GARD

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August 5, Session One (AM)
Climate Change, Biodiversity, and Pathogen Emergence

The roles of climate change and biodiversity in mediating amphibian disease risk

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Humans are modifying the planet in many ways, including changing the climate and biodiversity. Concurrent with these changes are increases in infectious diseases, such as those that are contributing to the declines of amphibians. Understanding the associations among changes in climate, biodiversity, and disease will be crucial for managing these global challenges. In this talk, I will present experimental and field studies linking climate change to amphibian declines associated with the fungus *Batrachochytrium dendrobatidis* (Bd). I will then present experimental and field studies that highlight how declines and changes to host and non-host biodiversity contribute to the transmission and persistence of Bd, *Batrachochytrium salamandrivorans* (Bsal), and other amphibian infections. Some of these studies will describe non-amphibian hosts of Bd and Bsal and their contributions to infection risk. Overall, this work underscores the importance of integrating experimental and field research to understand the interconnections among changes to climate, biodiversity, and infectious disease.

Using metabolic theory and thermal mismatches to model the temperature dependence of ectotherm resistance to an emerging disease

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Temperature has important effects on diseases of ectotherms, leading to unpredictable effects of climate change on emerging diseases. A major challenge is that pathogens and their hosts each have temperature-dependent physiological responses, such that pathogen growth rates in or on hosts depend on the thermal mismatch between pathogen and host thermal performance curves (TPCs). Although this thermal mismatch hypothesis is intuitive, it is hard to parameterize models based on separate pathogen and host TPCs due to inseparability of key parameters when fitting models to infection data. A proposed solution is to describe pathogen and host TPCs using dynamic models based on the metabolic theory of ecology (MTE) and assume that physiological rate processes, such as pathogen infectivity or host resistance, should be fundamentally limited by organism metabolic rates. We hypothesized that pathogen infectivity and host resistance to infection could be described using MTE-based models that are partially parameterized based on independently-measured pathogen and host metabolic proxies. We tested whether this approach could successfully describe how temperature affects frog infection with the emerging pathogen *Batrachochytrium dendrobatidis* (Bd). We conducted experiments quantifying temperature effects on Bd growth on experimentally infected frogs, a proxy for Bd metabolic performance (zoospore swimming speed), and a proxy for metabolic performance of uninfected hosts (respiration rate). This approach succeeded at describing temperature-dependent growth rates and equilibrium dynamics of Bd on frogs and yielded biologically reasonable outcomes and similar model predictions regardless of which organism's metabolic proxy (host or pathogen) our initial parameter estimates were derived from.

Comparing temperature dependence of experimental Bd infection dynamics at individual and population levels

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Amphibian populations are threatened by the temperature dependent disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (Bd). There has been extensive research into how temperature affects Bd infection in individual amphibians, but less is known about these patterns scale up to disease dynamics in whole populations. To address this question, we conducted controlled temperature experiments measuring Bd infection dynamics at both individual and population levels for the model species *Xenopus laevis*. We conducted a controlled temperature lab experiment to measure Bd infection on individually housed frogs at one of four temperatures (10, 15, 20, or 25 °C). We also conducted a large-scale controlled temperature mesocosm experiment tracking population-level Bd transmission dynamics for small populations of frogs maintained at one of three temperatures (10, 15, or 20 °C). In both experiments, frogs quickly developed high levels of infection in the 10 and 15 °C treatments; however, frogs experienced higher overall Bd loads in the population-level mesocosm experiment, especially in the 20 °C treatment. In the individual-level experiment, most frogs cleared the infection when held at 20 or 25 °C, whereas frogs experienced consistently high infections at 20 °C throughout the population-level mesocosm experiment. An important difference between individual- and population-level disease dynamics is that among-frog transmission is an important driver at the population level but not at the individual level. If transmission is higher at warm temperatures, for example due to faster zoospore production or zoospore swimming speeds, this might help account for the difference in our experimental results.

Modeling growth-immunity trade-offs in direct-developing frogs experiencing seasonal chytrid infections

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Activation of the immune response and growth are energy-demanding processes influencing fitness and survival. Understanding potential trade-offs between these processes is important for amphibian species that declined but persist in the presence of the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd). The coqui frog *Eleutherodactylus coqui* is a direct-developing species that recovered from population declines carrying a significant fitness cost as infected adults are typically smaller than non-infected ones. However, we know little about how these trade-offs affect juveniles, which become exposed to infection shortly after hatching and have an underdeveloped immune repertoire. We used dynamic modeling to predict energy allocation during seasons with differences in foraging success and exposure to infection using the state variables time from hatching, size (proxy of fitness), and pathogen burden. To evaluate whether hatching season affects fitness dynamics, we compared growth rates, time to maturity, and body size at the end of the growing season between different seasonality scenarios. We hypothesized that lower growth rates would indicate energy reallocation to immunity, whereas higher growth rates would allow rapid reproductive maturation if pathogen burden is tolerable. We found that the cost of the infection became exacerbated during the dry season because the additional environmental stress resulted in less growth and higher *Bd* burden. We discuss the mechanistic basis of our model in the context of metabolic processes affected by *Bd*. Our theoretical model can be extended to simulate disease-mediated trade-offs under future climate change scenarios and on different species.

Once a reservoir, always a reservoir? Seasonality affects the pathogen maintenance potential of amphibian hosts

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Host species that can independently maintain a pathogen in a host community and contribute to infection in other species are important targets for disease management. However, the potential of host species to maintain a pathogen varies within- and across-seasons. Here, we sought to understand the causes and consequences of seasonal infection dynamics in leopard frogs (*Rana sphenocephala* and *R. pipiens*) infected with the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). We addressed two questions. First, to what degree are observed seasonal patterns in infection driven by temperature-dependent infection processes compared to seasonal host demographic processes? Second, how does seasonal variation in maintenance potential affect long-term pathogen persistence in multihost communities? To answer these questions, we used field data collected over three years on >1400 amphibians across four geographic locations, laboratory and mesocosm experiments, and a novel mathematical model. We found that the mechanisms that drive seasonal prevalence were different than those driving seasonal infection intensity. Seasonal variation in *Bd* prevalence was driven primarily by changes in host contact rates associated with breeding migrations to and from aquatic habitat. In contrast, seasonal changes in infection intensity were driven by temperature-induced changes in *Bd* growth rate. Using our model, we found that the maintenance potential of leopard frogs varied significantly throughout the year and that seasonal troughs in infection prevalence made it unlikely that leopard frogs were responsible for long-term *Bd* persistence in these seasonal amphibian communities.

