history, unique genetic adaptations, and development of pathogenic traits within the genus. Live single trypanosome cells were successfully isolated from peripheral blood of wild-caught frog hosts. After whole genome amplification, short-read sequencing was performed, from which preliminary results indicate a partial trypanosome genome was obtained. Currently, the researchers are expanding upon the current dataset with increased sequencing depth and the combination of long-read sequencing to obtain the whole genome sequence of this species. Continued and future work is aimed at gaining a better understanding of host-parasite relationships, pathogenicity, vectors, and other ecological factors, using life cycle studies, haemocultures, and transmission experiments.

June 8th, 5:00 pm

Invasive pentastomes are a rapidly emerging circumglobal conservation issue for reptiles and amphibians

Farrell, TM[1]*; Palmisano, JN[2]
[1]Stetson University
[2]University of Central Florida
*tfarrell@stetson.edu

We discuss our unpublished findings and those from over 20 recently published accounts concerning pentastomes. Pentastome invasions have occurred on three continents (Australia, Europe, and North America) and islands in the Caribbean Sea, Mediterranean Sea and Pacific Ocean. The number of pentastomiasis cases documented in the pet trade is rapidly growing and involve localities (e.g. Belgium, Germany, Michigan) far from the parasite's previously known geographic ranges. Raillitiellids are more likely to become invasive than other pentastome taxa due to their use of synanthropic intermediate and definitive hosts and their ability to utilize novel hosts. In invasive ranges of North America and Australia, wild anurans and squamates serve as both intermediate and definitive hosts for invasive *Raillietiella spp*. The greatest health impacts and geographic range expansions involve *Raillietiella orientalis* infections in snakes. Chelonians are also at risk with three species of invasive pentastomes causing illness in wild and

captive individuals. Pentastomes may cause naïve host syndrome, whereby novel hosts, including snakes and chelonians, suffer higher prevalence, parasite intensity, and morbidity than host species with an evolutionary history with pentastomes. We discuss knowledge gaps and the geographic areas and species where we think *Raillietiella* poses particularly high probability of endangering reptiles.

June 9th, 8:45 am

Life stories of the harlequin toad: a tale told in biological collections

Vivas Barreto, D[1,2]*; Hernández-Poveda, M[3]
[1]Universidad de Los Andes
[2]Museo de Historia Natural C.J. Marinkelle
[3]University of Melbourne
*d.vivasb@uniandes.edu.co

Neotropical harlequin toads (*Atelopus spp.*) are among the most threatened amphibians worldwide, with 88 of 104 species listed as Critically Endangered and 38 considered possibly extinct. In Colombia, which hosts 40 species, most have not been observed in over three decades; although some populations may be extinct, others likely persist at very low abundances. A major driver of these declines is chytridiomycosis, an infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), which has devastated amphibian populations across the world. Understanding life-history patterns and Bd presence is essential to guide effective field monitoring and conservation planning. This study aimed to determine the reproductive seasonality of Colombian Atelopus using historical museum records. We examined 1,250 specimens representing 27 species, extracting information on sex, life stage, locality, and collection date. Meteorological data were used to correlate reproductive indicators, gravidity, tadpoles, and juvenile, with rainfall patterns across regions and years. Our results indicate that the transition between the dry and rainy seasons is the optimal period to detect tadpoles. These findings highlight the critical role of museums and biological collections in reconstructing species histories, informing conservation strategies for highly threatened amphibians, and identifying when and where Bd occurs.

June 9th, 9:00 am

From tadpoles to adults: Evidence of persistent circulation of ranid herpesvirus 3 (RaHV3) in common frogs (*Rana temporaria*) in Schleswig-Holstein, northern Germany

Grau Camps, S[1]*; Striewe, LC[1]; Leineweber, C[2]; Marschang, RE[2]; Winkler C[3]; Drews, A[4]; Steiner, N[1]; Rohner, S[1,7]; Seifert, R[5]; Pöhlmann, P[6]; Escobar Calderon, JF[1]; Siebert, U[1]
[1]University of Veterinary Medicine Hannover
[2]Laboklin GmbH & Co. KG. Bad Kissingen
[3]Faunistic-Ecological Study Group e.V. (FÖAG)
[4]State Authority for Environment (LfU)
[5]Alliance for Nature Protection in Dithmarschen e.V. Hemmingstedt
[6]Society for Outdoor Ecology and Conservation Planning mbH
[7]Zoologischer Garten Frankfurt am Main
*Sara.Grau.Camps@tiho-hannover.de

Diseases pose a major threat to amphibian populations worldwide. While chytridiomycosis and ranavirosis are widely investigated, herpesviruses – including R*anid herpesvirus 3* (RaHV3) – remain comparatively under-researched. Common frog (*Rana temporaria*) populations in Schleswig-Holstein, northern Germany, are declining. To monitor prevalences and dynamics of RaHV3 across multiple years and life stages, skin swabs from 87 postmetamorphic frogs were collected in 2022, 2023 and 2025 at four locations. In 2025, 35 tadpoles were sampled targeting keratinized tissues. All swabs were tested for RaHV3 via PCR. The factors sex, age class, location, nutritional score, and presence of skin lesions were evaluated for their association with virus detection. Prevalences varied between 30.3% to 63.6% in postmetamorphs depending on the year. Frogs with moderate to poor nutritional scores were significantly more likely to be infected than those with good body condition ($p = 0.04$), as were frogs with skin lesions ($p = 0.02$). Among tadpoles, 11.4% tested positive. Our results confirm the continued circulation of RaHV3 in common frogs in northern Germany and support reports of infection during larval or embryonic stages. However, the susceptibility of premetamorphic specimens, transmission pathways, and the impact of RaHV3 on common frogs warrants further investigation.

June 9th, 9:15 am

Living in a toxic world: can environmental contaminants reduce chytridiomycosis risk?

Meegamage, CP[1]*; Webb, RJ[1]; Berger, L[1]; Brannelly, LA[1]
[1]University of Melbourne
*cmeegamage@student.unimelb.edu.au

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), is one of the most severe wildlife diseases recorded and has driven major amphibian declines and extinctions worldwide. However, not all amphibian populations collapse after Bd arrives. In several regions, some species continue to persist even with the presence of Bd in their habitats. One explanation is that environmental contaminants may create conditions that suppress Bd while remaining tolerable for amphibians. This study investigated whether such contaminants could reduce Bd growth and viability. Using laboratory in-vitro experiments, we exposed Bd cultures to a range of heavy metals (Cu, Zn, Ni, Cd, Cr) and salt (NaCl) as individual contaminants and in mixtures that reflect natural conditions. Inhibitory concentration assays and zoospore viability tests were used to identify contaminant levels that inhibit or kill Bd. We also examined how contaminants interact in mixtures to determine whether their combined effects were additive, synergistic, or antagonistic. Our results demonstrate that metal–metal and metal–NaCl mixtures can modify Bd responses, with some combinations enhancing inhibition while others exhibit antagonistic effects. This work provides important insights for identifying disease-reduced refugia and underscores the need to carefully consider contaminant mixtures when selecting and managing amphibian reintroduction sites.

June 9th, 9:30 am

Assessment of sea turtle egg fusariosis (STEF) in *Dermochelys coriacea* nests in Puerto Rico

Rosado-Rodríguez, G[1]*
[1]University of Puerto Rico in Arecibo
*gualberto.rosado@upr.edu

Emerging fungal diseases pose a growing threat to global wildlife populations. Over the past decades, several pathogenic fungi have been implicated in significant declines of amphibian and reptile species, including the chytrids *Batrachochytrium dendrobatidis* and *B. salamandrivorans* in amphibians, *Ophidiomyces ophidiicola* in snakes, and members of the Fusarium solani species complex (FSSC) in sea turtle eggs. In Puerto Rico, FSSC was first documented in 2009, causing up to 100% egg loss in leatherback turtle (*Dermochelys coriacea*) nests on the beaches of Mayagüez and Añasco. In recent years, several conservation volunteer groups have reported continued egg mortality, with fungal involvement suspected. This study investigates the presence and composition of fungi associated with leatherback turtle nests and examines how fungal communities shift from oviposition to the end of incubation. Nocturnal monitoring at Playa Grande (Arecibo, Puerto Rico) enabled the collection of sand samples from egg chambers at the time of oviposition. Following the incubation period, unhatched eggs and sand from the chamber floor were sampled to characterize the post-incubation mycobiota. Fungi identified in pre-incubation sand included *Aspergillus, Penicillium, Cladosporium*, and *Fusarium*. Post-hatching samples revealed additional fungal morphotypes, distinct from *Fusarium*, suggesting that multiple fungal taxa may contribute to egg infection and subsequent reproductive failure.

June 9th, 9:45 am

Long-term monitoring reveals persistence of Chytridiomycosis in populations of stream anurans in the northern Western Ghats of India

Panwar, Y[1]*
Panwar, Y. S.[1*]; Sreedharan, G.[1]; Komanduri, K.P.K.[2]; Gupta, S.[1]; Dev, A. M.[1]; Vasudevan, K.[1]
[1]CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India
[2]The University of Newcastle, Australia
*Ysp.yashwant@gmail.com

1) Chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Bd), is a global threat to amphibians, yet in South-Asia it typically manifests as low prevalence, low loads, and no mortality. 2) Using seven years (2018–2024) of Capture–Mark–Recapture data from seasonal streams in the northern Western Ghats, we examined the roles of sub-clinical Bd and abiotic factors in population dynamics, survival, detection, and infection transitions in four anuran species. From 5214 anurans, 3769 swabs were collected, and Bd was diagnosed using SYBR-qPCR. 3) Bd prevalence fluctuated between high (0.43–0.94) and low levels (0.01–0.32), while infection load remained low. Prevalence exerted a weak but consistent adverse effect on population size. *Euphlyctis cyanophlyctis* (EC) and pooled frog community showed improvements in survival as prevalence declined, whereas in *Indosylvirana caesari* and

Nyctibatrachus jog, survival remained stable, albeit with large variance. Infection transition patterns suggesting disease resistance, characterised by a predominance of uninfected state, frequent recovery from infection, and reduced transitions to high-infection state in multistate robust design models. These findings demonstrate that anurans in northern Western Ghats inhabiting seasonal streams persist with Bd primarily through resistance mechanisms. Long-term monitoring reveals fluctuating Bd prevalence, with potential survival impacts on EC, informing South-Asian conservation strategies.

June 9th, 10:25 am

Leveraging host physiology to understand the impact of a changing climate on amphibian resilience to disease

Ohmer, ME[1]*; Zegar, JJ[1]; Anaya Morales, SL[1]; Diele-Viegas, LM[1,2]; and Cocciardi, JM[1]
[1]University of Mississippi
[1]Michigan State University
*mohmer@olemiss.edu

Both disease and climate variability have been identified as primary threats to amphibian populations worldwide, and while some species and populations are recently demonstrating resilience to disease, changing environmental conditions can result in new population vulnerabilities. Using a range of lab and field-based experiments and biophysical modeling to uncover the dynamic interplay between these biotic and abiotic stressors, we investigated the thermal biology and immune function of species demonstrating variable resilience to the disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) in Panama, Brazil, and North America. We demonstrate that thermal acclimation and Bd infection can have contrasting impacts on the thermal biology of infected and uninfected hosts. We then use these thermal physiology parameters from both infected and uninfected hosts to parameterize mechanistic environmental niche models and make spatial predictions of habitat suitability under current and future climatic conditions. Interestingly, species vary in how infection impacts their thermal limits and performance across temperatures, demonstrating increased susceptibility to thermal extremes. We can use this information to prioritize reintroduction and/or translocation sites that are climate and disease refuges. By leveraging physiological tools to understand mechanisms driving patterns of resilience to interacting stressors, we can future-proof conservation efforts.

June 9th, 10:40 am

Spillover dynamics and asynchronous temporal fluctuations in host density and competence drive Batrachochytrium dendrobatidis persistence in multi-host, seasonal amphibian communities

Schrock, SAR[1]; DeMarchi, JA[1]; Ohmer, MEB[2]; **Wilber, MQ**[1]*
[1]University of Tennessee
[2]University of Mississippi
*mwilber@utk.edu

Pathogens routinely persist in seasonal systems while infecting multiple species. However, in these systems we have little understanding of who drives pathogen persistence and when. We develop a model to decompose the temporal drivers of pathogen persistence in seasonal, multi-host communities. We combine this model with empirical data collected from amphibian communities infected with a *Batrachochytrium dendrobatidis* (Bd) to ask: i) do species trade-off in their contributions to Bd persistence across the year? and ii) How does seasonal variation in species contributions affect the robustness of long-term Bd persistence? We find that who contributes to Bd persistence is highly variable through time, with the dominant contributing species shifting throughout the year. Temporal variability in host density, and not host competence, describes most of the variability in species contributions to Bd persistence. Despite these species-level fluctuations, multi-year Bd persistence was often the result of a single dominant maintenance species, *Notophthalmus viridescens*, and subsequent spillover dynamics. In communities without N. viridescens, Bd persisted by leveraging temporal asynchrony in host species contributions that consistently allowed Bd fitness to remain high throughout the year. Our model reveals strategies pathogens use to persistence in complex environments and provides guidance for targeted pathogen control.

June 9th, 10:55 am

Thermal traits are resilient to immune manipulation but vulnerable to natural infection in temperate amphibians

Anaya Morales, SL[1]*; Trauffer, AG[2]; Reinert, L[3]; Rollins-Smith, L[3]; Woodhams, D[4]; Kearns, P[4]; Richards-Zawacki, C[2]; Ohmer, MEB[1]

[1]University of Mississippi
[2]University of Pittsburgh
[3]Vanderbilt University
[4]University of Massachusetts Boston
*slanayam@go.olemiss.edu

Amphibians rely on antimicrobial peptides (AMPs) secreted from their skin as a first line of defense against pathogens, such as *Batrachochytrium dendrobatidis* (Bd). AMPs play a critical role in mitigating infectious disease impacts. However, the interaction between AMPs and amphibian thermal physiology remains poorly understood. Using two North American frog species, *Rana clamitans* (green frogs) and *Rana pipiens* (northern leopard frogs), we investigated the effects of AMP depletion on multiple thermal traits and AMP profiles. Our findings revealed that AMP depletion did not significantly affect critical thermal limits (CTmax or CTmin) in either species. However, natural Bd infections reduced CTmax in green frogs in a load-dependent fashion and decreased thermal preference. After two weeks, green frogs fully recovered peptide quantities, while leopard frogs did not, suggesting potential links between AMP replenishment rates and species-specific susceptibility to disease. MALDI-ToF mass spectrometry identified species-specific AMP profiles, and skin microbiome community analysis revealed species specific assemblage and no effect of treatment on community composition. Although initially shaped by species and infection, communities converged after prolonged captivity, indicating strong laboratory effects. This work advances our understanding of how amphibian skin immune components influence thermal physiology in the context of disease and environmental stress.

June 9th, 11:10 am

Influence of natural chytrid fungal infection and thermal acclimation on the physiology of a common eastern North American amphibian

Zegar, JJ[1]*; Ohmer, MEB[1]
[1]University of Mississippi
*jzegar@go.olemiss.edu

Climate change and infectious disease are pervasive ecological stressors that can interact to reshape organismal physiology. The fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), has caused global amphibian declines, and recent evidence suggests a changing climate may worsen impacts. Quantifying how infection shapes thermal physiological traits that directly govern performance and survival can improve predictions of the impacts of endemic disease and rising temperatures on amphibian populations. Previous work in experimentally infected amphibians indicates that Bd infection can lower critical thermal limits, but it is unclear if naturally infected animals are also impacted. We investigated the combined effects of Bd infection and thermal acclimation (15°C and 25°C) on the critical thermal limits, thermal sensitivity of jumping performance, and resting metabolic rate of wild-caught *Pseudacris crucifer*. Preliminary data suggests that acclimation temperature is the primary driver of variation in these traits and may override effects of Bd infection, but given a low number of infected animals in initial trials, work is on-going to increase sample size. Together, this work will provide mechanistic insight into how host thermal physiology and natural history interact to shape disease impacts by linking thermal limits, locomotor performance, and metabolic rate in Bd-susceptible amphibians under climate warming.

June 9th, 11:25 am

Experimental evidence for behavioral cooling as a response to virus infection in a poikilothermic vertebrate

Herczeg, D[1,2,3]*; Bókony, V[1]; Herczeg, G[2,3]; Holly, D[1,4]; Kásler, A[1,3]; Ujszegi, J[1,3]; Papp, T[4]; Hettyey, A[1,2,3]
[1]HUN-REN Centre for Agricultural Research
[2]HUN-REN-ELTE-MTM Integrative Ecology Research Group
[3]ELTE Eötvös Loránd University
[5]HUN-REN Institute for Veterinary Medical Research
*herczegdavid88@gmail.com

Behavioral plasticity can enhance the ability of wild animals to survive disease outbreaks. In the absence of endogen heat control, poikilothermic animals adjust their body temperature behaviorally. While behavioral fever is well documented, its opposite, behavioral cooling - when infected animals actively seek cool microenvironments - remains poorly documented. Here, we report the first experimental evidence of behavioral cooling in a poikilothermic vertebrate as a response to pathogenic infection. We investigated thermoregulatory responses in tadpoles of agile frog Rana dalmatina, a cool-adapted amphibian, experimentally infected with a ranavirus capable of replicating at high temperatures. Tadpoles were kept for five days post-exposure in either a thermal gradient or a homogeneously cool

environment. In thermal gradients, all tadpoles reduced their preferred temperatures over time, but this decrease was steeper in infected tadpoles, and individuals with higher infection intensities preferred cooler temperatures. In contrast to non-infected conspecifics, infected tadpoles became increasingly precise in thermoregulation over time. Infection prevalence was similar between the two thermal environments, yet infection intensities were significantly higher in the thermal gradient. These findings indicate fine-tuned thermoregulation in infected tadpoles likely reflecting a trade-off between the pathogen-suppressive benefits of behavioral cooling and the physiological costs of maintaining low body temperatures.

June 9th, 11:40 am

Who is winning? Seasonal dynamics of skin-associated host defenses against *Batrachochytrium dendrobatidis* in *Notophthalmus viridescens*

Marquez, M[1]*; Miller, D1; Bletz, M[1]
[1]The Pennsylvania State University
*Mxm7163@psu.edu

Temperature shapes host-pathogen interactions in amphibians by differentially affecting pathogen growth and host immune function, altering infection outcomes. Innate skin-associated defenses, including the microbiome and mucosal defenses, play a key role in defense against the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). However, variation in these defenses within individuals across time in natural populations remains poorly understood. Here, we use a capture–mark–recapture approach to examine seasonal changes in skin bacterial communities and mucosal defenses in a population of Eastern red-spotted newts (*Notophthalmus viridescens*). From March through August of 2025, individuals were repeatedly sampled to quantify Bd infection via qPCR, characterize skin bacterial communities using 16S amplicon sequencing, and assess the ability of mucosal function against Bd. By pairing repeated individual sampling with fine-scale temporal data, this study disentangles within-individual temporal change from among-individual variation in skin defenses and infection. Our analyses reveal temporal shifts in microbial community composition and function, changes in mucosal function and Bd infection intensity, which are partially explained by temperature variation across the season. Together, these findings emphasize the importance of seasonal and temperature-dependent variation in skin defenses for predicting disease risk and host resilience in wild amphibian populations.