Experimental evolution of *Batrachochytrium dendrobatidis*, a lethal pathogen of amphibians, in climate change conditions

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Climate change is expected to negatively impact amphibian biodiversity globally. One ecological factor that is likely to be altered by climate change is infectious disease because both hosts and pathogens are influenced by environmental conditions. For example, some studies predict that increases in temperatures may alter pathogen replication rates, emergence, transmission, and spread such that disease is exacerbated with potentially negative consequences for host biodiversity. Yet, the effect of climate change on pathogens and their hosts is currently understudied. Amphibians face global population declines from the pathogen *Batrachochytrium dendrobatids* (Bd), which causes the disease chytridiomycosis. Because *Bd* is highly temperature-sensitive, one important question is whether climate change will alter *Bd* pathogenicity via adaptations to increased daily thermal conditions. The aim of our study was to investigate potential alterations in physiology of *Bd* lineages that are experimentally evolved under fluctuating thermal treatments that mimic climate change conditions. We hypothesized that when *Bd* is experimentally cultured in temperature conditions that simulate predicted climate change conditions, there will be increased growth and reproduction in the evolved lineages that experience higher ("future") temperatures relative to lower ("current") fluctuating thermal conditions. To test this question, we experimentally evolved the pathogen by culturing *Bd* at two simulated thermal climate treatments and one control thermal treatment for ~50 generations. Once the pathogen was experimentally evolved, we quantified physiological traits related to growth and reproduction to test changes in pathogen physiology and adaptation. After experimental evolution to modeled climate change conditions, the future evolved lineages had higher zoospore densities, but not higher population viability, than those of current evolved lineages. Our findings suggest that the higher temperature conditions (e.g., those that are predicted with climate change models) may alter *Bd* growth and reproductive traits with important implications for chytridiomycosis dynamics. This study provides insight into the effects of climate change conditions for *Bd* physiology and may help to aid in conservation of amphibians by predicting patterns of increased growth and reproduction in future evolved *Bd* lineages.

Seasonal changes in the mucosal defenses of leopard frogs (*Rana [Lithobates] sp.*)

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As wildlife fungal diseases continue to emerge, research increasingly focuses on host-microbiome interactions and links to disease. Certain skin-associated microbes may benefit hosts by protecting them from invading pathogens. Seasonal changes in the host environment can also result in shifts in the microbial community and pathogen virulence—potentially influencing disease dynamics. We investigated how cutaneous microbial communities differ across hosts, seasons, and infection status. These skin microbes reside in a protective mucus layer and may predict and mediate disease risk. I have sequenced the cutaneous bacterial communities of leopard frogs (four *Rana [Lithobates] sp.*) at five locations across the United States to explore seasonal microbial dynamics, including responses to naturally fluctuating *Batrachochytrium dendrobatidis (Bd)* infections. There were no differences in alpha diversity across time nor *Bd* infection status. Percent anti-*Bd* function varied across seasons and with infection status. Bacterial communities also varied across locations and time. We will further explore how individual microbes, that could be considered anti-*Bd*, seasonally shift in relative abundance to see how they correlate with *Bd* infection loads to better understand the defensive role of the amphibian skin microbiome. Better understanding of the complex interactions between host, microbes, and the environment can lead to elucidating disease transmission potential and more effective measures to combat wildlife pathogens.

Amphibian disease ecology: Are we just scratching the surface?

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Pathogen-induced population declines and extinctions are among the main threats to amphibian species worldwide. However, the ecological drivers underlying epidemiological patterns are still poorly understood. In an attempt to address this gap in knowledge, we identified 832 publications on the ecology of amphibian pathogens and diseases published between 2009 and 2019. The majority of publications investigated either chytrid and/or ranavirus infections (79% of the articles), while other pathogens such as bacteria and helminths received considerably less attention. Just over half of the studies included field research and 40% were experimental in nature; yet only 8% combined field and experimental approaches. More than half of the literature (56%) investigated post-metamorphic stages as compared to 23% for pre-metamorphic stages, and only 13% included both life stages. Susceptibility and mortality have been assessed in almost every study (91%) while 37% of them tested for cellular, physiological, and/or immunological responses. However, other host characteristics such as growth/development, behavior, and specific mucosome/microbiome were considered in only one out of four studies. Most research included at least one biotic factor (e.g., host- and pathogen identity, species diversity, genetic adaptations) but only one third considered environmental factors (e.g., temperature, landscape features, inorganic chemicals). Furthermore, there is no general consensus about the factors driving epidemiological patterns of pathogens in amphibian communities, making conservation implications difficult and management decisions challenging. To this end, our review identifies some research gaps and proposes future directions to better understand one of the major threats to this class of vertebrates.

Role of a disease-tolerant species in amplifying transmission of chytridiomycosis in tropical montane frog communities

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Many infectious diseases fade away once the density of susceptible hosts becomes too low to sustain new outbreaks. The amphibian fungal disease chytridiomycosis is remarkable due to its lack of host specificity for infection, but high virulence for some taxa and groups with aquatic reproductive modes. We have worked in montane forests hosting diverse frog communities in the Andes to Amazon region, where chytridiomycosis is associated with the loss of 35% of species at mid to high elevations. Why has chytridiomycosis not disappeared following the extirpation of most of the aquatic breeding species? We studied gladiator treefrogs, *Boana gladiator*, which are among the few aquatic-breeding species still common in the montane creeks. Using a combination of genomic, analytical, and experimental approaches, we examined how gladiator frogs tolerate chytrid infection, and we hypothesized that disease tolerance may help gladiator frogs amplify transmission to sympatric, susceptible frog species. We found that gladiator frogs might prevent the onset of symptomatic chytridiomycosis and repair skin disturbance with the help of symbiotic skin bacteria and a newly characterized skin peptide. Highly infected gladiator frogs and tadpoles shed zoospores at higher rates than sympatric species, and share the same strain of the global panzootic lineage of chytridiomycosis. Because the range distribution of gladiator treefrogs overlaps with the distribution of many threatened and highly endemic frogs, these tolerant hosts could contribute to the persistence of chytridiomycosis and continuance of detrimental effects on anuran biodiversity.

The role of monitoring and research in the Greater Yellowstone Ecosystem in framing our understanding of the effects of disease on amphibians

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Amphibians in the protected landscape of the Greater Yellowstone Ecosystem (GYE), one of the largest and most complete temperate-zone ecosystems on earth, are threatened by emerging infectious disease. Data from >20 years of monitoring, surveys, and population studies, provide insight into disease impacts, and the significance of disease in long-term persistence of amphibians. Although the amphibian chytrid fungus (Bd) has not been linked to die-offs in this landscape, there is evidence for reduced survival. Localized mortality events, consistent with disease from ranaviruses, are widespread in the GYE. We present the current state of knowledge about ranaviruses and Bd in this landscape. The significance of disease and persistence of amphibians in the GYE is linked to anticipated changes in climate, especially drought. Expected increases in visitor use and its associated impacts also have the potential to exacerbate the effects of disease. Long-term information from this large, intact landscape helps to frame our understanding of the effects of disease on amphibians and provides data that can contribute to management decisions, mitigation strategies, and forecasting efforts.

Surveys for chytridiomycosis in South Korean salamanders

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Amphibian chytridiomycosis, caused by infection of the chytrid fungi *Batrachochytrium dendrobatidis* (Bd) or *Batrachochytrium salamandrivorans* (Bsal), is a fungal disease spread among salamanders. We conducted a comprehensive field survey of South Korean salamanders, including Asian salamanders (family Hynobiidae) and endemic lungless salamanders (family Plethodontidae). Over three years, we swabbed and tested by nested or quantitative PCR (polymerase chain reaction) 218 *Hynobius leechii*, 156 *Hynobius yangi*, 15 *Hynobius quelpartensis*, 11 *Hynobius unisacculus*, 37 *Hynobius notialis*, 14 *Onychodactylus koreanus* (Hynobiiidae) and 154 *Karsenia koreana* (Plethodontidae). Study sites were widely distributed around South Korea. Mean Bd prevalence was 36.4 (15.1 – 64.6)% in *H. unisacculus,* 35.0 (22.1 – 50.5)% in *H. yangi*, 19.9 (13.2 – 28.6)% in *H. leechii*, and 14.5 (7.8-25.3)% in *K. koreana*. Infection loads generally were low (< 100 ZGE, zoospore genomic equivalents). We found no Bd-infected *H. notialus* or *H. qualpartensis*. Despite Bsal's reported presence elsewhere in Asia, we found no Korean salamanders infected by Bsal. Although our preliminary laboratory infection studies failed to find evidence that either *H. leechii* or *K. koreana* are susceptible to Bsal infection, should Bsal be introduced into Korea, endemic species might be particularly at risk.

Multi-scale occupancy of an endangered amphibian integrating disease dynamics and environmental DNA

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The chytrid fungus Batrachochytrium dendrobatidis (Bd) has swept through amphibian communities across the globe causing unparalleled declines. The infection risk posed by multi-host pathogens is tightly related to pathogen maintenance in the system by the host (and/or reservoir) community and the capability of the pathogen to live freely in the environment. In this study, we combined environmental DNA sampling and traditional visual surveys with amphibian capture and swabbing to investigate the effect of spatiotemporal variation in host assemblage and environmental conditions on resultant infection patterns. Amphibian species were assigned an aquatic index according to use of waterbodies across life stages as a measure of exposure to pathogen and host competency. We used the endangered Fleay's barred frog (*Mixophyes fleayi*) as a focal species to compare infection prevalence and intensity across disjunct populations. Multiple linear regression analysis was used to determine the relationship between air and water temperature, salinity, elevation, radiation, pH, rainfall, and forest quality with infection levels. A total of 509 adults of *Mixophyes fleayi* were sampled throughout the study period and 467 frogs of 10 other species. Individuals presenting positive results for Bd were smaller, lighter and having a negative body index. Both infection prevalence on frogs and Bd eDNA detection were related with a partial set of environmental variables (air temperature and humidity). Bd eDNA detection increased when radiation was higher, and stayed low once rainfall and temperatures increased. The highest percentage of Bd in water came from high elevation sites. Infection exposure risk to post-metamorphs frogs in lotic systems will vary according to drought, rainfall, and temperature regimes. Higher concentrations of Bd in streams in dry season when pools contract can be an important source of pathogen environmental transmission. Understanding the factors behind how species face a lethal disease across its distribution highlights the importance of local conservation strategies for disjunct populations of endangered amphibians.

The pathogenic chytrid fungus in the threatened admirable redbelly toad, *Melanophryniscus admirabilis*

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Bufonidae stands out among anuran families as one that includes numerous species that declined due to *Batrachochytrium dendrobatidis* (Bd) outbreaks in Central and South America. Thus, it is essential to improve our knowledge about *Bd* infections in toads of the family, mainly in threatened species. The genus *Melanophryniscus* (Bufonidae) is considered an important group for conservation efforts as several species are threatened. Hence, we quantified for the first time, the seasonal variation of *Bd* infections in *Melanophryniscus admirabilis*, a Critically Endangered and microendemic toadlet from southern region of the Atlantic Forest. We sampled individual toads between 2019 and 2021. The B*d* prevalence of the total sample was 11% (24/217), and the mean infection intensity was 115 zoospores genomic equivalents (g.e.). In 2019 spring, *Bd* prevalence was 14% (8/56), and mean of infection intensity of 32 g.e.. In 2020 winter, *Bd* prevalence was 9% (4/44) and mean infection intensity was 4.5 g.e.. In the spring of 2020, *Bd* prevalence was 1% (1/71), and in the winter of 2021, the prevalence was 24% (11/46) with mean infection intensity of 225 g.e.. Despite the general low pathogen prevalence and infection, studies that increase our knowledge about the effect of *Bd* on demographic and physiological parameters of *Melanophryniscus* spp. are important for developing effective conservation strategies. Also, the possible sublethal effects of *Bd* infections at the individual level can have important implications for the reproductive populations, also declines due to chytridiomycosis can still occur in populations historically infected with *Bd*.

Demographic compensation unlikely in montane amphibian populations challenged by *Batrachochytrium dendrobatidis*

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Emerging infectious diseases are often important drivers of host population dynamics as documented by recent outbreaks in human, domestic animal, and wildlife systems. While many diseases cause catastrophic harm to host populations, there often exists population-level variation in response to disease whereby some host populations are extirpated, some persist at lower densities or abundances, and others rebound to pre-disease levels. One such demographic mechanism of population persistence that has gained support in the amphibian-*Batrachochytrium dendrobatidis* (Bd) host-pathogen system is compensatory recruitment. Some populations may persist by increasing recruitment to compensate for reduced survival due to infection, thus limiting the negative effects of the disease on population trajectories. Montane amphibian populations may be limited in their ability to exhibit compensatory recruitment due to variation in life histories and inherently increased vulnerability to stochastic processes. We use 20 years of mark-recapture data on five populations of boreal toads (*Anaxyrus boreas boreas*) in Colorado before and after pathogen arrival to assess whether populations can persist with Bd via compensatory recruitment. Prior to Bd arrival, we found a life history trade-off between survival and recruitment across elevations, where high elevation toads have high survival but lower recruitment and vice versa at lower elevations. Bd arrival had a strong negative effect on apparent annual survival and recruitment, and led to negative population growth rates and host abundances. We did not find support for compensatory recruitment in our system, highlighting the strength of Bd in these populations and that demographic responses to pathogens may be context-dependent.

Transport of a lethal amphibian pathogen (*Batrachochytrium dendrobatidis*) through fog

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Amphibians are the most threatened animal group today. Among the main causes of decline and extinction are epidemics of infectious diseases, such as chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), which is associated with water bodies. The process by which species that have little or no contact with water are exposed to Bd remains unclear. Fog is an important water input between ecosystems and a carrier of microorganisms, including pathogens. Understanding how the contamination of these Bd-sensitive species occurs and identifying the environmental conditions that lead to outbreaks is important for the development of control measures and to prevent epidemics. We collected fog and rainwater from nine cloud forests in the Atlantic Forest, Brazil, and subjected them to qPCR analysis to investigate the presence of Bd in natural fog. We also conducted experiments on the infection of a species of direct development by Bd, through exposure to artificial and natural fog. Here we report the first evidence of Bd DNA in the fog. We also found that susceptible hosts can become infected and develop lethal chytridiomycosis through the passive transport of Bd zoospores through the fog. Our results extend the current knowledge about Bd transport pathways between environmental reservoirs. A new long-range dispersion pathway through fog may reflect patterns of pathogen occurrence and opens new avenues of investigation to elucidate mechanisms of exposure of amphibian species to the pathogen. Thus, we raise new questions and suggest that future epidemiological studies of Bd include passive transport airways.

August 5, Session Two (PM)
Emerging Reptile Diseases

Comparative ecology and evolution of reptile pathogens

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Microbes are essential for normal vertebrate functions, including digestion, nutrition, and defense. Koch's postulates are taught as criteria for establishing a microbe as a pathogen. Although they have their use, Koch's postulates frequently result in a false understanding of microbes as either pathogenic or nonpathogenic. There is no such thing as a microbe that is always either a pathogen or a nonpathogen. All life on earth has been selected for billions of years to reproduce successfully, and this is all that matters from an evolutionary standpoint.

Multiple factors influence evolutionary rates, including selective pressures, generation times, and fidelity of copying genes. Infectious disease is the largest selective factor on vertebrate evolution, and hosts are the largest selective factors on pathogen evolution. Microbes often have very short generation times. This is useful for rapid adaptation to novel selective pressures, such as immune selection and antimicrobial use. As a result, rates of evolution in microbes tend to be rapid, and evolution occurs in a clinically relevant time scale.