June 9th, 11:55 am

Determining the influence of temperature on in-host *Batrachochytrium dendrobatidis* growth in field systems

Flowers, J[1]*; Miller, DA[1]
[1]The Pennsylvania State University
*Jgf5234@psu.edu

Climate change is rapidly contributing to global temperature changes that could have drastic impacts on the conservation of species. We expect these changes to impact disease dynamics, particularly in the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd) system where we know that it exhibits a thermal growth optimum. However, we see differences between the observed Bd thermal growth optimum for in-lab studies and field studies. Simulations suggest this may be due to host immune response which is related to both temperature and time since infection. This may be exacerbated further due to unseen effects related to individual and site level differences seen in field systems. To better understand Bd growth in field systems, we seek to evaluate the relationship of Bd growth with temperature using longitudinal *Notophthalmus viridescens* field data while accounting for other factors such as time since infection, and individual-level and site level effects. Specifically, we approach this using an exponential population growth model. Our simulation studies show promising preliminary results. After incorporating real data, we expect that these findings will better inform our understanding of how Bd growth differs between field and in-lab systems, which will help to prepare conservation strategies in a changing world.

June 9th, 2:00 pm

The energetics of ophidiomycosis in a subtropical pit viper population

Lind, CM[1]*; Agugliaro, J[2]; Ortega, J[3]; Lorch, JM[4]; Farrell, TM[5].
[1]Stockton University
[2]Fairleigh Dickinson University
[3]University of Arkansas
[4]US Geological Survey National Wildlife Health Center
[5]Stetson University

*craig.lind@stockton.edu

Energetic coping costs are an important sublethal consequence of fungal infection. Coping costs may result from the upregulation of components of immune defense, the repair of damaged tissues, alteration of host thermoregulatory physiology or behavior, or other physiological processes that promote disease clearance (e.g. ecdysis). In ectothermic vertebrates, components of host coping effort may be sensitive to the thermal landscape, and linking environmental temperatures with host energetics may shed light on mechanisms that drive patterns of disease. We explored the energetics of coping with ophidiomycosis (or snake fungal disease) in pygmy rattlesnakes, *Sistrurus miliarius*, by measuring metabolic rates and body temperature in naturally infected individuals. We synthesize our findings from field, mesocosm, and laboratory studies to explain the role of energetics in host-pathogen dynamics. Specifically, we break down components of coping effort, link costs to behavioral fever and seasonal variation in the thermal environment, and explain seasonal costs as a potential driver of observed patterns in host energetics and disease severity in free-living S. miliarius populations. Our results indicate that components of coping effort are sensitive to the thermal environment and that energetic coping costs are a key sublethal mechanism that may explain seasonal disease dynamics in *S. miliarius* populations.

June 9th, 2:05 pm

Hemotological and Biochemical Parameters in Mexican Axolotls (*Ambystoma mexicanum*)

Yazbec, E[1]; **Knafo, SE**[1]*
[1]University of Tennessee
*sknafo@utk.edu

This study utilized 41 captive bred Mexican axolotls. Blood collection was performed with an insulin syringe (27g needle), 25g, 22g, or 20g needle and 1ml syringe using the ventral tail vein. Results were produced for these samples, though hemolysis made creating a valid reference interval impossible based on ASVCP guidelines. While hemolysis can be attributed in part to collection technique, another significant factor is the large size of axolotl red blood cells (RBCs). The average dog RBC is approximately 7um in diameter compared to 20-30um diameter in axolotls. Therefore, using a large gauge needle (>22g) may be necessary to minimize hemolysis and is important to keep in mind when collecting blood and evaluating results. Hematology and biochemistry panel mean and SD results were reported for the processed samples. A comparison of values for non-hemolyzed vs hemolyzed blood samples was performed to determine significance of hemolysis.

June 9th, 2:10 pm

Investigating protease function to reveal gene targets for innovative chytridiomycosis control strategies

Sumanasekera, KK[1]*; Webb, RJ[1]; Tham, W-H[2,3]; Sleebs, B[2]; Skerratt, LS[1]; Outram, M[3]; Berger, L[1]
[1]University of Melbourne
[2]Walter and Eliza Hall Institute of Medical Research
[3]Australian National University
*ksumanaseker@student.unimelb.edu.au

Chytridiomycosis continues to threaten amphibians worldwide, yet effective management in the wild populations remains limited. This study investigates the virulence mechanisms of *Batrachochytrium dendrobatidis* (Bd), focusing on the role of fungal proteases in growth, infection, and host invasion. Previous studies have shown increased protease gene expression in Bd upon exposure to amphibian skin, although the underlying mechanisms remain unclear. We tested a range of aspartyl and metalloprotease inhibitors on both zoospore and zoosporangia life-stages using standard TGhL culture conditions and an amphibian host cell infection model. Our findings demonstrate that proteases play a critical role in Bd growth and host cell infection. Notably, protease activity differed between experimental models (Bd cultured alone versus Bd within host cells) and between life stages. Zoospores were more sensitive to protease inhibition than zoosporangia, and substantially lower inhibitor concentrations were effective in the host cell infection model. Importantly, some inhibitors were only effective against Bd when in contact with host cells. Ongoing work is exploring development of inhibitor resistance followed by genome sequencing to identify the key protease genes involved. These efforts lay the groundwork for new intervention strategies, including novel antifungal drugs and potential vaccination approaches, to mitigate chytridiomycosis and support amphibian conservation.

June 9th, 2:25 pm

Differential immune responses correlate with Bsal-chytridiomycosis severity in Italian crested newts (*Triturus carnifex*)

Fieschi-Méric, L[1]*; Mulder, K[1]; Fernández Meléndez, E[1]; Van Praet, S[1]; De Bruyckere, S[1]; Fahrbach, M[2]; Pasmans, F[1]; Martel, A[1]
[1]Ghent University
[2]Niedernhaller Str. 8/2, 74653 Criesbach, Germany
*lea.fieschimeric@ugent.be

In the midst of the current biodiversity crisis, amphibians are severely threatened by emerging diseases such as chytridiomycosis. Characterizing the mechanisms that underlie susceptibility to this disease is fundamental to improve amphibian conservation. Using a comprehensive multi-omics approach, we investigated the impact of an exposure to the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) on the gene expression of Italian crested newts (*Triturus carnifex*) and on the bacterial symbionts that constitute their skin microbiota. Exposure to Bsal affected multiple components of the newts' immunity, from structural changes in their microbiota and decreased expression of keratin-encoding genes in the skin, to the upregulation of genes involved in inflammation and adaptive immunity both at the site of infection (skin) and in their primary lymphoid organ (spleen). Chytridiomycosis severity was positively correlated with the downregulation of basal metabolism and with the upregulation of immune responses and of tissue restructuration. Together, our results suggest that in *T. carnifex*, Bsal susceptibility may be linked to a reallocation of energy resources from the maintenance of basal metabolism and tissue integrity, towards elevated immunity and tissue restructuration.

June 9th, 3:05 pm

T cell receptor repertoire diversity across amphibian ontogeny and phylogeny

Vu, AL[1]*; Robert, J[1]
[1]University of Rochester Medical Center
*Andrea_vu@urmc.rochester.edu

T cell receptors (TCRs) are protein complexes on the surface of T cells that are important in the immune system. During development in the thymus, T cells undergo somatic recombination to produce many distinct TCRs made up of an alpha chain and beta chain. T cells in adult Xenopus laevis frogs, like all jawed vertebrates, have a diversified TCR repertoire. However, T cells in tadpole X. laevis have an overrepresentation of very limited innate-like TCR rearrangement reminiscent of human fetal T cells. Although variation in TCR repertoire diversity during different developmental stages has been characterized in the amphibian model *Xenopus laevis*, TCR repertoire diversity changes in other amphibian species during development is largely uncharacterized. Amphibian species can vary length of time in development from days to years to metamorphosis, which may play an important role in disease susceptibility. In this study, we sampled frog species across the amphibian phylogeny, to assess TCR alpha repertoire diversity over developmental stages. We additionally characterized the TCR alpha constant regions and expression preferences in frog species including *Rana catesbeiana* and *Rana sphenocephala*, among others. Preliminary results will be discussed.

June 9th, 3:20 pm

Cytokine-guided mast cell plasticity in an amphibian model of antifungal immunity

Tesega, H[1]*; Crow, R[1]; Grayfer, L[1]
[1]George Washington University
*h.tesega@gwmail.gwu.edu

Although traditionally studied in context of allergies, mast cells have broader roles in host defenses as tissue-resident immune sentinels. Mast cells store a plethora of immune-active molecules within prepackaged granules. Upon detecting infections or tissue damage, they rapidly release these mediators, driving immune responses and altering tissue physiology. Our previous work indicates that frog (*Xenopus laevis*) skin-resident mast cells confer antifungal protection by producing the interleukin-4 (IL4) cytokine. Because this cytokine is closely tied to mammalian mast cell functionality, presently, we explored the roles of IL4 in regulating frog mast cell responses. To this end, we treated X. laevis bone marrow- and peritonea-derived mast cells with a recombinant (r)IL4. rIL4-treated mast cells possessed substantially more granules, with greater baseline and stimulation-induced releases of these pre-packaged effector molecules. Pertinent to the antifungal nature of these cells, rIL4-treated mast cells also expressed greater levels of acidic chitinase, an enzyme critical for degrading fungal chitin. This reinforces the roles of X. laevis mast cells in combating fungal pathogens such as *Batrachochytrium dendrobatidis* (Bd). In turn, our findings suggest that as in mammals, IL4 plays a critical role in augmenting amphibian mast cell functionality.

June 9th, 3:35 pm

When protection becomes pathology: mast cell–driven susceptibility in the Panamanian Golden Frog

Crow, R[1]*; Ellison, A[2]; Osborne, O[2]; Grayfer, L[1]
[1]Department of Biological Sciences, George Washington University
[2]School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, Wales.
*ryley@gwmail.gwu.edu

Mast cells are key tissue-resident immune sentinels found across vertebrates. Upon activation, mast cells rapidly release potent bioactive molecules that confer immune responses and alter tissue microenvironments. Dysregulated mast cell responses cause severe tissue damage, reflected in several human and animal diseases. We recently demonstrated in the amphibian *Xenopus laevis* model, that skin-resident mast cells provided significant protection against the *Batrachochytrium dendrobatidis* (Bd) chytrid fungus. To extend these findings towards species susceptible to Bd, we sought to characterize mast cells of the critically endangered Panamanian Golden frog, *Atelopus zeteki*. We established in vitro cultures of *A. zeteki* mast cells from bone-marrow–derived precursors using a recombinant *A. zeteki* mast cell growth factor, which was also used to enrich skin mast cells *in vivo*. Our work indicates that *A. zeteki* mast cells robustly respond to Bd *in vitro*. However, and to our surprise, enriching *A. zeteki* skin mast cells culminated in significantly greater Bd susceptibility rather than the greater resistance that we previously observed in *X. laevis*. These findings reveal contrasting mast cell responses between Bd-resistant and Bd-susceptible amphibians, underscoring the need to understand the bases of these species-specific immune differences.

June 9th, 3:50 pm

Longitudinal gene expression of Southern Corroboree frogs (*Pseudophryne corroboree*) in response to chytridiomycosis, key insights into pathways associated with infection and disease progression

Guinto, DJ[1]*; Hernández-Poveda, M[1]; Davidson, MJ[1]; Aquilina, A[1]; Skerratt, LF[1]; Berger, L[1]; Torres-Sánchez, M[2]; Kosch, TA[1]
[1]University of Melbourne
[2]University of L'Aquila
*dguinto@student.unimelb.edu.au

The iconic Southern Corroboree frog (*Pseudophryne corroboree*), once abundant in alpine forests of Kosciuszko National Park now hangs on the brink of extinction resulting from the spread of the introduced amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). The persistence of P. corroboree currently depends on captive breeding programs; however, prospects for long-term survival in the wild remains unlikely since chytrid fungus is maintained on the landscape by the chytrid tolerant common eastern froglet (*Crinia signifera*). Investigating immune response to the pathogen through gene expression will help to better understand genetic potential for Bd resistance or tolerance by identifying genes and pathways associated with positive disease outcomes. Here, we used Illumina NovaSeq whole transcriptome sequencing of toe clip samples and applied differential gene expression analysis, weighted gene co-expression analysis, and enrichment analysis to identify key immune genes and pathways expressed in response to infection across four time points. Using disease phenotypes to inform gene expression comparisons, we classify specific gene networks and pathways of interests. We found an important interplay of inflammatory response and infection status as well as a relationship between ion transfer and disease progression. Our results will direct future efforts to increase resistance in the highly susceptible *P. corroboree*.

June 9th, 4:05 pm

The innate and adaptive immune response of the critically endangered *Pseudophryne spp.* to chytridiomycosis

Goodhill, C[1]*; Robert, J[2]; Skerratt, L[1]; Berger, L[1]
[1]The University of Melbourne
[2]The University of Rochester, Medical Center
*cgoodhill@student.unimelb.edu.au

Batrachochytrium dendrobatidis (Bd) is a fungal pathogen responsible for global amphibian declines, yet the mechanisms underlying host resistance remain poorly understood. The corroboree frogs (*Pseudophryne corroboree* and *Pseudophryne pengilleyi*), endemic to Australia's Mt. Kosciuszko National Park, have suffered severe Bd-driven population crashes and are now critically endangered. This study investigates host immune responses to Bd in corroboree frogs, with an initial focus on adaptive immunity. *P. pengilleyi* individuals were experimentally exposed to Bd, cleared of infection using antifungal treatment, and subsequently re-exposed. Survival analyses revealed no evidence of acquired immunity: neither prior exposure nor fungicide treatment improved resistance to secondary

infection. These findings indicate that adaptive immune priming is ineffective in this system, prompting a shift in focus toward innate immune mechanisms. Based on previous transcriptomic analyses in *P. corroboree* candidate innate immune genes associated with Bd resistance were identified in exposed but uninfected individuals. Expression of these genes will be analyzed in P. pengilleyi liver tissue and in infected and uninfected A6 cells to test whether shared innate pathways promote amphibian resistance to Bd. Candidate genes will then be genetically modified in *Xenopus* model species to evaluate their role in host defense against Bd.

June 9th, 4:20 pm

Nutritional immunity in amphibian–*Batrachochytrium* species interactions

Torres-Sánchez, M[1]*
[1]Amphibian Genomics Consortium
*torressanchez.maria@gmail.com

The invasive fungal species of the genus *Batrachochytrium* pose a major threat to global amphibian diversity. During interactions between these fungal symbionts and their amphibian hosts, amphibians can develop an infectious skin disease known as chytridiomycosis. Variation in infection outcomes across amphibian species and populations has driven extensive research aiming to mitigate the impact of the introduction of these fungi. Infection trials are a widely used approach to identify species' functional responses, which usually require the activation of differential genetic machinery. Using a multispecies comparative framework, I explored previously published and newly generated transcriptomes from experimentally infected individuals of 18 amphibian species with varying disease susceptibility that were exposed independently to *Batrachochytrium dendrobatidis* or *B. salamandrivorans*. I applied a dual transcriptomics technique to characterise gene expression profiles of both amphibians and fungi simultaneously. Multiple ion transporters and ion binding proteins for both amphibians and *Batrachochytrium* fungi showed increased expression in the infected skins. These proteins could restrict access to essential elements, such as metal and calcium ions, through a process known as nutritional immunity. Metals are critical for fungal virulence, serving as cofactors for numerous enzymes, while also being indispensable for amphibian physiology. Intracellular calcium is a universal signaling molecule, involved, for instance, in the activation of amphibian canonical immune responses and in the development of *Batrachochytrium* sporangia. Disruption of ion homeostasis can be detrimental to both amphibians and *Batrachochytrium* species, suggesting a competitive interaction mediated by nutritional immunity.

June 10th, 8:45 am

Integrated ranavirus surveillance in Gopher Tortoises of Florida

Hardman, R[1]*; Titterington, K[2]; Keel, K[1]; Orton, L[1]; Steen, D[1]; Subramaniam, K[3]; Adamovicz, L[4]; Allender, M[4]
[1]Florida Fish and Wildlife Research Institute
[2]Swamp Girl Adventures
[3]University of Florida
[4]University of Illinois
*rebecca.hardman@myfwc.com

Gopher tortoises (GTs) are an important keystone Chelonian species for upland coastal plain ecosystems of the southeastern USA. Although ranaviruses are documented to infect and cause disease in Chelonians, there is little information on their pathophysiology and epidemiology within GTs specifically, with little known health consequences of infection. The Fish and Wildlife Research Institute within the Florida FWC has collaboratively performed surveillance of ranaviruses in GTs in Florida from wild healthy and rehabilitated individuals (antemortem), and from necropsy cases (postmortem). Antemortem samples included oral/cloacal swabs with or without whole blood. Postmortem samples included swab and liver at minimum. Testing was via qPCR. We detected ranaviruses in a total of 14 out of 363 individuals representing wild healthy (0/70; 0.0%), rehab (6/268; 2.2%), and necropsy (8/25; 32.0%) cases. Positives were from swabs and tissue only, not blood. Most positive necropsy cases were associated with gross observations of multifocal hemorrhaging throughout the gastrointestinal tract and other coelomic organs. Blood smears were available in a few positive cases and some revealed cytoplasmic inclusions. Our findings document apparent severe ranavirosis in GTs and highlight the need for more research including better characterization of disease and creation of best antemortem and postmortem sampling protocols.

June 10th, 9:00 am

Life-stage structure and host competence shape pathogen persistence in multi-host communities

DeMarchi, JA[1]*; Lutz, KR[2]; Claymon, KA[2]; Searle, CL[2]; Schrock, AR[1]; Gray, MJ[1]; Wilber MQ[1]
[1]University of Tennessee

[2]Purdue University
*jdemarch@utk.edu

Predicting pathogen persistence in ecological communities remains a central challenge in disease ecology, particularly for generalist pathogens circulating among multiple host species that vary in infection competence. While host composition is often measured, how species-specific infection traits scale up to determine community-level persistence remains poorly understood. Here, we investigate how indirect environmental transmission and host competence traits interact to govern pathogen persistence in multi-host systems. We focus on the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd). We developed a multi-species, multi-life-stage host–pathogen integral projection model (IPM) parameterized using empirical infection, growth, and shedding data from amphibian exposure experiments. The model represents communities composed of adult and larval *Rana catesbeianus* and *Rana clamitans*, and adult *Notophthalmus viridescens*, and explicitly tracks load-dependent pathogen and environmental zoospore dynamics. Bd persistence strongly depended on community composition and life-stage structure. Under indirect transmission alone, single-species or adult-only communities rarely sustain Bd, except N. viridescens. In contrast, multi-species communities maintained Bd through complementary zoospore contributions, driven primarily by larval stages. These results demonstrate that Bd persistence emerges from load-dependent transmission and community structure, emphasizing the need to link host competence traits to environmental transmission when identifying maintenance hosts in multi-host systems.