Reptiles are rich and complex ecosystems of eukaryotes, prokaryotes, and viruses, and the wider temperature ranges at which they live adds a dimension to reptile medicine not present in mammals. This talk will provide an overview of reptile microbial disease ecology with comparison of pathogen clades and specific examples of pathogen ecology and evolution.

Genomic and *in vitro* characterization of ophidian serpentoviruses

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Ophidian serpentoviruses, positive-sense RNA viruses previously referred to as nidoviruses, are increasingly being identified as important infectious agents in both captive and free-ranging snake populations. While the clinical significance of serpentovirus infections in free-ranging snakes remains unclear, these viruses can cause severe and sometimes fatal disease in captive snakes associated with distinct pathologic changes. While much has been learned about these emerging reptile pathogens to date, significant gaps in our knowledge of these viruses remain. Using a combination of positive clinical samples and isolated viruses, genomic and *in vitro* characterization of select viruses was performed. Using Illumina MiSeq next generation sequencing technology, large portions of the viral genome were generated for 21 unique viruses. Phylogenetic analysis of these genomes alongside other previously published sequences identified multiple clades of ophidian serpentoviruses. For *in vitro* characterization of isolated viruses, a median tissue culture infectious dose (TCID50) assay was established and used to characterize viral environmental stability, disinfectant susceptibility, and growth kinetics. The *in vitro* host range of select viruses was also investigated by immunostaining on a broad and diverse panel of reptile tissue culture cell lines. The results generated not only increases our understanding of these emerging reptilian viruses, but also provide the basis for understanding the potential host range of ophidian serpentoviruses and mitigating the effects of viral outbreaks in captivity.

Detection of *Paranannizziopsis* spp. in wild snakes

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Reptile-infecting species of the fungal order Onygenales (many of which were formerly classified as “CANV” fungi) are known for causing severe skin infections in snakes, lizards, turtles, crocodilians, and tuataras. With the exception of three species (*Ophidiomyces ophidiicola* in snakes, *Nannizziopsis barbatae* in lizards, and *Emydomyces testavorans* in turtles), most infections caused by species of reptile-infecting onygenalean fungi have only been documented in captive reptiles. Thus, the origins of many of these fungi are unknown, which makes it difficult to develop management actions to prevent pathogen translocation and release into naïve populations. One such group of these fungi, *Paranannizziopsis*, consists of five species that have been documented to cause disease in captive snakes, lizards, and tuataras on two continents. We report the first cases of *Paranannizziopsis* infection in wild snakes in North America. Affected snakes exhibited clinical and histopathologic lesions consistent with those described in infected captive reptiles, and *Paranannizziopsis* was isolated in culture from all cases. These isolates represented *P. australasiensis* and two potentially novel species. Despite these detections, it is unclear whether *Paranannizziopsis* is native to North America or represents an introduced pathogen. Additional follow up sampling is needed to determine the distribution of *Paranannizziopsis* and whether the fungus poses a threat to wild reptile populations.

Infection experiments indicate some Florida anurans, lizards, and cockroaches can serve as intermediate hosts for the invasive pentastome parasite, *Raillietiella orientalis*

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The pentastome parasite, *Raillietiella orientalis*, from southeast Asia has successfully invaded peninsular Florida and utilizes some anurans, lizards, and snakes in Florida as hosts. This pentastome infects a wide range of species yet its complex life cycle remains poorly understood. At least 14 native Florida snakes serve as definitive hosts, as do at least three nonindigenous reptiles. We conducted laboratory infections to develop an understanding of potential intermediate hosts and to determine fitness consequences of visceral pentastomiasis caused by *R. orientalis*. Anoles and cockroaches, but not anurans, were readily infected with *R. orientalis* larvae through egg-exposure. Anurans and anoles were infected following consumption of *R. orientalis* larvae in roaches indicating a life cycle that involves a sequence of three hosts. Comparison with non-exposed control animals revealed no significant effects on survival or growth in these hosts. The lack of gross impacts on intermediate hosts is possibly a result of the small size of the larvae, surfactant-like materials secreted to evade detection or the fact that the larvae are encysted. Definitive hosts likely experience lethal and sublethal costs of infection given the large size and hematophagy of *R. orientalis* adults. Initial infection experiments and the rapid geographic expansion of *R. orientalis* populations suggest that suitable intermediate hosts are abundant in peninsular Florida. The diversity of species that can act as intermediate hosts, including synanthropic species like *Anolis sagrei*, may result in continued rapid range expansion of *R. orientalis* and the native species at risk of infection.

Are hatchlings emerging dehydrated? Preliminary packed cell volume and total solids data in leatherback (*Dermochelys coriacea*) sea turtle hatchlings and post hatchlings and their relation to incubation temperature

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All leatherback sea turtle (*Dermochelys coriacea*) populations are considered endangered under the Endangered Species Act, but isolated populations, such as the one in the Pacific Ocean, are critically imperiled. The cause of leatherback conservation status is multifactorial and ultimately resulting in negative impacts of health and survival. Climate change is one such factor as it results in elevated ocean and beach temperatures. Thus, there is an urgent need to characterize the impact of these elevated temperatures and identify key areas to mitigate. One essential management strategy to rescue the population may include maintaining this population of leatherbacks in a temporary human managed setting. For such an intervention to be successful baseline parameters for measuring hatchling and post hatchling health are needed. This study establishes preliminary baseline ranges for total solids (TS) and packed cell volume (PCV) in leatherback hatchlings and post hatchlings. Additionally, we assessed the effects of incubation temperature on PCV and TS at emergence and found a significant increase in both values at emergence from 'hot' nests. These data provide baseline values to assess hatchling health and begin to elucidate the cause(s) of decreased survival in hatchlings incubated at elevated temperatures.

Dysbiosis of the snake microbiome due to snake fungal disease results in loss of microbial diversity in both laboratory experiments and field studies

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To understand the effect of disease progression on the microbiome, we collected 22 Common Watersnakes (*Nerodia sipedon*) and inoculated a cohort with *Ophidiomyces ophidiicola*. Weekly skin swabs were taken of each snake to characterise the microbiome (16S amplicon sequencing) and pathogen load (qPCR). For the inoculated treatment we found a significant effect of disease progression (time) on microbial richness (GAMM, edf = 2.274, F = 3.864, p = 0.013) and Shannon diversity (GAMM, edf = 2.559, X2 = 20.747, p < .001) where values initially rose and then continually declined ~30 days after inoculation. When explicitly accounting for differences in assemblage richness, we found that β-diversity among snakes was affected by the interaction of time and treatment group, with assemblages becoming more dissimilar across time in the inoculated (GLS, int. = 0.293, coef. = 0.002, p = 0.022), but not the control group (GLS, int. = 0.283, coef. = -0.001, p = 0.457). These results suggest that disease progression has a destabilizing effect on the skin microbiome, consistent with conceptual models of pathogen-induced dysbiosis. Analysis of 791 microbiome samples collected from 31 snake species across 5 years in Tennessee has revealed similar trends to the live animal experiment. Increasing pathogen load correlated with a decrease in microbial richness (GAMM, edf = 1.920, X2 = 7.990, p = 0.032) and Shannon diversity (GAMM, edf = 1.935, X2 = 8.001, p = 0.027). Further research is needed to determine the identity and function of microbes which are excluded via pathogen induced dysbiosis.

The invasive pentastome parasite, *Raillietiella orientalis*, pervades the herpetofauna of central Florida habitats

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Introduced parasites with complex life histories have the potential to dramatically alter ecosystem dynamics, and the rapid spread of *R. orientalis* may be an emerging example of this phenomena. Experimental infection studies suggest several species of Florida anurans and lizards can serve as hosts for *R. orientalis*; however, captive infection may not accurately predict which species serve as hosts in the wild. To determine these natural intermediate and paratenic hosts, we made field collections of lizards and frogs at Lake Woodruff National Wildlife Refuge and several residential areas in central Florida. Our dissections indicated seven species of lizards and anurans harbored larval pentastomes. A significantly greater proportion of southern toads and Cuban treefrogs than brown anoles were infected by *R. orientalis*. The intensity of infection varied greatly, ranging from 1-78 larvae in toads and from 1-14 larvae in brown anoles. In field collections of snakes, we found that eight different species were infected with *R. orientalis* including three novel definitive hosts (*Lampropeltis elapsoides*, *Thamnophis sauritus* and *Micrurus fulvius*). Infected snakes were found in all five habitat types sampled. Over 50% of the sampled pygmy rattlesnakes and black racers were pentastome-infected. This invasive parasite is now widespread in central Florida and infects a large number of native species in both natural and urbanized habitats. We predict a rapid expansion in the geographic range of *R. orientalis* given the species it utilizes as intermediate and paratenic hosts, making it a major conservation issue for many snake species in the southeastern United States.

Snake parasite communities vary by region and habitat type in North America: A systematic literature review

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Parasite biodiversity generally follows patterns of host biodiversity, whereas parasite community composition is more difficult to predict. For example, are parasites with free-living stages less species rich in arid environments? If we can use habitat information to predict which types of parasites may exist in a given host species, we may gain important insights into the ecologies of cryptic or difficult to study host species. To that end, we conducted a systematic literature review of endoparasites that infect snakes using existing bibliographies for North America and additional database searches. We recorded information regarding snake hosts (e.g., species, size), parasites (e.g., species, prevalence of infection), and habitats (e.g., region, aquatic vs terrestrial habitat type). We found that parasite species richness follows patterns of snake species richness, as expected. For example, the Southeastern U.S. is home to more snake species than the Northeastern U.S., and correspondingly, the Southeast has more parasite species recorded from snake hosts. Aquatic snake species had proportionally more trematode parasites than terrestrial snake species, even though there were fewer aquatic snake species in our database. Terrestrial snakes had more nematode, acanthocephalan, and cestode parasites than snakes occupying other habitats. These results suggest that trematodes may be better bioindicators of aquatic snake species and that differences in snake diets among habitat types may influence snakes' interactions with natural enemies.

Identifying possible parasite bioindicators and patterns of trophically transmitted parasitism in four aquatic snake taxa

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Snakes are notoriously cryptic and difficult to study. Thus, snake conservation may be advanced by developing bioindicators of snake presence and health in ecosystems. Potential bioindicators include parasites with complex life cycles that use specific snake species as definitive hosts and more common or accessible host species as intermediate hosts (e.g., snails). To identify potential parasite bioindicators for aquatic snake species, we used existing bibliographies and a systematic literature review to collect snake, endoparasite, and location data for all snake species in the United States and Canada. We then compared parasite communities in four aquatic snake genera (*Agkistrodon*, *Farancia*, *Nerodia*, and *Regina*). Five out of the 18 species from these genera in the United States and Canada had no described helminth or pentastome species and thus require further study. Most described parasite species were trophically transmitted trematodes. Most of the trematode species were unique to each snake genus (42% for *Agkistrodon*, 100% for *Farancia*, 51% for *Nerodia*, and 83% for *Regina*). Furthermore, most (9/13) of the aquatic snake species had at least one specialist trematode species that is not known to infect any other snake species in the U.S. or Canada. These results suggest that several described trematode species may be worth pursuing as potential bioindicators of snake presence in aquatic ecosystems, but continued work on elucidating these parasites life cycles is necessary. Furthermore, for particularly rare snake species that have no known parasite species, like *Farancia erytrogramma*, future parasitological analyses may yield valuable conservation information.

Uncoiling the complex hiss-tory and impacts of snake fungal disease in North America

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Snake fungal disease (SFD) is often considered an emerging threat to snake populations in eastern North America. The disease is caused by the pathogen *Ophidiomyces ophidiicola*, which was formerly classified along with several other reptile-infecting fungi as the *Chrysosporium anamorph* of *Nannizziopsis vreisii* ("CANV"). SFD first gained attention in North America around 2010, but little was known about the history of *O. ophidiicola* in North America and its impacts on snakes. Recent examination of preserved specimens in museum collections indicates that SFD has been present in the United States since at least the 1940s, demonstrating that the disease had been overlooked for decades. Genetic analyses conducted using strains of *O. ophidiicola* collected throughout the eastern United States subsequently revealed that the pathogen population consists of three main clonal lineages and hybrids between those clonal lineages. Each clonal lineage shares a common ancestor within the last several decades, suggesting that *O. ophidiicola* was likely introduced to North America relatively recently. Some clonal lineages were estimated to have been introduced in the 1990s or early 2000s, consistent with the reported timeline for the emergence of severe cases of SFD. Due to a lack of historical population and disease data for snakes and the long-term monitoring needed to document effects from chronic disease processes such as SFD, the impacts of the disease are difficult to assess. However, the recent introduction of potentially virulent strains of *O. ophidiicola* into native snake populations is cause for concern and efforts are underway to better understand the risk posed by SFD.

Large-scale prevalence and host association with *Ophidiomyces ophidiicola* in Europe

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Emerging infectious diseases can have devastating consequences to species conservation on a global scale, leading to population declines, reduced host ranges, and population extirpations. Ophidiomycosis, an infectious disease in wild and captive snakes caused by the fungal pathogen *Ophidiomyces ophidiicola*, is considered a serious threat to snake biodiversity, and has been associated with declining snake populations in the USA. Although disease prevalence studies are relatively common in the USA, no such studies have been conducted in Europe, where widespread declines in snake populations have been documented. This study aimed to fill this gap by investigating pathogen occurrence in free-ranging snake populations, to better understand the prevalence, severity of infection, and variation in host susceptibility to *Ophidiomyces ophiidicola* throughout Europe. A total of 825 snakes were collected in 2020 and 2021 from 22 species across 10 countries. Snakes were swabbed in duplicate and when lesions were present, an additional lesion swab was collected. Presence of lesions was recorded, and each lesion was measured and photographed for further disease severity quantification. A total of 1786 swabs were analyzed by qPCR, revealing an overall 6.4% disease prevalence (n = 53 snakes). Lesion prevalence was recorded in 12.4% of snakes overall, and 64% of qPCR positive snakes had lesions. Ophidiomycosis was not detected in the Iberian Peninsula (Spain and Portugal) and was highest in Switzerland with 32% disease prevalence. Of the positive samples detected by qPCR, 77% were from the *Natrix* genus, possibly indicating higher susceptibility due to life history traits and habitat preference.