June 10th, 9:15 am

The importance of host species, size, and latitude on infection risk of the invasive parasite, *Raillietiella orientalis*

Kesselring, JH[1]*; Nelson, A[2]; Durso A[3]; Brennan M[5]; Hazelrig CM[5]; Nemeth NM[5]; Hopkins, S[2]; Savage AE[6]; Farrell T[4]; Palmisano, JN[6]
[1]University of South Florida
[2]North Carolina State University
[3]Florida Gulf Coast University
[4]Stetson University
[5]University of Georgia
[6]University of Central Florida
*jasminekesselring@usf.edu

Raillietiella orientalis (Ro) is an invasive pentastome that infects the lungs of snake and lizard definitive hosts in Florida. Ro is associated with morbidity and mortality in native snakes and has been detected in over half of Florida's counties. In 2022, Snake Lungworm Alliance and Monitoring (SLAM) was founded to track Ro's rapid expansion via opportunistic sampling of road-killed snakes. SLAM members have since dissected 1,556 snakes across the southeastern United States and documented an expansion 80 km northeast and 435 km northwest of Ro's previously known range. Ro infections were limited to Florida, where 29% of individuals (214 of 729) were infected. Eleven of 33 snake species sampled (33%) exhibited 20-60% prevalence with highest prevalences in *Coluber constrictor* (60%), *Sistrurus miliarius* (45%), *Thamnophis sirtalis* (41%), and *Nerodia fasciata* (39%). Lower latitude was a significant predictor of infection for *Agkistrodon conanti*, *C. constrictor*, *N. fasciata*, *Pantherophis quadrivittatus*, and *Thamnophis saurita*. Increased snout-vent length (SVL) was a significant predictor of infection for *S. miliarius*, *N. fasciata*, and *C. constrictor*. The influences of species, latitude, and SVL on Ro infection highlight potential risk factors for wild snakes and are important to consider in future pathogen and conservation assessments.

June 10th, 9:30 am

Novel computational pipeline for assessing microbe-mucin interactions and anti-chytrid compound production in amphibian microbiomes

Barnes, EM[1]*; Thompson, E[1]; Faust, EA[1]
[1]Rochester Institute of Technology
*embsbi@rit.edu

For decades the pathogenic chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and its relative *B. salamandrivorans* (Bsal), have threatened the survival of amphibians by infecting their skin. Recent research has shown that Bd/Bsal development is triggered by skin mucus, a complex mixture of immunomodulatory molecules and microorganisms embedded in a matrix of glycosylated proteins (mucins) which microbes can use for attachment or as a food source. To date, the use of mucin glycans by amphibian microbes remains poorly studied. To accelerate research related to microbe-mucin interactions, we have designed a bench-to-computation pipeline for generating and scanning large datasets of microbial genomes or metagenomes to identify candidate keystone microbes. The pipeline includes

genome assembly, annotation, metabolic modeling, and analysis focused on identifying biosynthetic gene clusters and pathways associated with chitin-binding/degradation, mucus adhesion and degradation, biofilm formation, siderophore biosynthesis, chemotaxis, and antifungal compound production/resistance. We demonstrate this pipeline using a dataset collected from *Ambystoma laterale* identifying several putative keystone members of their microbiome (*Serratia, Chryseobacterium, Acinetobacter sp.*) which likely contribute to the species' low Bd-infection prevalence. The in-silico nature of this pipeline will accelerate understanding of amphibian disease ecology to deliver high-throughput screening and simulation of microbiome dynamics.

June 10th, 9:35 am

Dose-dependent disease dynamics in eastern newt (*Notophthalmus viridescens*) exposed to *Batrachochytrium salamandrivorans*

Chowdhury, M[1]*; Carter, ED[2]; Gray, MJ[2]; Peace, A[3]
[1]Virginia Polytechnic Institute and State University
[2]University of Tennessee
[3]Queens University of Charlotte
*mohammc@vt.edu

In wildlife disease systems, exposure dose can strongly influence how outbreaks unfold and how severe they become. However, many standard epidemiological models assume equal infection risk across doses and often rely on a single infection stage. This simplification can miss important effects of initial pathogen load and environmental reservoirs. In this study, we examined how exposure dose influences disease progression and transmission. We hypothesized that higher doses speed up disease progression, increase mortality and pathogen shedding, and shorten the infectious period. We estimated key disease parameters—including latency period, disease-induced mortality, spore shedding rates, and transmission probability—using experimental data from eastern newt (*Notophthalmus viridescens*) exposed to different concentrations of the fungal pathogen *Batrachochytrium salamandrivorans* (Bsal). Using these estimates, we developed a mathematical model that incorporates dose-dependent transmission, multiple infection stages, and environmental pathogen accumulation. Our results show that exposure dose meaningfully alters disease dynamics, host outcomes, and environmental persistence. Incorporating dose-response effects into epidemiological models can improve outbreak prediction and support better management of emerging wildlife diseases.

June 10th, 9:40 am

Experimental manipulation of host trait diversity on predictability of disease outcomes and FV3 virulence

Campbell, M*[1]; Hua, J[1]
[1]University of Wisconsin-Madison
*mcampbell32@wisc.edu

Infectious diseases are an important contributor to amphibian declines. Host diversity is expected to be an important mechanism shaping disease outcome. Using a Wood frog-*Frog Virus 3* (FV3) model, we asked: How does host population trait diversity (FV3 tolerance) influence (1) host disease outcomes and (2) viral traits? To address these questions, we characterized baseline FV3 tolerance across 25 families and generated 250 single family (low diversity) and 250 mixed family (high diversity) experimental populations. Each artificial population had eight hosts and varied in magnitude of tolerance to FV3 (average time to death- TTD post FV3 exposure) and population variation in tolerance to FV3 (standard error in TTD). We serially and individually infected hosts in each artificial population with FV3 and documented TTD for each individual. Next, we evaluated whether viral traits differed between artificial populations following outbreaks. We found (1) In single family populations, disease outcome was harder to predict (higher TTD variability) in populations with higher baseline FV3 tolerance. (2) FV3 from these single-family populations experiencing larger variation in disease outcomes was less virulent. Collectively, this experiment informs how host diversity shapes our ability to predict disease outcomes and mediate future outbreaks via shifts in parasite traits.

June 10th, 9:45 am

Changing responses of susceptible frogs to chytrid infection revealed following three decades of wild amphibian monitoring

West, M[1,2]*; Gillespie, G[1]
[1]University of Melbourne
[2]Wild Research Pty Ltd
*matthew.west@unimelb.edu.au

When Chytridiomycosis emerged as a global threat to amphibians, conservationists urgently needed to understand individual and population-level responses of wild amphibians to chytrid fungus infection. Fortunately, we had already established an intensive mark-recapture study for two species in the 1990s to determine whether population changes were driven by natural or threatening processes. Building on this, we assessed infection responses by swabbing all individuals and determining chytrid status through quantitative PCR testing. Multi-state mark-recapture analysis was used to evaluate transitions in disease state, associated survival and population abundance. Amphibian population dynamics were influenced by complex interactions among natural processes, multiple threats and episodic extreme events such as bushfire. Although individual survival in both species was strongly affected by disease status, the species showed different population-level outcomes depending on their capacity to offset disease-induced mortality through recruitment. Ongoing monitoring now indicates that survival of some chytrid infected individuals is changing. These results provide hope that initially susceptible populations may persist despite chytrid infection and create opportunities to enhance populations chytrid resilience.

June 10th, 10:25 am

Pathogens in the domestic pet amphibian trade

Brunner, JL[1]*; Gray, MJ[2]; Warwick, A[3]; Poudyal, NC[2]; Pearhill, RAI[1], Viadanna, PHdO[1,2]
[1]Washington State University
[2]University of Tennessee, Knoxville
[3]Michigan State University
[4]University of Idaho
*jesse.brunner@wsu.edu

The trade of amphibians for research, food, and pets has aided the spread of dangerous pathogens, such as the chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), and *Ranavirus spp.* (Rv). Most research has focused on international trade (e.g., at borders, in imports) and so relatively little is known about pathogens within domestic trade networks. We conducted an anonymous pathogen surveillance study among hobbyists and businesses within the US. We found Bd and Rv were less common within participating businesses than has previously been observed at US borders, raising the question of what reduced prevalence? Aside from the potential biases in the types of businesses that volunteered to participate in our surveillance (i.e., this discrepancy may not be real), we consider the potential for typical housing temperatures and biosecurity measures to reduce the persistence and spread of pathogen within and between facilities in the pet amphibian trade. Data from several small studies show that simple biosecurity measures (e.g., quick disinfection baths) can appreciably reduce spillover risk and that warmer housing temperatures (e.g., those at which most tropical species are housed) can prevent Bsal infections, but may mask sublethal ranavirus infections. Practices in the domestic trade can diminish risk.

June 10th, 10:40 am

Susceptibility of ten common pet amphibians to the fungal pathogen *Batrachochytrium dendrobatidis*

Masecar, SC[1]*; Piovia-Scott, J[1]
[1]Washington State University
*susanna.masecar@wsu.edu

The pet trade is a recognized pathway for the spread of *Batrachochytrium dendrobatidis* (Bd), a fungal pathogen driving widespread amphibian declines. Understanding species-specific susceptibility to infection, disease, and mortality is instrumental in predicting Bd dynamics, yet susceptibility remains poorly characterized for many common pet species. We experimentally assessed the Bd susceptibility of ten frequently traded amphibian species (*Heterixalus alboguttatus, Dendropsophus marmoratus, Dendrobates tinctorius, Dyscophus guineti, Pyxicephalus edulis, Ceratophrys cranwelli, Lepidobatrachus laevis, Pleurodeles waltl, Rentapia flavomaculata*, and *Neurergus kaiseri*; n = 10 Bd-exposed and 5 controls per species). We exposed animals to Bd via water bath, monitored daily for clinical signs and mortality, and quantified infection intensity via qPCR of skin swabs every 6 days for 60 days. Susceptibility spanned a wide continuum from 0% infection to 100% mortality, highlighting striking interspecific variation. Many animals maintained chronic, high intensity infections. Our results were broadly consistent with previously established taxonomic patterns. Our findings expand Bd susceptibility knowledge to new genera and families and underscore the risk posed by infected but apparently healthy animals. Using targeted Bd surveillance could reduce pathogen persistence in captivity, and thus safeguard animal health as well as reduce spillover risk.

June 10th, 10:55 am

An investigation into consumer trends, attitudes and behaviour within the trade of pet reptiles and amphibians in the United Kingdom

Hesten, ACM[1]*
[1]Liverpool John Moores University
*a.hesten@2022.ljmu.ac.uk

The wildlife trade is a complex and controversial topic involving diverse stakeholders and affecting a wide range of species. Despite being a significant component of the wildlife trade, the exotic pet market remains under-researched, including in the United Kingdom (UK). Diseases spread through this trade can have serious welfare and conservation implications, particularly if transmitted to native species. Pathogens such as *Batrachochytrium salamandrivorans* (Bsal) and ranavirus have been documented spreading via the pet trade and are known to be present in UK captive collections. Through mixed-mode surveys of reptile and amphibian keepers (n = 643), we investigate disease management behaviours and attitudes in the UK, as understanding this transmission pathway is vital for preventing disease spread. Together, the quantitative and qualitative findings from this study indicate a disconnect between attitudes and actual behaviours relating to biosecurity and emphasise persistent barriers to effective information transfer, potentially compounding the occurrence of high risk biosecurity-related behaviours. Addressing these gaps is critical for future conservation strategies aimed at reducing the risk of pathogen transmission from captive reptiles and amphibians to wild populations. These findings have implications for targeted biosecurity outreach and policy, highlighting the need to better understand human dimensions of the trade.

June 10th, 11:10 am

Xenopus laevis, and *Batrachochytrium dendrobatidis*, two for one threat for French amphibians?

Lesbarrères, D[1,2,3]*; Bezandry, S[2]; Blanchais, S[2]; Forzán, M[4]; Pagano, A[2]; Pays, O[2]
[1]Université de Sudbury
[2]Université d'Angers
[3]Laurentian University
[4]University of Wyoming
*dlesbarreres@usudbury.ca

Invasive species and emerging pathogens are major drivers of biodiversity loss worldwide. Through global trade, travel, and climate change, organisms and microbes are transported beyond their native ranges, where they can outcompete native species, alter food webs, and introduce novel diseases. One such example is the African clawed frog, *Xenopus laevis*, an invasive species in many parts of the world. Widely traded for research, education, and the pet industry, *Xenopus* can act as asymptomatic carriers of Bd. Often resistant to disease, infected individuals may introduce Bd into naïve amphibian communities, contributing to population declines and extinctions. Upon studying the recent arrival of *Xenopus* in the Loire region in France, we recorded 24% to 100% mean Bd prevalence across six sites. While no other species were screened for Bd during this study, we recorded the absence of any successful breeding (e.g. eggs, larvae) from local species at these sites suggesting a lack of recruitment. Whether through predation, pathogen spread or both, these results confirm that *Xenopus* is a serious threat to amphibian populations and understanding its interaction with amphibian pathogens is crucial for designing effective conservation strategies and preserving native amphibian populations.

June 10th, 11:25 am

Crossing the taxonomic divide: Ranavirus transmissions from frogs to turtles

Dharwadkar S*[1]; Sundaram M[1]
[1]University of Georgia
*sneha.dharwadkar@uga.edu

Cross-species transmissions play a crucial role in shaping pathogen adaptation and host range expansion, yet the mechanisms facilitating taxonomic host jumps remain poorly understood. Using Ranaviruses as a study system, we investigated the ecological and evolutionary drivers of host jumps from frogs to turtles, two groups that differ in physiology, habitat use, and immune function, yet are both susceptible to the virus. We compiled geographic infection data across the USA and used niche overlap analyses to quantify environmental and ecological overlap in Ranavirus infections of turtles and frogs. Our preliminary results indicate a high degree of niche similarity between frog and turtle infections (Schoener's D = 0.97), despite turtle infections being restricted to the eastern US, where Ranidae frogs comprise 68% of recorded cases. These results suggest that Ranavirus transmission to turtles may not be environmentally constrained but is instead shaped by the composition of frog communities, particularly Ranidae clades. Future turtle infections are likely where key reservoirs such as *Lithobates sp*. and *Rana sp*. occur, suggesting that evolutionary mechanisms of cross-species transmission are a primary driver of Ranavirus host jumps.

June 10th, 11:40 am

Apparent Ophidiomycosis Alters Eastern Copperhead (*Agkistrodon contortrix*) Behavior and Habitat Use

Mark, M[1]; Christensen, TC[1]; Kwait, RE[1]; Eskew, EA[2]; Zoccolo, I[1]; Struck, EJ[3]; Maslo, B[1]
[1]Rutgers University
[2]University of Idaho
[3]Pacific Lutheran University
*mam1088@scarletmail.rutgers.edu

Pathogens not only cause mortality but also impose nonlethal fitness consequences. Snakes experience trade-offs associated with behaviors that combat disease but divert time and energy away from other critical activities. The impacts of such behaviors on fitness remain poorly understood, raising concerns amid the emergence of novel herpetofaunal diseases. Ophidiomycosis, caused by the ascomycete fungus *Ophidiomyces ophidiicola*, impacts free-ranging snakes across North America and has been implicated in declines of several imperiled populations. Although previous ophidiomycosis research has primarily focused on disease-related mortality, few studies have evaluated nonlethal impacts on snake fitness. To address this knowledge gap, we investigated the effects of apparent ophidiomycosis on the behavior, habitat use, and movement of snakes in central New Jersey, USA, from 2020 to 2021. Our focal species was the eastern copperhead (*Agkistrodon contortrix*), a state species of special concern with limited representation in the ophidiomycosis literature. Although we did not observe mortality in our study population, we found that copperheads with apparent ophidiomycosis (8/31 individuals) displayed significantly different thermoregulatory behaviors than snakes without ophidiomycosis. Specifically, individuals with apparent ophidiomycosis favored areas with less canopy cover, less rock cover, and more coarse woody debris. Our findings suggest that snakes with apparent ophidiomycosis select habitats conducive to initiating behavior-mediated fever, potentially facilitating recovery.

June 10th, 11:55 am

Elucidating the role of shed skins in the persistence of Ophidiomyces ophidiicola in soils

Adamski, J[1]*; Catenazzi, A[1]
[1]Florida International University
*Jonathan.m.Adamski@gmail.com

One of the major problems that wildlife face in the 21st century is that of emergent fungal pathogens causing widespread declines or mortality across numerous vertebrate taxa. The disease triangle has historically been used to assess the interactions of the host, the pathogen, and the environment, though there has been little work focused on the environment in the snake pathogen *Ophidiomyces ophidiicola* (Oo). Previous research has found that subsurface den soils allow for growth, while growth is inhibited in surface soils. Here, we explore the interaction of the environment (soil) and shed skins in the persistence of Oo by utilizing microcosm experiments. Microcosms consisted of 1.5mL vials with a silty loam mix. A three-by-three design was utilized to explore growth of Oo across microcosms with no shed, *Heterodon* shed, and *Drymarchon* shed against no spores, heat-killed spores, and live spores. These sheds were chosen due to ease of access and morphological differences. Growth was quantified across treatments through qPCR and analyzed using a generalized linear model. Treatment groups with shed skins had significantly higher growth than the control group. This work builds upon the literature and showcases how den sites may act as an environmental reservoir.

June 10th, 4:30 pm

Using antifungal treatments to investigate the effects of *Batrachochytrium dendrobatidis* on *Rana cascadea* through space and time

Wagner, RB[1]*; Piovia-Scott, J[1]
[1]Washington State University
*Ryan.wagner2@wsu.edu

Disease treatments can reduce infection intensity, infection prevalence, increase survival, and improve population viability for wildlife impacted by disease. Few studies, however, have examined how and why treatment effectiveness varies across space and time. The Cascades frog (Rana cascadae) has experienced severe declines due to the fungal pathogen Batrachochytrium dendrobatidis (Bd) in northern California. We conducted disease treatments using Itraconazole antifungal baths in four Cascades frog populations between 2012 and 2024 and compared treatment effectiveness by estimating overwinter survival for treated and untreated individuals. We observed variation in treatment effectiveness between sites and within sites across different treatment events. Differences in treatment

effectiveness may be due to site-level environmental differences, year-to-year environmental variation, or differences in host characteristics between sites.