Investigating the character of skin lesions caused by ophidiomycosis in the barred grass snake (*Natrix helvetica*), in eastern England

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The emerging infectious disease, ophidiomycosis, caused by the fungus *Ophidiomyces ophidiicola*, was recently identified in wild European snakes. Since its discovery, little research has been conducted in the affected species. Over 40 species of snakes inhabit Europe, with the implications of ophidiomycosis on their populations unknown. Opportunistic sampling of a population of barred grass snakes (*Natrix helvetica*) in eastern England in 2016 diagnosed skin lesions caused by ophidiomycosis, using a combination of real-time PCR, histology, and mycology. Over the period 2019-2021, this population was studied intensively in order to better understand the character and aetiology of skin lesions, and the demographic groups affected. Structured surveys using artificial cover objects were conducted over the period May to October each year. When captured, biometric data were collected from each snake, and if present, skin lesions were photographed, described and swabbed in duplicate. Every fifth snake captured without detected skin lesions was also swabbed, for comparative purposes. A customized scoring system was used to characterize and categorize the severity of skin lesions in each snake. Skin lesions were most commonly described as discolouration, crusting and scale margin erosion. 76.4% (207/271) of snakes with detected skin lesions were adults. Swabs were tested for the presence of *O. ophidiicola* using a rtPCR protocol. 79.3% (215/271) of snakes with skin lesions were PCR positive, compared to 2.4% (4/165) of snakes without skin lesions. Most skin lesions were scored as either mild (43.9%; 119/271) or moderate (37.2%; 101/271) severity and were consistent with ophidiomycosis.

Innate immune function in Lake Erie water snakes (*Nerodia sipedon insularum*) with ophidiomycosis

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Ophidiomycosis, caused by the fungus *Ophidiomyces ophidiicola*, poses a threat to the health of wild and managed snakes worldwide. Variation in snake innate immunity, the primary defense against infection in reptiles, may explain the observed variation in ophidiomycosis clinical disease severity among snakes. In this study, two components of the innate immune response were examined using snake plasma. We investigated whether complement activity, as measured by sheep red blood cell hemolysis, and chitotriosidase activity were associated with ophidiomycosis disease severity and time in captivity in Lake Erie watersnakes (*Nerodia sipedon insularum*). There was no difference in complement-mediated hemolysis or chitotriosidase activities between snakes with varying levels of ophidiomycosis clinical severity sampled in the field. However, among snakes with skin lesions kept in captivity, chitotriosidase activity was significantly higher in snakes with mild disease, compared to snakes with severe disease, and hemolysis activity increased with time in captivity. Overall, Lake Erie watersnakes had higher complement activity, but lower chitotriosidase activity, compared to other reptile species, and this is the first description of chitotriosidase activity in a snake species. These results provide mixed evidence of associations between innate immune function and ophidiomycosis severity, and more work is needed to investigate differences among snake species.

Morphological and molecular characterization of the fungal pathogen *Ophidiomyces ophiodiicola* in soil samples of cave habitats in Puerto Rico

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The fungal pathogen *Ophidiomyces ophiodiicola*, causative agent of Ophidiomycosis. It is considered an emergent disease and recent studies are investing efforts in disease surveillance and spatial distribution of it in the Caribbean. Reports have been published for North America, Europe, Australia, and Asia either from captive and/or wild snakes. Research has focused on the morphological and molecular characterization in temperate climates and not in tropical ecosystems such as PR. Our study aims to isolate and morphologically and molecularly characterize the pathogen from environmentally derived soils and surfaces of cave habitats commonly known for the presence of the endangered Puerto Rican Boa, Chilabothrus inornatus. Field surveys were done in two caves located at the Northern Karst of Puerto Rico. Soil and surface samples were collected at snake and random locations. Water samples were collected from small ponds, when found.
For initial isolation of fungi, all samples were inoculated in Dermatophyte Test Medium supplemented with gentamycin and chloramphenicol. All suspected dermatophytes were isolated in Sabouraud Dextrose Agar for morphological characterization. DNA extractions were performed, and PCR reactions were done for the nuclear ribosomal fungal barcode regions (ITS and 28S) and for the transcription elongation factor *TEF1*-α. Successful amplifications were sent for DNA sequencing. Suspect isolates were characterized by white to off-white colonies with no pigmentation on the colony reverse. Microscopically, candidates were characterized for its hyaline arthroconidia, blastoconidia and, in some isolates, aleuriconidia which are consistent features of *O. ophiodiicola*. These findings comprise the first isolation of the pathogen from Puerto Rico.

Study for the Spatial Analysis of *Ophidiomyces ophiodiicola* in snake species through surveillance and detection in Puerto Rico and US Virgin Islands

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Ophidiomycosis is an emergent disease in snakes caused by the fungal pathogen *Ophidiomyces ophiodiicola* (Oo). In the past decade it has increased in frequency globally. Recently, the pathogen was detected in a federally-listed species, *Chilabothrus inornatus*, in a military installation in Puerto Rico. This report, the first in the Caribbean, raises concern of whether the pathogen is present in other wild populations throughout Puerto Rico (PR) and US. Virgin Islands (USVI). Our study's main objective is to survey wild populations of snakes and museum specimens to detect the presence and spatial distribution of *Oo*. Field surveys were done in PR and USVI to capture snakes in different habitats with particular focus on two endangered species, *C. inornatus* and *C. granti*, and one exotic, *Boa constrictor*. Snakes were physically examined for clinical symptoms, samples were obtained by swabbing the skin and later analyzed with quantitative PCR (qPCR). Tissue samples were obtained from *C. inornatus* museum specimens for histological analysis. A total of 127 snake individuals representing six species were sampled throughout a variety of habitats in 15 municipalities in PR and USVI. Histology results of one snake might indicate the presence of the fungus as early as 1970's; however, results need to be confirmed with Immunohistopathology studies. qPCR analysis of 69 snakes showed 42% prevalence; a majority of the positive cases were found in cave habitats. The high prevalence of the pathogen in caves warrants further studies on fungal source and the environmental conditions in caves that trigger its higher incidence.

Ophidiomyces ophidiicola in wild snakes in Germany

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Ophidiomyces ophidiicola (*Oo*) is a well described pathogen in captive and wild snakes in North America. Detections in Europe have been more sporadic and were originally limited to cases in snakes in captivity. In 2017, *Oo* was reported in wild snakes in the United Kingdom and the Czech Republic. This was followed by a report in a grass snake (*Natrix natrix*) in Switzerland. Additional anecdotal reports have described lesions similar to those seen in *Oo* infected snakes in additional areas in Europe, but data on distribution and impact of this pathogen on wild European snakes is lacking. In 2018, skin lesions were observed in dice snakes (*Natrix tessellata*) along the Lahn river in Germany during routing population monitoring. In 2019, two snakes with similar lesions were captured for additional examination and testing. *Oo* was detected in shed skin from one of the snakes. Following this finding, additional testing was carried out on dice snakes in several populations in Germany as well as opportunistically in additional species in other parts of Germany in 2021. A total of 107 samples were examined and *Oo* was detected in samples from another 4 dice snakes, all from the same nature reserve in which the first cases were observed. In addition, *Oo* was detected in a sample from a clinically healthy Aesculapian snake (*Zamenis longissimus*). Additional testing on a larger number of snakes and in additional areas is currently underway to better understand the distribution and impact of this pathogen on wild snakes in Germany.