June 10th, 4:35 pm

First detection of *Batrachochytrium dendrobatidis* (Bd) in Baltics and molecular evidence for a novel *Batrachochytrium* lineage

Saare, L[1]*; Laasmaa, A[1]; Anslan, S2; Rannap, R1; Tedersoo, L1,2
[1]University of Tartu
*linda.saare@ut.ee

The pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (Bd) is a primary driver of global amphibian biodiversity loss. While widespread, the distribution limits of this pathogen are poorly understood, particularly across Northern Eurasia. To address this knowledge gap, we surveyed 243 native amphibians from eight species in Estonia and 68 amphibians from captive collections in Estonia and Latvia. Pathogen infection was assessed using metabarcoding of the Internal Transcribed Spacer 2 (ITS2) marker. Despite the hypothesis that the harsh northern climate might preclude chytrid establishment, Bd was found in 10 wild amphibians across Estonia and three specimens from captivity. The detected Bd sequences largely match strains linked to the global pet trade. However, our survey revealed a clade of nine Operational Taxonomic Units (OTUs) originating from a single wild *Rana arvalis* specimen captured in North Estonia. Phylogenetic analysis places this clade as a molecular sister group to Bd, exhibiting significant divergence in the ITS2 region, which suggests an undescribed sister species in the genus *Batrachochytrium*. Exploring this phylogenetic diversity is critical, as the presence of low-virulence chytrid lineages may protect local amphibian populations against the hypervirulent Bd Global Panzootic Lineage (BdGPL) responsible for the majority of chytridiomycosis-driven declines.

June 10th, 4:40 pm

Prevalence and intensity of invasive *Raillietiella orientalis* infections in native Everglades snake species

Arnashus, A[1]*; Aleuy, OA[1]
[1]Florida Atlantic University
*aarnashus2020@fau.edu

Raillietiella orientalis is an old-world pentastomid lung parasite that has established itself in Florida native snakes, likely as a result of the Burmese python invasion. This parasite has been documented in 19 species of Florida native snakes and spread as far north as the Florida panhandle, despite the pythons' limited thermal tolerance restricting them to South Florida. In native snakes, *R. orientalis* prevalence can exceed 50%, accompanied by high infection intensities. To assess the threat from *R. orientalis* on native snake species and on the Everglades, native snakes were collected as roadkill from the last exit off I-75 traveling west in Broward County. These snakes were dissected for morphometric measurements and to detect *R. orientalis* infection. Across common species, including banded water snakes, brown water snakes, corn snakes, ribbon snakes, garter snakes, and mud snakes, infection prevalence met or exceeded 50%. The highest infection intensities were observed in banded water snakes. As mid-level predators in an aquatic ecosystem, banded water snakes can be used as bioindicators, meaning that the impacts from *R. orientalis* on this species can reflect the impacts on the Everglades. Future research will assess the health impacts of *R. orientalis* on these infected native snake species.

June 10th, 4:45 pm

Environmental factors influencing Bd prevalence and intensity in a terrestrial-breeding frog community in a montane forest of Peru

Diaz, MI[1,2,3]*; Catenazzi, A[1,4,5]
[1]Florida International University
[2]Universidad Nacional de San Antonio Abad del Cusco
[3]Museo de Biodiversidad del Perú
[4]Centro de Ornitología y Biodiversidad
[5]Instituto Peruano de Herpetología
*mdiaz608@fiu.edu

Amphibians face one of the most virulent global threats, chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd). This pathogen has driven declines and extinctions in hundreds of species worldwide. Bd is commonly associated with aquatic-breeding frogs, but terrestrial-breeding frogs may also play a crucial role in disease transmission. We hypothesized that soil pH would be the principal driver of Bd prevalence and infection intensity in a

terrestrial breeding frog community, given the importance of pH for fungal growth and persistence. To test this, we evaluated vegetation structure factors such as canopy coverage, tree cover, shrub and herbaceous cover, leaf litter and moss cover, and number of trees with a DBH > 10 cm, as well as soil variables including soil pH, and concentrations of C, N, and P. We ran a Bayesian GLMMs using the brms package in R. The best supported model was identified based on the lowest LOOIC value. Contrary to our hypothesis, our results indicate that local vegetation and habitat characteristics show a stronger relationship with Bd dynamics. These variables likely shape microclimatic conditions that affect Bd persistence and transmission. Understanding the environmental factors associated with Bd infection can provide valuable insights to mitigate the spread of the pathogen, inform conservation strategies, particularly for endemic species, and support more effective management plans.

June 10th, 4:50 pm

Antimicrobial Resistance and Stewardship Gaps in Reptiles and Amphibians Across Clinical and Zoological Settings

Sallaberry-Pincheira, N[1,2]*; Maboni, G[1]; Sanchez, S[1]; Rivera, S[3]; Chastain, I[1]; McKinney, A[1]; Temples, H[1]; Divers, SJ[1]
[1]University of Georgia
[2]Universidad Andres Bello
[3]Zoo Atlanta
*Nicole.sallaberry@uga.edu

Reptiles and amphibians presented to wildlife rehabilitation centers, zoological institutions, and veterinary hospitals, frequently suffer from infectious disease or trauma, with secondary bacterial infections. We evaluated causes of admission, bacterial isolates, antimicrobial susceptibility profiles in a southeastern U.S. veterinary teaching hospital, and antimicrobial stewardship practices through an international survey of zoological institutions (83 institutions, 26 countries). Free-ranging reptiles were most commonly admitted due to vehicular trauma and dog attacks, whereas captive reptiles presented primarily for respiratory and reproductive disease associated with suboptimal husbandry. Gram-negative bacteria predominated (77%), and nearly 45% of isolates were not identified by MALDI-TOF, suggesting substantial undercharacterized microbial diversity. High resistance rates were observed, particularly in previously treated captive animals. Only 9.6% of institutions reported formal antimicrobial stewardship programs. European institutions were significantly more likely to have stewardship protocols than North American institutions (Europe: 4/20, 20%; North America: 1/41, 2.4%; Fisher's Exact Test p = 0.036). Third- and fourth-generation cephalosporins and second- and third-generation fluoroquinolones were commonly used as first-line agents in reptiles and amphibians, whereas mammalian practice were typically dominated by Tier 1 antibiotics (aminopenicillins, sulfonamides, tetracyclines). These findings underscore the urgent need for culture-guided therapy, stewardship implementation, and integrated One Health surveillance strategies.

June 10th, 4:55 pm

An Ostracod a day keeps the chytrid at bay? Ostracod predation of *Batrachochytrium dendrobatidis* zoospores *in vitro*

Webb, RJ[1]*; Poh, A[1]; Berger, L[1]; Skerratt, LF[1]; Brannelly, LB[1]
[1]University of Melbourne
*Rebecca.Webb@unimelb.edu.au

Batrachochytrium dendrobatidis (Bd) has impacted global biodiversity by causing amphibian declines and extinctions. Studies from Europe and the America's have found that aquatic micropredators such as Daphnia will prey on Bd zoospores and could offer a biological control option. However, the Bd predation of Australian micropredators is unknown. Here we use a Bd isolate genetically modified to express a red fluorescent reporter to track zoospore predation in an Australian ostracod and assessed their ability to eradicate Bd from the environment under a range of conditions. Ostracods quickly consumed hundreds and thousands of zoospores, and the ability to remove Bd from the environment increased with increasing ostracod density, and was not affected by the presence of an alternative food source. Ostracods reduced Bd concentration over a range of environmental conditions, including salinity, temperature and heavy metal and herbicide contamination. Exposure to Bd did not affect ostracod survival. In these controlled laboratory experiments, ostracods exceeded Daphnia for their ability to remove zoospores from the environment. We suggest that ostracods might be an important predator of Bd and their impact on disease dynamics in wild frog populations and potential for biological control in combination with habitat manipulation should be further explored.

June 10th, 5:10 pm

Reading the river: Environmental drivers of chytrid fungal eDNA in a changing climate

Sasso, T[1,2]*; McCallum, H[1]; Newell, D[3]; Grogan, LF[4]
[1]Griffith University
[2]University of New South Wales
[3]Southern Cross University
[4]University of Queensland
* Thais.sasso@austmus.gov.au

Batrachochytrium dendrobatidis (Bd) has a free-living zoospore that can remain viable in moist environments. Despite over two decades of surveillance of Bd in hosts, a better understanding of the ecology of Bd also requires examining how the environment shape its dynamics in waterbodies. We used environmental DNA to quantify Bd across 31 rainforest streams in Australia under shifting climatic conditions, providing rare insight into Bd dynamics during unforeseen drought followed by record-breaking rainfall. We collected water samples across four periods during frog breeding season. There was a high geographic variation in detection and quantity of Bd. eDNA was detected in 75 out of 222 water samples, with the greatest percentage of streams testing positive during spring. Detection proportion and Bd eDNA quantity were both positively influenced by elevation and cumulative rainfall, with detection greatest at higher elevations and following increased rainfall. eDNA quantity was positively related to water temperature but declined at temperatures above 19.5°C. Bd eDNA detection also varied within samples, with low correlation between positive qPCR results. Through sampling Bd eDNA, we can expand our understanding of the ecology of a lethal pathogen, but our results highlight the need for multiple spatial, temporal and technical replicates to improve reliability of eDNA.

June 11th, 9:30 am

Seasonal dynamic and individual determinants of *Amphibiocystidium sp*. infection in the palmate newt during the aquatic breeding season

Lorrain-Soligon, L[1,2]*; Brischoux, F[1]
[1]La Rochelle Université
[2]Doñana Biological Station
*ariana.aronis@sazoo.org

Amphibiocystidium sp., a mesomycetozoean parasite, is emerging as a potential contributor to amphibian health deterioration. We investigated the seasonal dynamics of Amphibiocystidium infection in the palmate newt (*Lissotriton helveticus*) during its aquatic breeding period in western France. Host traits exhibited pronounced seasonal variation, with body mass and condition declining over the breeding season, particularly in females. Infection prevalence and cyst intensity peaked in late March and were undetectable after May. Smaller individuals showed higher infection probability, while infected females harbored greater parasite loads than males. Notably, infection probability increased with body condition in males only. The marked temporal restriction of infection suggests that environmental conditions and host aggregation during breeding play key roles in transmission dynamics. These findings provide new insights into the ecology of Amphibiocystidium and underscore the importance of seasonal host dynamics and individual traits in shaping parasite transmission and infection outcomes.

June 11th, 9:45 am

Health effects of gastrointestinal parasitism by the nematode *Skrjabinoptera phrynosoma* in managed Texas horned lizards (*Phrynosoma cornutum*)

Aronis, AR[1]*; Kruse, NK[2]; Hadley, TL[2]; Thompson, ME[3]; Coke, RL[4]; Fenolio, DB[1]
[1]Center for Conservation & Research at San Antonio Zoo
[2]Animal Health Center at San Antonio Zoo
[3]Texas Veterinary Pathology Associates
[4]South Texas Avian & Exotic Hospital
*ariana.aronis@sazoo.org

The Texas horned lizard (*Phrynosoma cornutum*) is a threatened reptilian species, with several zoological institutions managing groups for breeding and reintroduction programs. Harvester ants (*Pogonomyrmex sp.*) are their primary food source, as well as the mode of transmission for the parasitic nematode, *Skrjabinoptera phrynosoma*. Although these two species have coevolved, few references describe effects on the host. From 2019 to 2024, the Texas Horned Lizard Reintroduction Project at the Center for Conservation & Research (CCR) at San Antonio Zoo documented 31 lizards with gastrointestinal nematode infections. Of these lizards, 14 (45%) died and were submitted to Texas

Veterinary Pathology, LLC, for histopathologic evaluation. Evidence of health decline related to gastrointestinal parasitism was reported in 12 of the 14 (86%) deceased lizards. Predominant findings included gastrointestinal impaction with nematodes, and a negative energy balance causing fat and hepatic atrophy. Due to the absence of any other substantial abnormalities, these findings were most likely secondary to heavy endoparasitism. These results suggest that infection with *S. phrynosoma* can be more detrimental to the health of managed Texas horned lizards than previously reported.

June 11th, 10:00 am

Novel records of *Paranannizziopsis spp.* fungal infections and associated coinfections in the pygmy rattlesnake, *Sistrurus miliarius*

Palmisano, JNP[1]*; Savage, AE[1]; Lorch, JM[2]
[1]University of Central Florida
[2]United States Geological Survey
*Jenna.Palmisano@ucf.edu

Diseases including mycosis and raillietielliasis are associated with severe pathology, mortality, and population declines in wild snakes. The fungus *Ophidiomyces ophidiicola* (Oo) has been detected in at least 60 species globally. Infected snakes display a range of clinical signs, from subtle dermal abnormalities to severe lesions and systemic infections. *Paranannizziopsis spp*. (Pz), another fungal pathogen, has recently been reported in wild snakes in North America and Europe. Its clinical presentation resembles that of Oo, suggesting that Pz may have been present historically but under-recognized in wild snake populations. The pygmy rattlesnake (*Sistrurus miliarius*) exhibits both raillietielliasis and mycosis caused by Pz and Oo. Here we report novel findings of Pz in wild S. miliarius in Florida based on three years of field surveys. Pz was detected at five locations, and in 26% (18/68) of cultured lesions, while Oo was found in 74% (50/68) of lesions. In this talk we will compare the clinical signs, severity and progression of disease caused by Oo and Pz and examine the occurrence and implications of coinfections with raillietielliasis. Although the consequences of Pz remain unclear, its clinical overlap with Oo and evidence of co-infections highlight its emerging importance for snake populations.

June 11th, 10:15 am

A decadal assessment of *Batrachochytrium dendrobatidis* (Bd) and ranavirus (RV) in Oklahoma's amphibians

Shafer, AM[1]*; Watters, JL[2]; Siler, CD[1,3]
[1]University of Oklahoma
[2]Oklahoma Department of Transportation
[3]Oklahoma Biological Survey
*ashafer@ou.edu

Emerging infectious diseases are a major driver of global biodiversity loss, with amphibians among the most heavily affected vertebrate groups. Two widespread pathogens, *Batrachochytrium dendrobatidis* (Bd) and ranaviruses (RV), have contributed to global population declines. However, many disease surveillance efforts are spatially or temporally limited, restricting our ability to detect long-term and large-scale patterns in amphibian disease dynamics. The aim of this study is to synthesize existing long-term surveillance data to evaluate how Bd and RV infection patterns vary across time, taxa, and habitats. We conducted a meta-analysis of a decadal dataset comprising Bd and RV screening data from 19 anuran and 14 caudate species sampled across 9 of Oklahoma's 12 ecoregions. Preliminary analyses indicate Bd and RV prevalence and infection intensity vary among taxa, with site-specific patterns persisting through time. While overall prevalence of Bd, RV, and coinfection decline over time, spatial heterogeneity persists across years, suggesting that site-specific factors may play a key role in shaping disease dynamics. Ongoing analyses will explore the environmental and ecological drivers of these patterns, including the role of anthropogenic exposure and community diversity in infection dynamics. This study will provide a regional baseline for understanding multi-pathogen dynamics under changing environmental conditions.

June 11th, 10:35 am

Alpine frogs like to be cozy too: Thermal body preference in critically endangered alpine frogs

Hernández-Poveda, M[1]*; Berger, L[1]; Aquilina, A[1]; Vivas Barreto, D[2]; Guinto, DJ[1]; Skerratt, LF[1]; Kosch, TA[1]; Waddle, AA[3]
[1]University of Melbourne
[2]Universidad de Los Andes
[3]Macquarie University

*mhernandezpo@student.unimelb.edu.au

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), is a major driver of amphibian declines. Temperature strongly influences disease outcomes in the nature, with lower mortality typically observed in warmer conditions. As a result, conservation strategies include artificial warm shelters to enhance overwinter survival. However, the effectiveness of thermal interventions varies across species due to differences in host temperature selection and physiological responses. In this study, we examined the temperature selection of two critically endangered species, the Southern Corroboree Frog (*Pseudophryne corroboree*, SCF), ecologically extinct, and the Northern Corroboree Frog (*P. pengilleyi*, NCF), that still persist in nature despite population declines. We experimentally assessed 20 individuals of each species, including adults and juveniles, to investigate age and species differences in thermal preference. SCFs showed slightly higher preferred temperatures than NCFs, with a difference between age. In contrast, NCF juveniles preferred warmer temperatures than adults. These species- and age-specific patterns may influence Bd susceptibility. Although mean preferred temperatures are below those thought to reduce Bd impacts, both species can tolerate brief exposures above 25 °C, suggesting short-term warm refugia could still improve disease outcomes. Understanding these thermal preferences can inform conservation interventions and help mitigate chytridiomycosis in declining frog populations.

June 11th, 10:50 am

Late winter storms delay amphibian development and increase susceptibility to pollutants and pathogens

Gajewski, Z[1]*; Campbell, M[1]; Affek, K2; Dugan, H1; Wilkinson, G1; Brunner, I3; Shi, Y1; Hua, J1
[1]University of Wisconsin-Madison
[2]Warsaw University of Technology
[3]Ohio State University
*gajewskizach@gmail.com

Winter is undergoing rapid and unprecedented change (e.g., false springs or late winter storms). These changing winter conditions expose amphibians that rely on predictable seasonal transitions to uncharacteristic cold conditions during sensitive life stages with consequences later in development. In this study, we exposed eggs of *Anaxyrus americanus* and *Pseudacris maculata* to temperature treatments simulating no winter storm vs. a late winter storm. Next, we conducted tolerance assays and compared the susceptibility tadpoles from the different winter treatments to a common environmental pollutant (road salt- NaCl) and a pathogen (Frog Virus 3- FV3). Lastly, we generated indoor pond microcosms, placed tadpoles from the different winter treatments into microcosms, tracked survival and evaluated whether late winter storm exposed-tadpoles had indirect ecological consequences on algal communities and nutrient cycling. Results: (1) Late winter storms altered development in both species and increased tadpole susceptibility to NaCl, but not to FV3. (2) Later in development, *A. americanus* but not *P. maculata* recovered from winter storm-induced effects. (3) In microcosms, *P. maculata* exposed to winter storms had higher survival leading to impacts on algal communities. These results demonstrate that late winter storms can generate immediate and lasting consequences for amphibians from individual to ecosystem levels.

June 11th, 11:05 am

Rising temperature and chytrid infections' role in shaping microbial composition and interaction

Thompson, EG[1]*; Barnes, EM[1]
[1]Rochester Institute of Technology
*egt5318@rit.edu

Chytrid fungi, Bd (*Batrachochytrium dendrobatidis*) and Bsal (*Batrachochytrium salamandivorans*) are one of the largest threats to amphibian populations worldwide. Research has shown that the amphibians skin microbiome can inhibit chytrid infection, but that microbial composition can vary drastically with host and environmental factors. In addition to species-specific microbiome manipulation, habitat-wide mitigation methods that capitalize on the thermal mismatch between Bd/Bsal and their hosts (e.g., warming the host or environment) have been proposed to combat infection. These methods have proven successful so far, but they do not consider the indirect effects that warming has on the microbiome. In this study, we used synthetic microbial communities of ten core members to simulate the Ambystoma laterale skin microbiome and exposed them to Bd/Bsal at five different temperatures, reflecting mitigation methods and a climate change scenario, resulting in a factorial experiment. Microbial community composition and gene expression were assessed via high-throughput amplicon sequences and metatranscriptomics. We found that the families Burkholderiaceae and Enterobacteriaceae were the most abundant in the microcosm. In addition, time plays a larger role in shaping community composition and function than temperature, and temperature

does not significantly affect chytrid inhibition in vitro, potentially suggesting that the host is a key player in temperature-pathogen interactions.

June 11th, 11:10 am

Health assessment and multipathogen surveillance in free-ranging snakes to the southeastern United States

Hazelrig, CM[1]*; Farrell, TM[2]; Palmisano, JN[3]; Ossiboff, RJ[4]; McCray, M[4]; Haynes, E[1]; Maerz, JC[1]; Meichner, K[1]; Zhang, J[1]; Garrett, KB[1]; Yabsley, MJ[1]; Ortega, J[2,5]; Cleveland, CA[1]; Nemeth, NM[1]
[1]University of Georgia
[2]Stetson University
[3]University of Central Florida
[4]University of Florida
[5]University of Arkansas
*corinna.hazelrig@uga.edu

Concerns associated with ophidiomycosis, caused by *Ophidiomyces ophidiicola* (Oo), have brought needed attention to snake conservation. We evaluated native snake health in the southeastern United States through surveillance targeting *Cryptosporidium spp., Hepatozoon spp., Mycoplasma spp*., Oo, *Raillietiella orientalis* (Ro), *Salmonella spp.*, and serpentoviruses. We assessed pathogen and disease prevalence through monthly sampling from 2022 to 2024 (via swabs, blood, feces, and physical examination) at wetlands in Volusia County, Florida and Jasper County, South Carolina. We opportunistically sampled snakes from Athens-Clarke County, Georgia and diagnostic cases submitted to the Southeastern Cooperative Wildlife Disease Study from 2021 to 2024. We sampled 509 snakes with 49 recaptures representing 29 species. We detected *Salmonella enterica* in 62.6% (306/489), *Hepatozoon spp*. in 53.4% (205/384), *Mycoplasma spp*. in 17.5% (78/445), Oo in 16.1% (82/508), and *Cryptosporidium spp*. in 2.0% (10/489). Detection of Ro was limited to Florida (12.7%; 37/292). No serpentoviruses were detected (n=447). Coinfection was a strong predictor of apparent ophidiomycosis. Detections of Oo or Ro were negatively associated with nutritional condition score, and increased detection risks were observed in pygmy rattlesnakes (*Sistrurus miliarius*). Our health assessment enhances the understanding of infectious agents of free-ranging snakes and establishes methods for snake health evaluation.

June 11th, 11:25 am

***Emydomyces testavorans*, mycoplasma, and a novel herpesvirus in the European pond turtle (*Emys orbicularis*)**

Marschang, RE[1]*; Schneeweiss N[2]; Leineweber C[1]; Dietz J[1]
[1]Laboklin GmbH & Co. KG, Bad Kissingen
[2]Landesamt für Umwelt, Land Brandenburg
*Rachel.marschang@gmail.com

The European pond turtle (*Emys orbicularis*) is considered Threatened by Extinction in Germany. Multiple conservation efforts are underway to help support turtle populations. Despite this interest, relatively little is known about infectious disease threats to the species. Juvenile turtles in an established conservation breeding group in northeastern Germany had been experiencing increased mortality, especially among juvenile turtles. To evaluate the possible role of infectious agents, ten carcasses (six in formalin, three in ethanol, one fresh) were examined pathohistologically. Oral and cloacal swabs from an additional nine turtles and pooled tissues from four of the carcasses were tested for the presence of *Emydomyces testavorans*, mycoplasmas, ranaviruses, herpesviruses, and adenoviruses by PCR. A chronic hyperplastic dermatitis associated with fungal hyphae was observed in nine of the ten animals examined histologically. Pneumonia was noted in three, stomatitis in three, and enteritis in six animals. *E. testavorans* was detected in nine, mycoplasmas in four and a novel herpesvirus in three samples. These finding expand the known host and geographical range of *E. testavorans*. The finding of multiple pathogens in this conservation breeding group, including the association of *E. testavorans* with severe skin lesions and mortalities, is concerning and deserves further study.

June 11th, 11:40 am

Retrospective analysis of diagnostic PCR testing reveals pathogen diversity and prevalence across reptile and amphibian host groups

Tillis, SB[1]*; Childress, AL[1]; Selden, JN[1]; Ossiboff, RJ[1]; Wellehan, JFX[1]
[1]University of Florida
*Reptillis@ufl.edu

The University of Florida College of Veterinary Medicine Zoological Medicine Diagnostic (ZMDX) Laboratory specializes in molecular pathogen detection using polymerase chain reaction (PCR) assays for diverse non-domestic animal species. Since 2005, the ZMDX laboratory has performed more than 40,000 PCR tests targeting hundreds of pathogens from reptile and amphibian samples. Despite this extensive diagnostic history, comprehensive synthesis of these data has not been conducted. This study aims to retrospectively standardize and analyze historical diagnostic records to characterize pathogen diversity and prevalence across major reptile and amphibian host groups. Historic diagnostic records were standardized, centralized into a searchable database, and cleaned prior to analysis. Samples were categorized into major taxonomic groups including chelonians (Order Testudines), squamates (Order Squamata), anurans (Order Anura), caudates (Order Caudata), and crocodilians (Order Crocodylia). Analyses also evaluated subclades of reptile or frequently tested individual species including ball pythons (*Python regius*), bearded dragons (*Pogona vitticeps*), corn snakes (*Pantherophis guttatus*), box turtles (*Terrapene carolina*), and desert tortoises (*Gopherus agassizii*). Results provide ranked summaries of pathogen diversity and prevalence across host groups and species. These findings identify commonly detected pathogens, establish a framework to guide targeted surveillance, and support future epidemiological investigations for captive and wild reptile and amphibian populations.

June 11th, 11:55 am

Pharmacokinetics of two doses of ceftiofur crystalline free acid in Mexican axolotls (*Ambystoma mexicanum*)

Figueroa, R[1]; Yazbec, E[1]; Knafo, SE[1]*
[1]University of Tennessee
* ccy577@utk.edu

Pharmacokinetics of ceftiofur crystalline-free acid (CCFA) at two doses was performed in 11 healthy Mexican axolotls (*Ambystoma mexicanum*). Blood samples were collected at 0, 24, 4,8, 72, 96, 120, 144, and 168 hours following administration. Analysis of plasma ceftiofur was performed and pharmacokinetic parameters determined. Slow clearance of CCFA is consistent with pharmacokinetics of this drug in other poikilothermic species although no pharmacokinetic studies of CCFA have been performed in amphibians. Plasma concentrations are consistent at least a seven day dosing interval.

June 11th, 2:00 pm

Microbes are friends? How early life exposure to antifungal bacteria impacts disease susceptibility of *Dendrobates tinctorius* post-metamorphosis

Cuomo, RG[1]*; Bletz, MC[1]
[1]Pennsylvania State University
*rgc5241@psu.edu

Early life exposure to microbial diversity can impact immune development and susceptibility to disease later in life, which suggests that microbial exposure across tadpole developmental stages in amphibians may play a critical role in immune capacity. We examined how introducing bacteria that inhibits the growth of *Batrachochytrium dendrobatidis* (Bd) as well as a dietary additive of carotenoids into the aquatic environment of *Dendrobates tinctorius* tadpoles affects development and Bd susceptibility as juveniles. Tadpoles were raised in one of two water treatments: (i) water with Bd-inhibitory microbes added, and (ii) 'semi-sterile' water with no microbes added. This was crossed with one of two diet treatments: (i) carotenoid addition, and (ii) no carotenoid addition. Individual development metrics, including gosner stage, weight, and length, were taken. Skin microbiomes were sampled via swabbing throughout development and after pathogen exposure to characterize bacteria communities through 16S amplicon sequencing and quantify Bd loads via qPCR. Ongoing analysis suggests pre-metamorphic treatment may change post-metamorphic microbiome composition, but this community shift may not lead to changes in survivability or infection load. Disentangling the relationship between early life microbial exposure and immune development may help us understand trends in disease susceptibility and work towards disease mitigation strategies.

June 11th, 2:15 pm

Skin lipid chemistry influences host-microbiome-pathogen interactions in snake fungal disease (ophidiomycosis)

Dallas, JW[1]*; Murphy, KM[1], Ghotbi, M[1]; Vargas-Gastélum, L[2]; Van Moorleghem, C[1,3]; Phillips, JL[4]; Ludwig, D[4]; Sun, T[4]; Mason, RT[1]; Spatafora, JW[2]; Niedzwiecki, JH[5]; Parker, MR[6]; Leblond, JD[1]; Miller, JM[7]; Wilson, I[1]; Foster, E[1]; Crick, J[1]; Ramazan, S[1]; Stalzer, C[1]; Bergman, O[1]; Chue Donahey, MS[8]; Chevrette, MG[9]; Walker, DM[1]
[1]Middle Tennessee State University
[2]Oregon State University

[3]University of Antwerp
[4]Middle Tennessee State University
[5]Belmont University
[6]James Madison University
[7]Middle Tennessee State University
[8]University of Florida
[9]University of Wisconsin-Madison
*Jason.dallas@mtsu.edu

Within host-microbiome-pathogen systems, the host chemical microenvironment is often overlooked despite its inherent role in host physiology. Snakes represent an ideal study system as they are at risk of an emerging fungal pathogen, *Ophidiomyces ophidiicola* (ophidiomycosis, snake fungal disease), which closely interacts with the skin microbiome, and snakes exhibit a complex skin lipid profile. We used a multifaceted experimental approach encompassing culture-dependent and independent methods, metagenomic and genomic data to assess the impact of host skin chemistry and the bacterial microbiome on the growth of O. ophidiicola. Results suggest that host skin lipid chemistry (e.g., oleic acid, squalene) and bacteria isolated from wild snake skins (e.g., *Chryseobacterium sp*. and *Stenotrophomonas maltophilia*) suppress *O. ophidiicola* growth. Notably, the *O. ophidiicola* genome contains biosynthetic gene clusters (BGCs) that encode metabolites that may suppress host lipid production, facilitating fungal pathogenicity. BGCs from bacterial genomes isolated from snake skin overlap with metagenome profiles from wild snakes and correlate with disease state. We highlight antifungal activity found in the diverse lipid milieu of snake skin and bacterial-fungal interactions that structure the skin microbiome. Our results illustrate a strong relationship among a fungal pathogen, the microbiome, and host skin lipid chemistry.

June 11th, 2:30 pm

Microbial responses to antibiotics shift the direction of Bd-wood frogs disease outcomes

Velasquez, I[1]; Ricci, K[1]; Milusich, E[1]; Tuthill, B[1]; Sauer, K[2]; Hernandez-Gomez, O[3]; **Hua, J**[1]*
[1]University of Wisconsin-Madison
[2]Binghamton University
[3]New Mexico State University
*jhua23@wisc.edu

Diseases are emerging at unprecedented rates, prompting global efforts to understand factors shaping disease outcomes. Microbes associated with hosts and the environment influence disease by secreting antipathogenic metabolites, hindering pathogen success. However, antibiotic contamination and costs associated with microbial responses to antibiotics may reduce antipathogenic efficacy. We investigated how antibiotic tolerance influences the antipathogenic properties of metabolites produced by *Pseudomonas aeruginosa* using in vitro and in vivo experiments. We found that metabolites from antibiotic-non-tolerant *P. aeruginosa* strains retained their antipathogenic properties and inhibited the growth of *Batrachochytrium dendrobatidis* (Bd), an amphibian fungal pathogen responsible for amphibian declines worldwide. In contrast, metabolites from *P. aeruginosa* strains that developed antibiotic tolerance promoted Bd growth and exacerbated infection severity. These patterns were consistent *in vitro* and *in vivo*. We also ran toxicological and small molecule analyses of microbial metabolites, and findings suggest the divergent experimental outcomes may stem from differences in metabolite toxicity and profiles between strains. These findings underscore the hidden consequences of interactions between microbial responses to environmental changes and pathogens on infectious disease dynamics and highlight the need to integrate environmental change and eco-evolutionary perspectives into disease research.

June 11th, 2:45 pm

Small predators, big impact: How predation by protists influences microbiome-mediated defense against amphibian chytridiomycosis

Venezia, J[1]*; Hill, A[2]; Bletz, M[1]
[1]Pennsylvania State University
[2]Tennesse Tech University
*jcv5194@psu.edu

Chytridiomycosis (chytrid) is a devastating fungal disease that has resulted in record-breaking losses of global biodiversity. Both strains of chytrid, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal), shed zoospores which infect amphibian skin. Importantly, the amphibian skin microbiome may be able to prevent infection and suppress disease. Thousands of skin-associated bacterial isolates have been screened for their

antifungal function, but how the eukaryotic members of these microbiomes impact chytrid infection is poorly understood. We investigated how top-down pressure by predatory protists influences the structure and disease-suppressive function of the amphibian skin microbiome. Through 16S and 18S amplicon sequencing of skin swabs from eastern newts (*Notopthalmus viridescens*) and slimy salamanders (*Plethodon glutinosus*), we constructed a cross-domain correlation network, demonstrating a complex web of positive, negative, and neutral associations between bacteria, protists, and Bd. Additionally, we show through in vitro predation assays that predatory protists found on amphibians, namely *Acanthamoeba spp.*, consume Bd zoospores, which may reduce infection load and improve disease outcomes in wild salamanders. Through this preliminary investigation, we highlight how cross-domain microbial interactions may influence defense against disease and urge further investigation into how both prokaryotic and eukaryotic members of the skin microbiome may protect amphibians from chytridiomycosis.

June 11th, 3:00 pm

Adaptive microbiome may explain enhanced survival of Eastern Newts after first and second exposures to the chytrid fungus, *Batrachochytrium salamandrivorans*

Le Sage, M[1]*; McCartney, JA[2]; Woodhams, D[2]; Rollins-Smith, LA[1]
[1]Vanderbilt University School of Medicine
[2]University of Massachusetts, Boston
*lesmitc@gmail.com

The pathogenic chytrid fungus, Batrachochytrium salamandrivorans (Bsal), has caused devastating losses of fire salamanders (Salamandra salamandra) in Europe, and there is great concern for survival of unique endemic salamanders in North America if Bsal is accidentally introduced. The Eastern Newt (Notophthalmus viridescens) is an abundant salamander that has previously been shown to be susceptible to Bsal. To determine whether newts could develop an effective defense, we examined survival, pathogen burden, and two measures of immune defenses after first and second exposures to Bsal zoospores. Survival probability was greatly increased in the newts that survived a prior infection and heat clearance, and the zoospore burden was significantly reduced in comparison with newts that were exposed only once. These results suggest that prior exposure and clearance provides some protection of survivors from a second exposure. Changes in microbial communities in the skin and defensive skin secretions were assessed to determine possible mechanisms of enhanced survival. The results showed that the functions of the microbiome shifted through time toward a more resistant microbial community supporting predictions of the adaptive microbiome hypothesis.

June 11th, 3:15 pm

Pathogen-induced dysbiosis alters the snake skin microbiome across space and environmental gradients

King, T[1]*; Dallas, JW[1]; Grisnik, M[2]; Rubin, R[1]; Foster, E[1]; Rurik, AJ[1]; Ghotbi, M[1]; Hanscom, R[3]; Clark, R[3]; Walker, DM[1]
[1]Middle Tennessee State University
[2]Coastal Carolina University
[3]San Diego State University
*tmk3w@mtmail.mtsu.edu

Emerging infectious diseases are a major threat to global biodiversity. For example, ophidiomycosis (snake fungal disease; SFD; *Ophidiomyces ophidiicola*) affects phylogenetically and geographically diverse free-ranging snake populations. To understand how this pathogen affects snakes, research on the snake skin microbiome and its interactions with O. ophidiicola and the environment is needed. In the Southeastern United States, extensive work has been done to explore relationships among host snake species, the skin microbiome, and the presence of O. ophidiicola. However, a study including snakes from Western North America and bacterial-fungal interactions across various environments has not yet been completed. By using metabarcoding and high-throughput amplicon sequencing of bacterial 16S rRNA and fungal ITS rDNA markers for ~1,000 snake individuals, we investigated skin micro- and mycobiomes across diverse hosts and environments. To assess O. ophidiicola status and quantification, we performed quantitative polymerase chain reaction. This study demonstrates that host genus, ecoregion (a geographic area defined by its environmental conditions), and O. ophidiicola status are significant predictors of microbiome community structure. Future exploration involving fine-scale environmental factors will elucidate the interrelationships in this understudied host-microbiome-pathogen-environment system and will help to introduce ecosystem-relevant components to ophidiomycosis management.

June 11th, 3:20 pm

Host-mediated microbial assembly confers disease resistance in turtle eggs

Carranco, AS[1,2]*; Romo, D[2]; Torres, ML[2]; Wilhelm, K[1]; Sommer, S[1]
[1]Institute of Evolutionary Ecology and Conservation Genomics
[2]Universidad San Francisco de Quito
*anasoficarranco@gmail.com

Fungal emerging diseases threaten wildlife species globally, particularly amphibians and reptiles. Among the most concerning are the *Fusarium solani* species complex (FSSC) pathogens, causing mass hatching failures in sea and freshwater turtles worldwide. Although vertebrate eggs harbour distinct microbial communities, the role of these microbiomes in mediating disease susceptibility and immune defence, particularly through host-mediated microbial assembly, remains poorly understood. Understanding how hosts select and maintain protective microbial communities could reveal novel strategies for combating emerging fungal diseases. Using longitudinal sampling, we investigated microbial community assembly across eggshells and the hatchling cloaca. Our findings revealed that eggshell microbiomes experience dramatic shifts from diverse initial communities during oviposition to uniform communities after incubation, with FSSC pathogens detected only post-incubation. Successfully hatched individuals maintain tightly organised microbial communities despite pathogen pressure, suggesting active host-filtering for protective microbial communities. Moreover, microbial communities differ significantly between external and internal eggshells, indicating multiple selective barriers that may confer disease resistance. This study shows that host-mediated microbial assembly is essential for pathogen resistance during incubation, offering insights into pathogen-host interactions in early life. These findings open new possibilities for microbial-based conservation strategies to protect threatened reptile populations from emerging fungal diseases.