Prevalence of *Ophidiomyces ophiodiicola* in *Nerodia harteri paucimaculata*, a threatened species candidate

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Ophidiomyces ophiodiicola (*Oo*) is the causative agent of snake fungal disease (SFD), which has been found to cause dermatitis, skin lesions, and even mortality in snakes. Studies have shown higher fungal prevalence in aquatic snakes such as those within the genus *Nerodia*. The Concho water snake (*Nerodia harteri paucimaculata*) is a highly aquatic species endemic to the Colorado and Concho rivers of Texas. The species was delisted from protective status in 2011, however, post-delisting surveys indicate there could be on-going population declines and range contractions. Our study aimed to determine if SFD could be contributing to population declines. To examine the impacts of *Oo*, we estimated prevalence in *N. h. paucimaculata* and its sympatric congeners. Sites were sampled throughout the distribution of *N. h. paucimaculata* for the presence of *Oo*. Snakes were captured using strategically placed minnow traps and visual encounter surveys. For every capture, snakes were swabbed along the entirety of their body and examined for clinical signs of the disease. Swab samples were tested using qPCR analysis to determine the presence of *Oo* DNA on the snakes. Prevalence in *N. h. paucimaculata*, *N. e. transversa*, and *N. rhombifer* was 23.1%, 32%, and 36.4% respectively. When examining only adults of each species, prevalence mostly increased (32%, 38.5%, and 35.3%). This study determined the first prevalence data for *Oo* in *N. h. paucimaculata* and provides confirmed *Oo* presence in four previously undocumented counties.

Batrachochytrium salamandrivorans in Europe: Here to stay

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Since 2004, the *Batrachochytrium salamandrivorans* (Bsal) epidemic has been spreading at a slow pace in Europe, affecting at least 5 urodele species. Human mediated pathogen dispersal is likely to have brought Bsal near the European urodele hotspots in Italy and the Iberian peninsula. The hypersusceptible fire salamander (*Salamandra salamandra*) appears currently most affected. These salamanders remain defenseless against infection: concurrent infection with low virulent *B. dendrobatidis*, immunization attempts or their microbiome and thermal biology all fail to elicit any protective response. Pronounced association with the salamander's skin galactose and subsequent induction of pathogen virulence precede unrestrained intra-epidermal pathogen proliferation, with loss of the skin barrier function. Fire salamander populations show no sign of recovery but persist in disease refugia that protect against population extirpation. Pathogen persistence is mediated through reservoir hosts and the potential to adopt a saprotrophic lifestyle. Rapid diversification of Bsal in Europe suggests pronounced adaptive potential to the pathogen's invasive niche. Risk analyses allow prioritizing conservation efforts but are hampered by a lack of knowledge of Bsal behaviour in urodele communities. A European network for passive surveillance promotes the early detection of outbreaks, which allows rapid interventions. While emergency action plans have been developed and there has been some success in containing Bsal outbreaks, the destructive nature of current mitigation efforts calls for the urgent development of more sustainable control measures on the long term.

Broad host susceptibility of North American amphibian species to *Batrachochytrium salamandrivorans* suggests high invasion potential and extinction risk

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Batrachochytrium salamandrivorans (Bsal) is a novel fungal pathogen of amphibians that is believed to originate from Asia, is emerging in Europe, and could be introduced to North America through international trade or other pathways. Previous Bsal risk analyses ignored host susceptibility to Bsal infection and chytridiomycosis. Thus, we performed dose-response experiments on 36 North American species from 10 families, and estimated indices of Bsal infection and disease susceptibility. Using these data, estimates of environmental suitability of Bsal zoospores, and amphibian species distributions, we modeled invasion potential and predicted biodiversity losses of salamanders in the United States (US) if Bsal is introduced. Overall, we discovered that Bsal caused infection in 72% and mortality in 36% of species tested. Geographically, there were no regions in the country where Bsal could not invade into resident amphibian communities. Predicted biodiversity loss is expected to be greatest in the Appalachian Mountains and along the West Coast, where salamander species richness is greatest and amphibian communities are composed of moderately to highly susceptible species. We estimated that >60 salamander species in the US could experience population declines and extinction if Bsal is introduced, which would result in the greatest loss to US vertebrate biodiversity in recorded history. Our results suggest that emphasis should be placed on preventing Bsal introduction in the US. Given that international trade of pet amphibians is the most likely pathway of introduction, the US government should aggressively seek partnership with the US pet amphibian industry to establish a subsidized clean trade program that reduces the likelihood of Bsal spillover from captive to wild amphibian populations.

EXPERIENCES from the field, ten years after the first ever recorded *Batrachochytrium salamandrivorans* outbreak

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The chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) was first detected in 2008 in a population of fire salamanders (*Salamandra salamandra*) in the Netherlands (Europe). The fungus was isolated and characterized in 2013, and since its discovery nearly ten years ago many studies, legal regulations and surveys have been initiated. Since its discovery, Bsal has been detected in other urodele populations (a.o. *Triturus cristatus*) in the Netherlands, with various impact. In this presentation I would like to share our experiences with Bsal in the Netherlands and expand on its current distribution, present our projects studying the impact of Bsal on species and populations and our citizen science project. As this is work in progress, the results will be preliminary, yet will provide useful for other scientists, Bsal- surveillances and urodele monitoring projects.

Incorporating species susceptibilities and climate change into models of *Batrachochytrium salamandivorans* risk in the United States

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Worldwide, amphibian populations are threatened by several factors including climate change, habitat destruction, and emerging pathogens. Within emerging pathogens, the fungal pathogen *Batrachochytrium salamandivorans* (Bsal) has been associated with recent European salamander die-offs. This emerging pathogen has led to increased concern of spread to the United States, which is a world hotspot for salamander diversity. While Bsal has not been detected in the United States, the first step in disease-risk analyses is to predict areas of potential spread of the pathogen. Previous work has attempted to predict the risk of Bsal in the United States, however the effects of climate change on the Bsal niche, as well as the variability in susceptibility of salamander species were not incorporated. The objective of this work was to incorporate variation in salamander susceptibility and changing environmental conditions driven by climate change to create a predictive map of potential Bsal emergence in the United States. To generate this prediction, we used a combination of layers to represent introduction risks, variation in species susceptibility, and climate change driven climatic niche shifts. To model introduction risks we used a combination of distance to wildlife trade and park visitation rates. Species susceptibility and changes in risk due to climate change were modeled using MaxENT and randomforest algorithms to create climatic niche models for all United States salamander species as well as Bsal under current and predicted climatic conditions. These layers were then combined to form a map predicting risk associated with Bsal emergence in the United States.

Electrolyte imbalances and dehydration play a key role in *Batrachochytrium salamandrivorans* chytridiomycosis

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One of the most important emerging infectious diseases of amphibians is caused by the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*). *Bsal* was recently discovered and is of global concern due to its potential to cause high mortality in amphibians, primarily salamander species. To date, little has been reported on the pathophysiological effects of *Bsal*; however, studies of a similar fungus, *B. dendrobatidis* (*Bd*), have shown that electrolyte losses and immunosuppression likely play a key role in morbidity and mortality associated with this disease. In this study, we aimed to investigate pathophysiological effects and immune responses associated with *Bsal* chytridiomycosis using 49 rough-skinned newts (*Taricha granulosa*) as the model species. *Taricha granulosa* were exposed to a 1 x 107 per 10 mL dose of Bsal zoospores and allowed to reach various stages of disease progression before being humanely euthanized. At the time of euthanasia, blood was collected for biochemical and hematological analysis as well as protein electrophoresis. Ten standardized body sections were histologically examined, in which *Bsal*-induced skin lesions were counted and graded on a scale of 1-5 based on severity. Results indicate that electrolyte imbalances and dehydration induced by damage to the epidermis play a major role in *Bsal* chytridiomycosis in this species. Additionally, *Bsal*-infected, clinically diseased *T. granulosa* exhibited a systemic inflammatory response identified through alterations in complete blood counts and protein electrophoretograms. Overall, these results provide integral information on the pathogenesis of this disease and highlight the differences and similarities between *Bsal* and *Bd* chytridiomycosis.

From the early stages of infection to the grave: How does *Batrachochytrium salamandrivorans* transmission probability shift throughout infection?