POSTERS

Molecular characterization of the *Ranavirus ambystoma1* 21L methyltransferase protein

Alan, HG[1]*; Jancovich, JK[1]
[1]California State University San Marcos
*alan001@csusm.edu

Ranavirus ambystoma1, formerly known as Ambystoma tigrinum virus (ATV), is a large dsDNA virus originally isolated from the Sonora tiger salamander. ATV's genome is approximately 106 kbp in length and is highly methylated. After ranavirus replication is initiated, newly synthesized viral genomic DNA is transported from the nucleus to the cytoplasm where it is methylated by a virus-encoded cytosine-specific DNA methyltransferase (DMTase) methylating each cytosine within CpG dinucleotides. In ATV, the 21L open reading frame encodes a DMTase. Ranavirus DNA methylation has been suggested to play a role in preventing viral genome degradation and improving host immune evasion. However, the comprehensive function of ranaviral DMTases in viral replication remains poorly understood. The goal of the study is to further understand and characterize the role of the ATV 21L DMTase during a viral infection by (i) generating a recombinant virus where ATV 21L has been deleted; (ii) further characterizing the 21L expression pattern in virally infected cells; (iii) investigate the ATV DMTase's ability to methylate host DNA; and (iv) determine ATV 21L's ability to modulate host immune responses. Collectively, this body of work will help further our understanding of the role of ranavirus methyltransferase genes in viral replication and pathogenesis.

Evaluating the impacts of habitat complexity on the susceptibility of common pet amphibian species to Ranavirus, *Batrachochytrium dendrobatidis*, and *B. salamandrivorans*

Ambelang, J[1]*; Galvin, DJ[1]; Miller DL[1]; Gray MJ[1]
[1]University of Tennessee
*jamblan@vols.utk.edu

Emerging pathogens including Ranavirus (Rv), *Batrachochytrium dendrobatidis* (Bd), and *B. salamandrivorans* (Bsal) have been detected in the global amphibian trade. Despite the presence of these pathogens in the amphibian trade, little is understood regarding the transmission of these pathogens in captive amphibians. One factor of interest is how husbandry conditions, specifically habitat complexity, influence host susceptibility and disease progression. To investigate the affect of habitat complexity on host susceptibility to Rv and Bd, we used blue dyeing dart frogs (*Dendrobates tinctorius*) and yellow bellied toads (*Bombina variegata*). To investigate the affect of habitat complexity on host susceptibility to Bsal, we used Eastern newts (*Notophthalmus viridescens*) and Cuban treefrogs (*Osteopilus septentrionalis*). For these challenges, animals were placed into one of two pathogen treatment groups (control (unexposed) and exposed) and into one of four habitat treatment groups (simple, ABG only, unaged bioactive, and aged bioactive). Each pathogen challenge lasted 30 (Rv) or 60 (Bd, Bsal) days. We collected skin swab samples every 6 days to monitor infection. Photographs were collected regularly to track signs of infection. The results indicate that pathogen type and host species identity may have a greater impact on pathogen susceptibility and disease progression than habitat complexity.

Report of *Kircephalus coarctatus* in the Eastern mud snake (*Farancia abacura*) from Florida

Arnashus, AA[1]*; Aleuy, OA[1]
[1]Florida Atlantic University
*aarnashus2020@fau.edu

The Eastern mud snake (*Farancia abacura*) has been documented with the invasive pentastome *Raillietiella orientalis* in Florida. The North American snake pentastome, *Kiricephalus coarctatus*, exists in Florida's native snake communities via infectivity from intermediate hosts. Reports in Florida of *K. coarctatus* do not extend to *F. abacura*, possibly due to a specialized diet of aquatic salamanders. A *K. coarctatus* infection in *F. abacura* from Florida is reported. An adult female *F. abacura* from Broward County, Florida, was collected during road mortality surveys and examined via necropsy. A single immature female *K. coarctatus* measuring 36 mm was discovered encysted in the right lung. The *K. coarctatus* specimen possessed morphological characteristics associated with Florida's native snake pentastomes, such as a globular head, two sets of hooks, external annulations, and an oval mouth shape. Molecular confirmation revealed the identity as *K. coarctatus*. This finding represents the first documented case of *K. coarctatus* in *F. abacura* from Florida, and within an Everglades habitat which also hosts the invasive snake pentastome *R. orientalis*. Further investigation is needed to determine infection dynamics in an ecosystem where a native and an

invasive snake pentastome exist in sympatry, and the extent to which both pose a conservation risk to Florida's native snakes.

Detection and characterization of microorganisms in aquatic habitats of turtles

Bantha, P[1]*; McIver, AM[1]; Neuman-Lee, LA[1]
[1]Arkansas State University
*pallavi.bantha@smail.astate.edu

The freshwater turtles are in contact with a wide range of microorganisms in their habitats, among which many are non-pathogenic and serve as commensal for them. However, some may be pathogenic and contribute to disease in turtles, potentially causing declines in populations. Human impacts on ecosystems have caused or exacerbated disease outbreaks in turtle populations. Therefore, understanding the diversity of microorganisms, including those that are pathogenic, is essential for turtle health management and conservation. This study aims to detect and characterize the potential bacterial pathogens present in aquatic habitats of turtles in a natural setting in Arkansas by collecting water and environmental samples. Microbial isolation from the collected samples was performed using standard culture-based techniques using Spread plate method, followed by morphological and biochemical characterization of the isolates. The common types of bacteria found in the collected samples were mostly environmental, some were opportunistic and few were pathogenic. Some of the identified isolates of bacteria from collected samples include *Escherichia coli, Staphylococcus aureus, Bacillus, Salmonella* and *Pseudomonas*. The identified pathogens were then further evaluated for their potential virulence based on known pathogenic traits and their associations with turtle diseases. This study contributes to a better understanding of the bacterial diversity present in freshwater turtle habitats and identifies potential pathogenic microorganisms that may cause health risks in populations of turtles. It encourages people to focus on disease surveillance, turtle health management, and conservation efforts. Additionally, the study highlights the potential for zoonotic transmission, emphasizing the importance of monitoring aquatic ecosystems influenced by human activity.

Limited evidence for the proliferation of inhibitory skin microbes in response to *Batrachochytrium dendrobatidis* infection in a population-level experiment with *Xenopus laevis*

Bermudez, SA[1]*; Theis, KR[2]; Greenburg, JM[2]; Noelker, JE[3]; Raffel, TR[1]
[1]Oakland University
[2]Wayne State University
[3]Madonna University
*sbermudez@oakland.edu

The Adaptive Microbiome Hypothesis (AMH) proposed by Douglass Woodhams (2023) suggests that when amphibians are infected with *Batrachochytrium dendrobatidis* (Bd), the skin microbiome recruits Bd-inhibitory microbes as an adaptive response. This leads a proliferation of inhibitory microbes and a subsequent decline in microbial dispersion. However, it does not address how temperature contributes to this dynamic despite Bd's thermal sensitivity. To test the AMH and evaluate the effect of temperature on microbial dynamics, we examined skin swabs from a controlled-temperature Bd infection experiment conducted by the Raffel Lab in 2022. Bacterial DNA was obtained using 16S rRNA amplification and next-generation sequencing. Data analysis was done in R using the DADA2, vegan, lmer, and piecewiseSEM packages. We found that Microbial diversity decreased in response to infection but this decease in diversity was driven by a reduction in inhibitory microbes. In addition, Structural equation modeling showed a strongly significant negative bidirectional effect on Bd-inhibitory microbes and Bd Infection Load. There were no statistically significant effects of temperature on its own without accounting for interactions with Bd infection intensity. These results suggest that factors such as temperature and inhibition of microbes by Bd can drive host-microbiome-pathogen dynamics in ways not outlined by the AMH.

Immune System Development Across Species and Gosner Stages in Tadpoles: Implications for Susceptibility to Emerging Infectious Diseases

Burchfield, EDR[1]*; Savage, AE[1]
[1]University of Central Florida
*elana.burchfield@ucf.edu

Emerging infectious diseases are widespread in anuran populations, yet the developmental and life-history characteristics that shape their vulnerability to these pathogens remains poorly understood. A contrast in life-stage-specific immune defenses appears conserved across taxa, where juveniles tend to rely more heavily on innate immune defenses and adults on adaptive defenses. However, we hypothesize that frog species with extensive tadpole stage

durations may exhibit more robust adaptive immune characteristics as juveniles. Here, we compare splenic gene expression in tadpoles of the American bullfrog (Rana catesbiana) and southern leopard frog (Rana sphenocephala) using PacBio Iso-Seq to recover full-length transcripts. Given the longer tadpole stage duration of R. catesbiana, we expect to see a higher investment in innate and adaptive immune capacity at similar Gosner stages compared with R. sphenocephala. We also characterize immune gene expression across Gosner stages 26 to 41 to determine whether consistent immune benchmarks align with ontogeny across taxa. This study aims to address a critical gap in our understanding of tadpole immune system plasticity and evolution, and the degree to which variation in larval development time among species predicts relative investment in highly diversified adaptive immune defenses.

Skin lipids may play an important role underpinning species-specific susceptibility to Ophidiomycosis (snake fungal disease)

Crick, JE[1]*; Dallas, JW[1]; Munafo, JP[2]; Martinez, T[1]; Wilson, I[1]; Foster, E[1]; Hines, L[1]; Cheng, M[1]; Avalos Madera, M[1]; Rigby, R[1]; Catlett, T[1]; Benz, H[1]; Moolman, A[1]; Ramazan, S[1]; Johnson, K[1]; Gamez, V[1]; Davis, A[1]; Termuhlen, E[1]; Walker, DM[1]
[1]Middle Tennessee State University
[2]University of Tennessee
*jec9y@mtmail.mtsu.edu

Ophidiomycosis is an emerging infectious disease caused by *Ophidiomyces ophidiicola* (Oo). It affects a wide diversity of snake species and causes a range of clinical signs, from mild skin lesions to systemic infections. Lipids are a major component of snake skin and have a multifaceted role in snake physiology and social interactions, but are rarely considered when studying ophidiomycosis. We examined how snake skin lipids, both non-volatile and volatile, affected the growth of Oo. Non-volatile lipids were extracted from the skin of three snake species susceptible to Oo (*Crotalus horridus, Thamnophis sirtalis*, and *Nerodia sipedon*) and added to a biologically relevant carbon source (keratin) in a medium. Volatile lipids were extracted from snake sheds colonized by Oo or a control using solvent assisted flavor evaporation and GCMS used to profile the skin chemistry. Using headspace jar assays, we tested for the effect of eight volatile short-chain fatty acids (SCFAs) on Oo growth. We found that non-volatile skin lipids suppressed fungal growth, but this effect differed among species. Eight SCFAs were absent on Oo-colonized sheds, and four of these volatile SCFAs promoted fungal growth. Results suggest that skin lipids may play an important role underpinning species-specific susceptibility to snake fungal disease.

Assessment of sea turtle egg fusariosis (STEF) in Olive Ridley (*Lepidochelys olivacea*) nests at Abacoa Beach, Arecibo, Puerto Rico

Cuevas-Soto, YA[1]*; Rosado-Rodríguez, G[1]
[1]University of Puerto Rico in Arecibo
*yahir.cuevas@upr.edu

Lepidochelys olivacea (Olive Ridley) is the most abundant sea turtle species worldwide and one of four that nest along the coasts of Puerto Rico. Despite its global abundance, the species is listed as Endangered due to factors such as climate change, habitat degradation, pollution, and predation. Infections caused by microorganisms, including fungi (particularly *Fusarium*-associated diseases) have been shown to significantly reduce hatching success in sea turtles. However, information on Olive Ridley nesting in Puerto Rico remains limited, constraining our understanding of these pathogenic threats. This study characterizes the mycelial fungal diversity associated with L. olivacea nests and unhatched eggs along Puerto Rico's northern coast, with emphasis on fusariosis and its effects on embryonic development. Through fungal isolation and combined morphological and molecular identification, *Fusarium solani* species complex (FSSC) members were identified from nest sand and the exterior and interior surfaces of egg samples. These findings provide critical insight into the role of fungi in hatching success and the broader health of sea turtle populations. By elucidating the relationship between fungal infection and embryonic mortality, this research enhances our understanding of Olive Ridley ecology and informs evidence-based conservation strategies aimed at ensuring the long-term viability of this species in the Caribbean.

Investigating the trypanosoma spp diversity within South African frogs across the Soutpansberg Mountain Range

Du Buisson, J[1]*; Du Preez, LH[1]; Netherlands, EC[2]
[1]North-West University
[2]University of the Free State
*jorethadb@gmail.com

The Soutpansberg mountain range forms part of the Vhembe Biosphere Reserve in the Limpopo province of South Africa. This area is known to harbour at least 35 species of anurans. Anurans are known to host a variety of haemoparasites. A larger study for this area showed that the rich anuran diversity found at the Soutpansberg Mountain also hosts a variety of haemoparasite species, specifically trypanosomes. Blood samples were collected, prepared, and screened for the presence of haemoparasites from 387 individual frogs. Overall, 89% (31/35) of expected anuran species were observed, of which blood samples were collected from 25 species of anuran. The preliminary data show 22 morphologically different Trypanosoma spp. infecting 32 specimens belonging to four species of anurans. However, little significant molecular data has been generated in the current study due to the high prevalence of mixed trypanosome infections in the anuran hosts. Future work includes reviewing and refining the phylogenetic methods that are used to be able to identify species accurately within mixed infections. These results demonstrate a diverse parasite community across the mountain range, providing insight into understanding the diversity and distribution of South African anuran blood parasites.

Comparing bacterial growth derived from *Ambystoma laterale* skin in standard vs. mucin-based media

Faust, EA[1]*; Stearn, K[1]*; Olawuyi, T[1]; Barnes, EM[1]
[1]Rochester Institute of Technology
*eaf5094@rit.edu; kas3494@rit.edu

Batrachochytrium dendrobatidis (Bd) is a chytrid fungus that causes fatal disease in many amphibian species. Amphibian skin is covered in mucus which provides niches for diverse microbial communities. Research has shown that commensal skin microbes act as an extension of the immune system combatting Bd infections, but that mucus is likely key to this interaction as Bd can use it to accelerate encystation. In this study, we isolated bacteria from *Ambystoma laterale* in New York, USA. We identified 20 isolates via full-length 16S rRNA sequencing representing common phyla Actinomycetota, Bacteroidota, and Pseudomonadota. We assessed their growth (nreplicate = 3) in both 0.3-1% tryptone, a common media used in amphibian microbiome studies, and 0.3-1% mucin-based media (i.e., mucin as the sole carbon source). Results indicated that several core amphibian bacteria show differential growth in mucin than in tryptone often resulting in some isolates previously identified as putative probiotics for amphibians showing minimal to no growth in a mucin-based environment including *Janthinobacterium lividum* and *Lelliottia amnigena*. Further investigation into differences in these phenotypes and how they affect metabolic interactions between the bacteria and the pathogen could allow for the development of further potential solutions to stop worldwide amphibian loss due to chytrid.

Local and systemic immune responses to lungworms in experimentally infected cane toads (*Rhinella marina*) over time

Floreste, FR[1,2]*; Chen, H[2]; Chan, TCL[2]; Brown, GP[3]; Assis, VR[1,4]; Gomes, FR[1]; Shine, R[3]; Rollins, LA[2]
[1]University of São Paulo
[2]University of New South Wales
[3]Macquarie University
[4]University of West Alabama
*felipe.floreste@gmail.com

Amphibians host a plethora of parasites, including lungworms of the genus *Rhabdias*. In vertebrates, helminths elicit a T-helper type 2 (Th2) immune response with upregulation of proteins such as IL-4Ra, IL-13Ra1, IL-25, IL-6, IL-10, while inhibiting the release of Th1 stimuli, such as IFN-γ. Parasite presence impacts host's fitness by disrupting central endocrine axes and mediating important trade-offs. Currently, amphibians face critical threat from emerging pathogens worldwide, and much attention has focused on Th1 responses and natural plasma antibodies, while Th2 responses have been overlooked in this context. Herein, we experimentally infected cane toads (*Rhinella marina*) with lungworms (*Rhabdias pseudosphaerocephala*) and tracked the local (lung) and systemic (spleen) gene expression of Th2 proteins, in addition to systemic IFN-γ at 1, 3, 14, and 30 days post-infection (DPI). In the spleens, lungworms caused an upregulation of IL-4Ra at 3 DPI, and marginal upregulation of IL-25. In the lungs, IL-6 was the only upregulated cytokine, and IL-4Ra decreased at 14 DPI. IL-13Ra1 and IL-10 were not altered in either tissue, and IFN-γ mRNA was not detected. These results indicate that the most pronounced immune responses occurred systemically rather than at the site of infection. Despite evidence of Th2 activation in the spleen, lungworms remained in the lungs through 30 DPI, suggesting a mismatch between systemic signaling and local immune activity. This pattern suggests immunomodulation or tolerance mechanisms that may limit host responses in lung tissue and facilitate parasite persistence. Further studies are needed to clarify the mechanisms underlying this tissue-specific immune regulation.

Detection of *Batrachochytrium dendrobatidis* in three native amphibian species within an invasive *Xenopus laevis* hotspot in Western France

Gharnit, E[1]*; Lesbarrères, D[1,2]; Kosciolek, C[1]; Moreau, E[1]; Jeauffre, J[3]; Pagano, A[1]
[1]Université d'Angers
[2]Université de Sudbury
[3]Institut Agro
*elouana.gharnit@univ-angers.fr

Batrachochytrium dendrobatidis (Bd) has been identified as a major driver of global amphibian declines. Its rapid spread is largely attributed to asymptomatic carrier species such as the invasive African clawed frog (*Xenopus laevis*) acting as a historical reservoir host and facilitating pathogen dissemination. Despite increasing concern in Europe, epidemiological data remains limited for many amphibian species, particularly their susceptibility to infection. Here, we assessed Bd occurrence and loads in *X. laevis* and three co-occurring native amphibians, the green frog complex (*Pelophylax* spp.) and two urodele species, *Lissotriton helveticus* and *Triturus cristatus*. Bd was detected in 20 out of 22 sites but no association with *Xenopus* occurrence was observed. Prevalence was highest in *X. laevis* (82%, 90/110), intermediate in *Pelophylax* spp. (35%, 50/142), and low in urodeles (<14%), with no clinical signs observed. Infection loads were generally low (<10 DNA copies μL^{-1}) and were not explained by body condition, but rather by species identity, while decreasing slightly with host density, suggesting that local environmental conditions, dilution effects, and host-specific factors may play primary roles in shaping infection dynamics. These results also highlight that *Pelophylax* spp. may function as asymptomatic reservoir host, suggesting a more limited role for *Xenopus* as a carrier in this invasion hotspot.

Incorporating a citizen science project for surveillance of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* into an undergraduate curriculum

Goodman, RM[1]*; Starr, AM[2]
[1]Hampden-Sydney College
[2]Longwood University
*rgoodman@hsc.edu

The Student Network for Amphibian Pathogen Surveillance (SNAPS) is a North American citizen-science program that integrates authentic research into undergraduate education, providing shared protocols, centralized DNA testing, and teaching resources to 62 participating institutions. Students collect skin swabs that are screened by qPCR for *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal). We joined SNAPS in 2023 and contributed samples from 89 amphibians in 2024-2025. Additional samples will be collected in April 2026. While no Bsal has been detected, 7–10% of amphibians have tested positive for Bd within each collecting period. Participation in this project has provided hands-on field experience and training in biosecurity techniques for students in four undergraduate courses and supported conservation-relevant surveillance efforts. In 2026, we will utilize institutional summer programs to conduct additional research on duplicate swab samples and tissue samples collected during the SNAPS project. DNA testing (qPCR) will be conducted to screen all samples for Ranavirus (Rv) and Perkinsea (Pr), and a subset of samples will be sequenced using Oxford Nanopore MinION to characterize skin microbiomes of amphibians carrying Bd, Rv, and Pr. These laboratory studies will offer students deeper training in molecular methods, data management, and scientific communication.

Scale-dependent predictors of disease, integrating species distribution models with telemetry to predict snake fungal disease

Grisnik, M1*; Alter, A1; Grosse, A2; Haberman, K2; Brubaker, R3; Hanscom, R4; Walker, D5
[1]Coastal Carolina University
[2]South Carolina Department of Natural Resources
[3]Amphibian and Reptile Conservancy
[4]University of South Carolina Beaufort
[5]Middle Tennessee State University
*mgrisnik@coastal.edu

Emerging fungal pathogens are increasingly recognized as major drivers of wildlife declines. Among these, *Ophidiomyces ophidiicola* (Oo), the causative agent of snake fungal disease (SFD), poses a significant conservation concern due to its wide host range and potential for environmental persistence. While SFD has been reported across much of North America, landscape-level factors associated with its distribution remain poorly understood. The

objective of our work was to understand how landscape level factors influence the prevalence of this pathogen. We used a species distribution modeling (SDM) framework to model the potential distribution of Oo. To evaluate how these model patterns relate to real world infection dynamics, we overlaid telemetry data from individually tracked snakes with known Oo status. Results demonstrate a scale dependent role of climate in SFD risk, with broad climatic conditions constraining where Oo can occur across the landscape. However, once snakes occupy climatically suitable environments, additional variation in finer scale habitat characteristics does not explain individual infection status, highlighting the role of host level factors in disease outcomes. Together findings provide a multiscale perspective on the ecological and environmental drivers of SFD and demonstrate how integrating SDMs with field-based infection data can improve understanding of wildlife disease dynamics.

Investigating the anti-chytrid mechanisms of curcumin on *Batrachochytrium dendrobatidis* zoospores

Hammontree, IG[1]*; Galvin, DJ[1]; Silva, S[2]; Miller, DL[1]; Gray, MJ[1]
[1]University of Tennessee Knoxville, School of Natural Resources
[2]Glenville State University, Science and Mathematics Department
*Ihammont@vols.utk.edu

Chytridiomycosis has contributed to the decline of >500 amphibian species globally. There is a critical need for an alternative treatment that offers less negative side effects and can be used in the pet trade. Our study aims to identify concentrations of curcumin and itraconazole that will inactivate Bd zoospores. We hypothesise that curcumin exerts inhibitory effects on Bd by affecting cell membrane structure and function and that the effective dose of curcumin and itraconazole will be lower when used in combination. To enhance the solubility of curcumin we created nanoparticles via ultrasonication. Membrane integrity was assessed using a Propidium Iodide Influx Assay, Ethidium Bromide Uptake Assay, and DPH Assay. The next step is to look at whether ergosterol really is the target of anti-chytrid activity through minimum inhibitory concentrations (MIC) and measure ergosterol content using gas chromatography-mass spectrometry. Finally, checkerboard assays and fractional inhibitory concentration will determine whether curcumin and itraconazole act synergistically. This research is still in the beginning stages of determining the MIC for curcumin nanoparticles and performing assays to assess cell membrane damage. Our findings can offer a better understanding into how Bd interacts curcumin and may offer a less toxic treatment.

Immune and skin microbiome associations with skin infections in snakes

Haydt, NT[1]*; Neuman-Lee, L[1]
[1]Arkansas State University
*natalie.haydt@smail.astate.edu

Fungal skin infections are affecting reptile populations worldwide. To better predict their impact we must understand how immune responses to pathogens vary between species and populations. The skin microbiome can be considered part of the innate immune response and is especially vital for defense against fungal skin pathogens. By conjunctly investigating associations between the skin microbiome and innate immune response to multiple pathogens, we can obtain a more comprehensive understanding of immunocompetence. We examined the skin microbiome and innate immune responses in multiple snake species from sites in Arkansas, including individuals with and without signs of dermatomycosis. We quantified the innate immune response against multiple pathogens using microbial killing assays. To characterize the skin microbiome, we swabbed snakes and ran targeted sequencing to quantify both bacterial and fungal taxa. We identified the presence of known dermatophytes associated with reptile fungal infections (in the order Onygenales). We then examined associations between dermatophyte presence and abundance, additional skin microbiome metrics, MKA metrics, snake species, sampling site, and skin infection symptoms. Significant predictors of fungal skin infections can be used in future conservations efforts to identify populations more at risk for fungal disease impacts which can inform prioritization of populations for management.

Amphibian population monitoring and pathogen surveillance in the Great Smoky Mountains National Park: 2015-2026

Haynes, K[1]*; Galvin, DJ[1]; Miller DL[1]; Gray MJ[1]
[1]School of Natural Resources, University of Tennessee
*khayne21@vols.utk.edu

The southeastern United States has the greatest diversity of salamanders in the world. The Great Smoky Mountains National Park, in particular, is home to over 30 species of salamander, including the endemic species *Plethodon jordani* (Red-cheeked salamander). 25 species of lungless (Plethodontid) salamanders can be found within the park. To monitor population trends over time and to evaluate pathogen presence in the GSMNP salamander populations, we

have collected data for more than 10 years from three sites: Sugarlands, Chimney Tops, and Indian Gap. Salamanders were captured using nets and morphometric data (SVL, mass) were collected. A skin swab and a tail snip were collected for pathogen surveillance. All salamanders were released near the point of capture following data collection. Pathogen samples were extracted using QIAGEN DNEasy spin columns and tested for *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, and ranavirus using Taqman-based qPCR. The prevalence of these pathogens varied from year-to-year. Bsal has not been detected during these sampling events. This data provides important insights for salamander populations and amphibian pathogen surveillance in the salamander capital of the world.

A pathologic mortality assessment in free-ranging eastern diamondback rattlesnakes (*Crotalus adamanteus*)

Hazelrig, CM[1*]; Gray, E[2]; Waldron, J[2]; Yabsley, MJ[1]; Lorch, JM[3]; Holloway, J[4]; Langer, E[2]; Nemeth, NM[1]
[1]University of Georgia
[2]Marshall University
[3]United States Geological Survey
[4]Marine Corps Recruit Depot Parris Island
*corinna.hazelrig@uga.edu

The eastern diamondback rattlesnake (EDB, *Crotalus adamanteus*) is a focal species of conservation concern in the southeastern United States with declines exacerbated by human persecution and infectious diseases. We aimed to evaluate threats to EDB populations through postmortem examination. To do so, we examined 23 EDBs submitted to the Southeastern Cooperative Wildlife Disease Study from 2023 to 2026. We reviewed gross, histologic, and laboratory findings from 21 EDBs from Parris Island, South Carolina and two EDBs from Onslow County, North Carolina. The most common diagnosis was blunt force trauma (60.9%, 14/23) and the most common infectious disease being ophidiomycosis (33.3%, 7/22). Three had systemic fungal infections with hyphae and *Oo* detected in internal organs (lungs, ova, and kidney). We also documented two dermatomycosis cases with unidentified fungal agents (*Oo* DNA not detected) and one case with *Oo* detected without concurrent fungal lesions. Additional postmortem findings included multisystemic bacterial infections (n=1), goiter (n=6), severe gastrointestinal *Kalicephalus* sp. parasitism (n=2), poor nutritional condition (n=8), thermal burn injury (n=1), reproductive tract disease (n=1), and gastric foreign body obstruction (n=1). Opportunistic, complete diagnostic examination in wildlife, including EDBs, provides vital information that informs conservation management actions such as reintroduction and release programs.

Temperature-dependent patterns and infection dynamics are similar among FV3-like ranaviruses infections in larval wood frogs (*Lithobates sylvaticus*)

Keller, EL[1]*; Brunner, JL[1]
[1]Washington State University
*Erin.keller@wsu.edu

Ranavirus infections can produce extremely divergent outcomes, presumably due to differences in virus isolates, host genetics, and the environment (e.g., temperature). The diversity of these factors and complexity of their interactions makes it challenging to form even general predictions for infection outcomes. Thus, we sought to 1) test whether temperature-dependent outcomes were consistent across Ranavirus rana1 isolates and 2) begin to understand how important isolates are to outcomes of infection. We experimentally infected wood frog (*Lithobates sylvaticus*) larvae at 16°C and 20°C with five virus isolates expected to vary substantially in their likelihood of causing infection and death, including knock-out recombinants deficient in key putative immune evasion and regulatory genes. We found that infection prevalence was higher at 16°C than 20°C, presumably due to slower innate immune responses. Case mortality was similar among temperatures but occurred more rapidly at 20°C likely reflecting faster viral replication. Contrary to expectations, the Δ64R recombinant virus lacking a putative immune evasion gene had the highest infection prevalence and was most virulent while other strains were very similar. Our results suggest that infection dynamics and outcomes in larval wood frogs are surprisingly homogenous among Rv. rana1 isolates and have consistent temperature-dependent patterns.

How different amino acids impact the growth and development of the amphibian fungal pathogen *Batrachochytrium dendrobatidis*

Lehmkuhle, JR[1]*; Thekkiniath, J[1]
[1]Purdue University Fort Wayne
*Lehmjr02@purdue.edu

Batrachochytrium dendrobatidis is an increasingly important pathogen to study due to its global presence. It is a major cause of the decline of global amphibian populations and has not been extensively studied. It forms a monolayer to help attach to amphibian skin, making it an important part of pathogenicity to understand. Identifying the mechanisms of monolayer formation may be key to understanding and preventing fungal disease in amphibians. This may be done first by enhancing monolayer formation in *Batrachochytrium dendrobatidis* (BdVM1) using amino acids then analyzing the different genes that may be enhanced or over expressed compared to normal fungus. My research has found that both L-glutamic acid and L-methionine can increase monolayer formation in *Batrachochytrium dendrobatidis*. These amino acids may be useful in helping to identify important genes in monolayer formation to be used as targets for inhibition in future studies.

Diagnosis of *Ophidiomyces ophidiicola* in snakes in managed care in Europe over a six-year period

Marschang, RE[1*], Schüler, L[1], Leineweber, C[1]
[1]Laboklin GmbH & Co. KG
*marschang@laboklin.com

This retrospective study evaluated the results of PCR testing for *Ophidiomyces ophidiicola* (Oo) in captive snakes in Europe between January 2020 and December 2025. A total of 338 samples from 53 species from eight families were tested. Oo was detected in 59 samples (17.5%) from 11 species in five families. This is significantly higher than detection rates in wild snakes in the same period (10.03%, p=0.002). Detection rates differed significantly between species and families (both p<0.001), with the highest detection rates found in anacondas (*Eunectes* spp.; 47/87, 54% positive). Anaconda samples were submitted from 13 different owners, and samples from ten of these (76.9%) were Oo positive. Higher detection rates were found in snakes from zoos and rescue centers compared to private owners (P<0.001), driven by the higher number of anacondas in zoos. All positive samples were submitted from France and Germany, the two countries from which the highest sample numbers were submitted. The results presented underline the wide host range of Oo and document a high prevalence in anacondas in managed care. Reasons for this high infection rate in this species deserve further study as does the possibility of spillover between wild and captive snake populations.

Sensitivity of Pacific tree frog larvae (*Pseudacris regilla)* to 6PPDQ, a ubiquitous stormwater toxicant

Masecar, SC[1]*; Des Roches, S[2]; Smith, R[3]; Piovia-Scott, JSmith, JA[1]
[1]Washington State University-Vancouver
[2]Washington State Department of Fish and Wildlife
[3]Washington State Department of Ecology
* susanna.masecar@wsu.edu

6PPD-quinone (6PPDQ), a transformation product of the tire rubber antiozonant 6PPD, is a potent environmental contaminant with acute lethality in coho salmon (*Oncorhynchus kisutch*). As a ubiquitous constituent of road runoff, 6PPDQ reaches high concentrations in stormwater ponds, where many amphibians deposit eggs. The toxicity of 6PPDQ to larval amphibians remains uncharacterized. We conducted a repeated exposures laboratory experiment to evaluate the effect of 6PPDQ on the survival, growth, and development of a native amphibian, the Pacific tree frog (*Pseudacris regilla*), during the larval period. After eclosion of field-collected eggs, we assigned tadpoles to one of four 6PPDQ exposure levels (10,000 ng/L, 1,000 ng/L, 100 ng/L, or sham; n = 20 per group). We exposed tadpoles to 6PPDQ for 24 hours every 9 days for 60 days or until metamorphosis. We monitored for mortality daily, measured developmental stage and body length every 9 days, and collected body mass at metamorphosis or at 60 days. This experiment is ongoing. Preliminarily, 6PPDQ does not affect survival or growth during early development. These results will inform risk management for amphibian conservation and stormwater pond management. Expanding this work to additional species will be critical for characterizing the sensitivity of amphibians as a group.

"AMPing up" the microbiome: Interactions of innate defenses and protective microbiota on the skin of northern leopard frogs (*Rana pipiens*)

McDonnell, NB[1]*; Reinert, LK[2]; Rollins-Smith, LA[2]; Bletz, MC[3]; LaBumbard, BC[1]; Woodhams, DC[1]
[1]University of Massachusetts Boston
[2]Vanderbilt University
[3]Pennsylvania State University
*nina.mcdonnell@umb.edu

The resident microbiota on amphibian skin can be a key constitutive defense against invading pathogens. This community is often enriched in antimicrobial producers, such as *Pseudomonas, Janthinobacterium*, and *Chrysobacterium*. Yet, the processes influencing host-microbe associations have not been well defined, limiting our ability to leverage microbial interventions for disease. This dissertation work explores the multiple roles of host-produced antimicrobial peptides (AMPs) in shaping community assembly, abundance, and protective function on the skin of the northern leopard frog (*Rana pipiens*). First, we assess how naturally occurring peptide secretions influence the growth and *Batrachochytrium*-inhibiting capacity of skin-associated and environmental microbes, testing whether AMPs selectively promote defensive symbionts. Second, we experimentally perturb AMP stores in vivo to evaluate how disrupting this regulatory system alters microbiome structure and predicted function. Our results suggest that AMPs maintain protective resident microbiota through multiple modes of action, including effects on community total abundance, composition, and protective function. Additionally, our work underscores the importance of naturalistic conditions in amphibian microbiome research. These results inform our understanding of host-microbe symbioses and associated health conditions.

Characterizing seasonality of the innate immune function in Red-Eared Slider Turtles

McIver, AM[1]*; Neuman-Lee, LA[1]
[1]Arkansas State University
*Abbymciver8@gmail.com

The immune system is composed of both innate and adaptive components to defend the body against pathogens and parasites. This complex, multidimensional approach is well studied in many taxa, but less is known about the reptilian system. Reptiles are facing multiple conversation challenges, including novel disease threats. Therefore, to ensure the best conservation outcomes, it is vital to establish the natural cycles of immunity. Both circannual changes and life-history stages can cause immunological shifts due to energetic needs and temperature fluctuations. We plan to assess innate immune function of red-eared slider turtles (*Trachemys scripta elegans*) each month in the spring and early summer of 2026. Microbial killing assays will be conducted using serum+buffy layer. We will manipulate the fresh serum+buffy layer through freezing and introduce the mixture to three microbes, a gram-negative bacterium (*Escherichia coli*), a gram-positive bacterium (*Staphylococcus aureus*), and a fungus (*Candida albicans*), each activating a specific immune pathway of the system. We expect to see variation in immune function in response to temperature differences in both sexes and variation between sexes during the reproductive season. This study can provide a deeper insight into how we can target mitigation efforts to support natural defenses.

Hiring a Molecular Bounty Hunter: A CRISPR-based Assay for Rapid Detection of *B. dendrobatidis*

Muñoz, DA[1]*; Nagarajan, RP[1]; Kleeman, PM[2]; Jenkinson, T[3]; Halstead, BJ[2]; Schreier, AM[1]
[1]University of California, Davis
[2]U.S. Geological Survey
[3]California State University
*damunoz@ucdavis.edu

Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd), has been associated with amphibian population declines worldwide. Currently, noninvasive diagnostic methods often rely on amphibian capture, swabbing, and quantitative PCR (qPCR) assays. However, processing samples via qPCR requires molecular biology experience, expensive instrumentation, and several hours of benchwork to produce results. Outsourcing to laboratories can further increase project costs and wait times. CRISPR-based diagnostics have enabled the development of novel methods for pathogen detection. Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) assays use the CRISPR-Cas13a enzyme complex to detect target nucleic acids and produce a measurable fluorescent signal. These assays are rapid (<1 hr) and can be performed by individuals with little to no molecular ecology experience. We developed a SHERLOCK assay to detect a molecular barcode of the Global Panzootic Lineage of Bd (BdGPL) from swabs and eDNA samples. Our assay positively detected culture-derived DNA from strains within the BdGPL-1 and BdGPL-2 subclades within 30 minutes of SHERLOCK reaction initiation in the laboratory. SHERLOCK can be performed by non-geneticists with minimal equipment and training, which can facilitate the monitoring of Bd in captive and wild environments.

Environmental predictors of metabolic EA to parameterize thermal mismatch models of disease across amphibian phylogeny

Noelker, JE[1]*; Bermudez, SA[2]; Six, VM[2]; Sckrabulis, JP[3]; Vassallo, M[4]; Pell, B[5]; Craig, HM[2]; Raffel, TR[2]
[1]Madonna University

[2]Oakland University
[3]University of Florida
[4]Detroit Zoological Society
[5]Lawrence Technological University
*jnoelker@madonna.edu

The combined threats of climate change and emerging multi-host diseases have led to broad interest in predicting temperature dependent infection dynamics across taxa. Amphibian infection by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) is of particular concern. We can use host metabolic thermal performance curves (TPCs) to derive a key activation energy (EA) parameter for metabolic theory (MT)-based thermal mismatch models of Bd infection. However, we lack metabolic data for many Bd-threatened hosts and want to know if species level EA relates to available environmental datasets. We compiled O2 consumption TPCs for more than 85 species using experimental respirometry and published data. For each species, we estimated metabolic EA and compiled 19 WorldClim variables (plus latitude and elevation) within its native range. We used phylogenetically corrected regression to investigate environmental predictors of EA. We hypothesized a priori that EA is influenced by environmental temperature. Surprisingly, we were unable to identify any significant linear or curvilinear relationships between temperature predictors and EA. Instead, we found highly significant relationships with total precipitation and precipitation seasonality that were strengthened with phylogenetic correction. This suggests metabolic EA evolves in response to environmental conditions, but precipitation may be more important than previously assumed.

Coinfections over space and time in the dusky pygmy rattlesnake, *Sistrurus miliarius*

Palmisano, JNP[1*]; Savage, AE[1]
[1]University of Central Florida
*Jenna.Palmisano@ucf.edu

Coinfections are increasingly recognized as important drivers of disease dynamics. In snake populations, diseases including mycosis and raillietielliasis have been linked to severe pathology, mortality, and population declines. The pygmy rattlesnake (*Sistrurus miliarius*) in Florida exhibits infection with mycosis caused by *Paranannizziopsis* spp. and *Ophidiomyces ophidiicola* along with raillietielliasis caused by the invasive pentastome, *Raillietiella orientalis*. The spatial and temporal patterns of coinfection and their potential impacts on survival are unknown. As part of a long-term study, we surveyed *S. milarius* across 12 sites in the Florida panhandle and peninsula to assess variation in infection prevalence, severity, and coinfection dynamics. Here, we discuss findings from the first three years of the study. *Raillietiella orientalis* is present in nine sites and in 19% of snakes (n=451), while clinical signs consistent with mycosis were observed at all sites and in 40% of individuals (n=831). Coinfections were detected at six sites, with prevalence ranging from 3 to 50% (mean=8%). Temporal patterns suggest that mycosis prevalence and severity may increase following wetter conditions associated with active hurricane seasons, while declining during drought periods. Ongoing analyses will evaluate how environmental variation shapes coinfection risk and disease outcomes. Given the empirical and anecdotal evidence of declines in *S. miliarius* along with the diversity of host species affected by these pathogens and the potential for co-infection, it is critical to investigate the dynamics of coinfections in *S. miliarius* to understand the conservation implications.

Skin microbiome-derived siderophores influence colonization resistance against *Batrachochytrium dendrobatidis*

Paschalidis, C[1]*; Murphy, E[2]; Read, E[3]; Ho, FKH[2]; Dumas, ME[2,4]; Bates, K[3]; Cunrath, O[1]
[1]Université de Strasbourg
[2]Imperial College London
[3]Queen Mary University of London
[4]University of Lille
*c.paschalidis@unistra.fr

Batrachochytrium dendrobatidis (Bd) is a fungal pathogen that has led to catastrophic declines in amphibian populations worldwide. Bd zoospores infect amphibian skin, disrupting its function and ultimately leading to death. Iron competition is a well-defined battleground in host-pathogen interactions across diverse systems, ranging from the human gut to aquatic vertebrates. However, its role in amphibian skin defense remains unexplored. In parallel, we know that skin commensal bacteria can produce iron-chelating molecules, called siderophores. We hypothesized that the skin microbiome may drive this iron competition, through siderophores, acting in synchrony with the host to restrict nutrient availability to Bd. We used a combination of culture-dependent assays and LC-MS to screen and identify siderophores produced by skin bacterial isolates from *Alytes obstetricans*. To evaluate the anti-fungal potential, we challenged Bd with these siderophores in in vitro growth assays. Our research suggests that siderophore

production is prevalent within *A.obstetricans* skin microbiome. These secondary metabolites displayed potent anti-fungal activity against Bd, in an iron-dependent manner. Consequently, we are currently designing siderophore producing synthetic communities (SynComs) of native skin bacteria to screen *in vivo* as probiotics. Our findings suggest that microbiome-derived siderophores may provide a critical layer of colonization resistance against Bd though iron limitation.

First detection of Ranavirus infection in threatened Brazilian anurans

Pontes, MR[1]*; Trafford, J[3,4]; Rosa, GM[3,5,6]; Ruggeri, J[1,7]; Toledo LF[1]
[1]*Universidade Estadual de Campinas (Unicamp), Brazil*
[3]*Institute of Zoology, Zoological Society of London, UK*
[4]*University College London, UK*
[5]*IMIB Biodiversity Research Institute (CSIC, Universidad de Oviedo, Principado de Asturias), Spain*
[6]*Faculdade de Ciências da Universidade de Lisboa, Portugal*
[7]*Universidad Nacional de Misiones, Posadas, Argentina*
*maah.retuci@gmail.com

Amphibians are the most threatened vertebrate group worldwide, with emerging infectious diseases playing an important role in their ongoing declines. While the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has received considerable attention, *Ranavirus* (*Rv*) remains less explored, particularly in South America. In this study, we report the first detection of *Rv* in two threatened amphibian species from Brazil. Specifically, we found infected individuals belonging to the genus *Melanophryniscus* (Redbelly toads) with highly restricted distributions in the southern Brazilian Atlantic Forest. Importantly, these findings contributed to recent updates of threatened species assessments in the region. Our findings highlight the need for expanded surveillance and pathogen monitoring in threatened anuran populations, particularly those with limited geographic distributions and threatened with extinction. Furthermore, understanding *Rv* dynamics and its potential interactions with *Bd* and other stressors is essential for informing conservation and management strategies aimed at safeguarding *Melanophryniscus* species and other vulnerable amphibians in the Atlantic Forest biome.

Building an in vitro amphibian skin model to interrogate early host–microbiome–pathogen dynamics

Read, E[1]*; Bates, KA[1]
[1]Queen Mary University of London
*emily.read@qmul.ac.uk

Although chytridiomycosis remains one of the most damaging wildlife diseases globally, the mechanisms surrounding early events in *Batrachochytrium dendrobatidis* infection, particularly how the skin microbiome shapes disease outcomes, are not well defined. In vitro systems that preserve amphibian skin structure and function, including the components of the native microbiota, offer a powerful tool to dissect host–microbiome–pathogen dynamics under controlled conditions and reduce reliance on live infection studies. To address this need, we developed transwell-based skin explant models from hosts with varying Bd susceptibility (*Alytes obstetricans*, susceptible and *Xenopus laevis*, relatively resistant). These models maintain apical–basal polarity, tissue organization and mucus production. This is complemented by a phylogenetically representative culture collection (>300 isolates) of core skin bacterial taxa. By colonizing explants with candidate bacterial taxa prior to infection, we show the ability of specific microbes to influence early disease dynamics in a tissue relevant setting. We demonstrate changes in epithelial integrity (transepithelial resistance and fluorescent dye leakage), tissue architecture (histology), and expression of key inflammatory pathways (RT-qPCR). Our explants support the discovery of protective microbial functions and early immune signatures that have the potential to inform probiotic approaches to disease mitigation.

Lymphocyte inhibition by cell wall components of *Batrachochytrium dendrobatidis* (*Bd*)

Rollins-Smith, LA[1]*; Lee, J[2]; Linney, K[3]; Mirchandani, RD[1]; Killian, KE[1]; Reinert, LK[1]
[1]Vanderbilt University School of Medicine
[2]Louisiana State University
[3]University of Tennessee
louise.rollins-smith@vanderbilt.edu

The chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), is linked to continuing global amphibian population declines. Bd causes chytridiomycosis when swimming zoospores attach, encyst, and invade epidermal skin cells. Disruption of the host skin results in death. Although amphibians have robust immune defenses, Bd has evolved

mechanisms to evade immunity. Previous studies revealed three small metabolites released by Bd that inhibit lymphocyte responses. We hypothesized that another molecule or molecules associated with the fungal cell wall is also inhibitory for lymphocytes. To investigate the role of cell wall components, we fragmented mature zoosporangia, isolated the cell wall components lacking other soluble factors, and cultured them with proliferating frog spleen cells (*Xenopus laevis*) or Jurkat T cells. Enriched cell wall fragments alone inhibited proliferation of both frog spleen cells and Jurkat T cells in a dose-dependent manner. Boiling of the cell wall fragments or treatment with carbohydrase enzymes reduced their capacity to inhibit proliferation. These results suggest that the active inhibitory factor in the *Bd* cell wall is a glycosylated protein or a protein-carbohydrate complex. Ongoing studies aim to further understand the nature of the lymphocyte inhibitory factors. Support: Vanderbilt SyBBURE Searle Undergraduate Research Program and NSF IOS-2147467.

Engineering resistance to chytrid fungus: A transgenic, nanobody-mediated solution

Sawyer, C[1]*; West, M[1] ; Morales, R[1] ; Youngson, N[1] ; Pask, AJ[1,3] ; Frankenberg SR[1]
[1]The University of Melbourne
[2]Australian National University
[3] Colossal Biosciences
* susan.sawyer@rch.org.au

Chytridiomycosis, caused by the fungal pathogens *Batrachochytrium dendrobatidis (*Bd*)* and *Batrachochytrium salamindrivorans* (Bsal) has had a devasting impact on global amphibian populations, but few solutions exist to mitigate this disease. The responsible pathogenic chytrids present a unique challenge to conservationists, particularly due to their broad host range and unique immuno-evasive mechanism of infection. In recognizing these challenges, our research aims to provide the foundations of a transgenic, nanobody-mediated solution. Nanobodies are short-chain antibodies produced by camelids. By identifying nanobodies which attenuate chytrid pathogenicity-associated proteins, chytrid-neutralizing nanobody sequences can be edited into a broad range of vulnerable amphibians to confer innate, transgenic immunity to chytridiomycosis. To identify proteins that play a role in early chytridiomycosis infection, we isolated proteins from whole Bd zoospores and Bd zoospore membrane fractions. We analyzed these proteins using mass spectrometry and subsequently constructed a whole-zoospore proteome alongside a membrane-specific proteome. Our study presents a Bd zoospore specific membrane proteome, which builds on published chytrid proteome and transcriptomic data and can all help identify accessible nanobody targets. We hope this proteome can become a resource for the chytrid research community, providing insights into Bd's mechanism of infection and potential targets for therapeutic solutions to the chytrid pandemic.

A Multi-Omic Approach to Exploring the Amphibian Microbiome

Thompson, E[1]*
[1]Rochester Institute of Technology
*egt5318@rit.edu

Chytrid fungi, Bd (*Batrachochytrium dendrobatidis*) and Bsal (*Batrachochytrium salamandivorans*), are one of the largest threats to amphibian populations worldwide. Research has shown that the amphibian's skin microbiome can inhibit Bd/Bsal infection, but that microbial composition can vary drastically with host and environmental factors. Habitat-wide mitigation methods that capitalize on the thermal mismatch between Bd/Bsal and their hosts (e.g., warming the host or environment) have been proposed to combat infection. These methods have proven successful so far, but do not consider the indirect effects that warming has on the microbiome. Synthetic microbial communities of ten core members to simulate the *Ambystoma laterale* skin microbiome were exposed to Bd/Bsal at five different temperatures, reflecting mitigation methods and a climate change scenario. Microbial community composition and gene expression were assessed via high-throughput amplicon sequences and metatranscriptomics. It was found that time plays a larger role in shaping community composition than temperature, and that although temperature affects Bd/Bsal inhibition in vitro, it is not to a large degree, potentially suggesting that the host is a key player in temperature-pathogen interactions. When examined at the transcript level, temperature influences gene expression in these bacteria, resulting in a clear response to mitigation methods and Bd/Bsal infection.

The hypothalamic-pituitary-interrenal axis and the immune-pineal axis crosstalk regulates immunity in amphibians

Titon, SCM[1,2]*; Assis, VR[3]
[1]University of Rochester Medical Center, Rochester
[2]University of São Paulo
[3]University of West Alabama
*jec9y@mtmail.mtsu.edu

Amphibians have experienced severe global population declines associated with infectious diseases and anthropogenic environmental changes. We demonstrated that environmental stressors, including elevated temperatures and heatwave events, activate the hypothalamic-pituitary-interrenal (HPI) axis and increase corticosterone release in anurans. HPI activation modulates cellular and humoral immune responses in several amphibian species. Thermal stress also impairs immune function and reduces the ability to respond to subsequent stress challenges, potentially compromising disease resistance. We further showed that intraperitoneal injection of lipopolysaccharide or heat-killed *Aeromonas hydrophila* in *Rhinella* toads activates both the HPI and immune-pineal (IP) axes, indicating that corticosterone and melatonin modulate inflammatory responses in amphibians. Experimental evidence demonstrated that corticosterone influences phagocytosis and leukocyte transmigration. More recently, we found that inflammatory stimuli modulate ocular melatonin production and that immune cells synthesize melatonin during phagocytic activity in *Rhinella* toads. Understanding neuroimmune-endocrine interactions associated with environmental stressors and infectious diseases is essential for elucidating mechanisms underlying amphibian susceptibility and for developing effective conservation strategies.

Can we estimate infection history in wild animals?

Torres-Sánchez, M[1]*
[1]Amphibian Genomics Consortium
*torressanchez.maria@gmail.com

Wild animal populations are exposed to a growing diversity of infectious diseases, driven in part by the spread of invasive pathogens and anthropogenic change. These emerging pressures can alter host health, survival, and population dynamics. A major limitation in wildlife disease ecology is the lack of rapid, non-lethal methods to reconstruct individual infection histories, particularly when infections are repeated or subclinical. Such tools are essential for understanding cumulative disease burden and its long-term effects on hosts. For this purpose, I proposed to explore telomere and epigenetic changes in association with infections. Telomeres and epigenetic profiles could be sentinels of animal resilience. Changes in both could generate heritable adaptive variation preceding selection, which could help animals overcome infections, leading to the adaptation to their new impacted environments. Ultimately, telomeres and epigenetic profiles, such as methylation profiles, would help to describe host–pathogen coevolution, capture individual infection history and enable better predictions of disease outbreaks, being, in other words, the wildlife disease clocks.

Parental care and microbiome transmission in amphibians: assessing the current state of knowledge and disease research and the potential role of parental care in the vertical transmission of microbiomes in amphibians

Vonhoff, DJ[1]*; Galvin, DJ[2]
[1]Vrije Universiteit Amsterdam
[2]University of Tennessee
* Dylan.vonhoff@hotmail.com

Amphibians are among the most threatened vertebrate groups, facing global declines partly driven by infectious diseases. Their skin microbiomes are increasingly studied for their role in disease resistance. Despite growing interest in amphibian microbiota, the potential for vertical microbial transmission via parental care remains underexplored. In contrast, such transmission is well documented in other vertebrates. We assessed how amphibian care strategies may facilitate vertical microbiome transmission and identify candidate taxa for future research. To evaluate data availability and transmission potential, we compiled literature on microbiome presence, disease testing (Bd, Bsal, and Ranavirus), and parental care for 8,895 amphibian species. This broad synthesis reveals substantial gaps across lineages. Our analysis focused on 1,264 species with recorded parental care across the amphibian phylogeny. Care strategies for these species were reclassified by their intensity and mapped onto a pruned phylogenetic tree of 394 representative species from 347 genera. To evaluate transmission potential, we compared the care behaviour intensity of known vertical microbiome transmission cases in amphibians to similar care intensity situations in our dataset. From this comparison, we identified 61 genera where care intensity aligns with conditions that may facilitate vertical

microbiome transmission. Our findings suggest that vertical microbiome transmission in amphibians may be more widespread than currently recognized, a pattern consistent with the widespread underrepresentation of amphibians in global research efforts. Understanding where vertical microbiome transmission is most likely to occur may inform future conservation strategies and provide a framework for targeted experimental studies on these interactions.

Addressing amphibian disease spread through the exotic pet trade: opportunities and challenges for U.S. state and federal agencies

Warwick, AR[1]*; Drouillard, A[1]; Sherwood, H[1]; Ying, C[1]; Toppen, S1
[1]Michigan State University
*awarwick@msu.edu

Globalization facilitates spread of pathogens via trade, which in turn can negatively affect human health and biodiversity. Managing disease in live animal trade networks is challenging because many factors, including state and federal regulations, influence the decisions businesses make about species traded and biosecurity practices used. In North America, the spread of Bsal is of particular concern for native salamanders. To better understand how U.S. state and federal agencies manage amphibian diseases and interact with pet amphibian trade networks, we conducted virtual focus groups with agency professionals (N = 44). Questions addressed agency actions, concerns, Bsal response, and potential solutions. We found many states conduct wild amphibian disease monitoring and most only have regulations on native species possession, which was a primary concern. Some of the suggested solutions were better communication and coordination among states, rapid response funds, and rapid disease testing.

Gut check: Pond water impacts the tadpole gut microbiome, immune priming, and development

Westcott, RG[1]*; Woodhams, DC[2]; Woodley, SK[1]
[1]Duquesne University
[2]University of Massachusetts
*westcottr@duq.edu

Tadpoles acquire many of their microbiota from the environment in which they develop. In other animals, these host-associated microbiota serve to “train” the immune system, but little is known about the role of the microbiome in immune priming in tadpoles. Here, we analyzed the gut microbiome, immune priming via immune gene expression, and development in *Xenopus laevis* (African clawed frog) tadpoles raised in either autoclaved or natural pond water to establish low-diversity and high-diversity microbiomes, respectively. We used a known quantity of a bacterial spike-in during DNA extraction to allow for integrated bacterial density and absolute abundance analysis after 16S sequencing. We also analyzed key immune gene expression via direct mRNA detection with a custom NanoString code set and correlated expression with gut alpha diversity. We found that tadpoles raised in autoclaved pond water have: 1) lower gut bacterial diversity but similar bacterial density, 2) altered immune gene expression, 3) more severe scoliosis compared to those raised in natural pond water. Establishing this microbiome manipulation model in this species will allow for the further use of molecular tools to better understand the effects of the microbiome on tadpole immunity at the molecular level.

The snake fungal disease pathogen (*Ophidiomyces ophidiicola*) influences the evolution of the skin microbiome

Wilson, IB[1]*; Martinez, TA[1]; Dallas, JW[1]; Ghotbi, M[1]; Murphy, KM[1]; Chevrette, M[2]; Walker, DM[1]
[1]Middle Tennessee State University
[2]University of Wisconsin-Madison
*ibw2g@mtmail.mtsu.edu

Identifying evolutionary and ecological influences on host-microbiome interactions and their role in host health represents an effective means for wildlife conservation. Exploring how pathogen-induced dysbiosis influences a host’s microbiome over time can be challenging given a lack of experimental control. Using a controlled experimental evolution design, we characterized variation in bacterial fitness, morphology, and system relationships for two snake skin microbiome residents (*Stenotrophomonas maltophilia* and *Chryseobacterium sp.*) in response to metabolites of the snake fungal disease pathogen (*Ophidiomyces ophidiicola*). Strains of *S. maltophilia* and *Chryseobacterium sp*. were evolved on either a 10,000 ppm keratin control or a fungal spent keratin media. Using a fully factorial design, we passaged replicate strains independently and in coculture for ~250 generations to allow for genomic changes to accumulate in response to the experimental medium. A differential effect of fungal metabolites on the growth of derived bacterial strains was observed, as well as variable fitness effects in strains grown in coculture on media simulating dysbiotic snake skin. These findings lay the foundation for future targeted experimentation to gain a deeper

understanding of the snake host-microbiome-pathogen tripartite interactions and the impact of pathogen-induced dysbiosis on the evolution of the snake skin microbiome.