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Batrachochytrium salamandrivorans (*Bsal*) poses a major threat to global amphibian biodiversity. It is essential we understand *Bsal* transmission so better-informed management strategies are developed. We measured the latency and infectious period of *Bsal*-infected eastern newts (*Notophthalmus viridescens*) using controlled transmission experiments. We estimated latency by measuring transmission probability given host contact by simulating three one-second contact types (venter-venter, dorsum-venter, or venter-dorsum) between 10 infected and 10 unexposed newts at four periods post-exposure (3-, 6-, 9- or 10-days PE). Our results indicate transmission probability correlates with infection load of infected individuals ($P<0.001$), and infected newts become infectious within 3 days PE. In a separate experiment, we evaluated whether infected newt carcasses could contribute to transmission dynamics. We cohoused 10 infected carcasses at three time periods post-death (1 – 24, 24 – 48, or 48 – 72hrs) with susceptible newts within two cohousing chamber types (partitioned or non-partitioned). The partitioned chamber allowed only indirect transmission of zoospores from infected carcasses to occur. Our results indicate carcasses are capable of effectively transmitting *Bsal* to susceptible newts at least 72hrs post-death, even without hosts directly contacting the carcass. All susceptible newts in each cohousing chamber type and post-death period became infected. *Bsal* DNA copies/uL in skin swabs taken from infected carcasses and water samples collected from cohousing chambers were high (Mean/SD = 2743±3157 among time periods) and not statistically different, suggesting carcasses may remain infectious for longer than 72hrs post-death. These results indicate *Bsal* transmission occurs rapidly between live and dead newts, and carcasses may prolong outbreaks.

Parameterizing a Multi-Stage Infection Model of the Emerging Fungal Pathogen *Batrachochytrium salamandrivorans* (Bsal)

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We develop and parameterize a novel mathematical model to help identify critical transmission pathways and conditions under which Bsal is likely to emerge in salamander host populations. We consider multiple transmission pathways, allow transmission probabilities to change as infection progresses to disease, and consider multiple life stages across a range of temperatures biologically realistic to the hosts. Our system of ordinary differential equations considers susceptibility of juvenile and adult life stages across a range of biologically realistic temperatures, pathogen latency, multiple stages of infection, a recovered state for host populations, as well as the shedding, persistence and encystment rates of zoospores. The probability of transmission given contact is estimated via density-dependent contact rate. Model parameters are estimated using empirical data from controlled experiments, and numerical simulations are explored to identify transmission pathways that drive Bsal epidemics in newt hosts with aquatic life histories. We calculate the invasion potential (R0) of Bsal into populations under differing conditions and explore various mitigation strategies. This integration of empirical data and theoretical modeling allows us to ask questions such as: What are the most important transmission pathways that facilitate pathogen invasion, diseased-induced population declines, and pathogen persistence? What role does life stage play in emergence and persistence of Bsal? How do changes in environmental conditions and mitigation strategies affect transmission and disease outcomes?

A Standardized Method for Observing Amphibian Behavior in Climate Controlled Chambers to assess changes with fungal disease (*Batrachochytrium salamandrivorans*) inoculation

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The chytrid fungus has decimated populations of amphibians around the world. A related fungus, *Batrachochytrium salamandrivorans* (Bsal), is also deadly to amphibians, especially salamanders. Originating in Asia, it has destroyed populations in Europe, but has not yet reached the Americas. Although researchers are trying to determine which native amphibians are at risk, behavior changes accompanying the progression of the disease are less studied. We developed a method to monitor behavior of individually housed control and infected animals (5 x 103 to 106 fungal spores/10 mL) in environmental chambers at 15 C. Cameras were programmed to take time-lapse photos at 60 second intervals inside the secured chambers periodically over the time course during which susceptible animals will develop symptoms and die. Photos were viewed to assess number of behavioral changes and whether they were or in or out of the provided cover object. Inter-rater reliabilities were high. Data on seven species representing six genera will be reported. Based on movement and exposure data gathered from the photos, we found that changes in movement and time undercover were detected, often before animals exhibited physical signs of the disease. For example, *Eurycea wilderae* showed both increased time under cover and decreased locomotion and posture changes with increasing infection load. Behavior may be useful in future studies of this potentially devastating threat to American, and especially Appalachian, hotspots of salamander diversity.

Management strategies to reduce invasion potential of *Batrachochytrium salamandrivorans*

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The emerging fungal pathogen, *Batrachochytrium salamandrivorans* (Bsal), has the potential to negatively impact global salamander diversity. Identifying strategies that impact transmission pathways is one approach to managing disease in wild populations. We undertook a series of controlled experiments to: (1) evaluate if reducing host density and increasing habitat structure reduced host contact rates, (2) estimate the transmission function of Bsal in aquatic mesocosms across a range of host density and infection prevalence treatments, and (3) estimate the effectiveness of plant-derived fungicides at inactivating Bsal zoospores. We found that contact rates of eastern newts (*Notophthalmus viridescens*) was significantly reduced by decreasing host contacts and increasing habitat structure; however, the functional form of Bsal transmission was frequency dependent. We also found that small quantities (<60 ug/mL) of plant-derived fungicides (e.g., allicin, curcumin, thymol) were effective at inactivating Bsal zoospores. Our results suggest that reducing densities of eastern newts to 2 newts per m2 may not be an effective management strategy to prevent invasion of Bsal; however, strategies that prevent aquatic zoospore transmission or increase host resistance to infection may have greater success.

Risk of bacteremia associated with probiotic treatment of *Batrachochytrium salamandrivorans*

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Batrachochytrium salamandrivorans (Bsal) is a recently described fungal pathogen that has caused declines of wild salamanders and been detected in captive populations in Europe. One potential preventative and/or treatment option for Bsal chytridiomycosis is the application of probiotic bacteria. Previous studies have shown that certain bacteria present on amphibian skin have anti-fungal properties. This study aimed to determine the safety and efficacy of using a probiotic bacteria (*Pseudomonas fluorescens*) to prevent and treat chytridiomycosis. Alternatively, some bacteria can become pathogenic when given the opportunity. Thus, a secondary aim was to determine if bacteremia plays a role in the pathogenesis of chytridiomycosis. Eastern newts (*Notophthalmus viridescens*) were exposed either to Bsal, probiotic bacteria, pathogenic bacteria (*Aeromonas hydrophila*), or a combination of Bsal and one of the bacterial species. They were monitored throughout the course of disease development and samples were collected for PCR, blood cultures, and histopathology. Our results indicate that Bsal loads on the skin measured by PCR were not lower in groups that were exposed to probiotic bacteria, and mortality ranged from 67-100% and did not differ among treatment groups. We also found evidence of bacterial infiltration into the blood and organs in animals that were exposed to Bsal in probiotic-exposed and -unexposed newts. Thus, our results suggest that the *P. fluorescens* isolate was not an effective probiotic, and future studies should examine the potential for probiotics to contribute to bacteremia and Bsal pathogenesis.

Combination strategies boost eastern newt survival to the salamander chytrid fungus

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Arrival of the chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal) looms over the United States – a salamander diversity hotspot rivaled by no other around the world. Eastern newts are one of the most susceptible species to 'salamander-eating' fungus and currently have a wide distribution within the Eastern US. Identifying strategies to support population resilience through invasion is essential. Management strategies can target both the host to boost immunity and the environment to reduce the infectious zoospore pool. We performed factorial experiments to test efficacy of multiple strategies singly and in combination in eastern newts at both the adult and larval lifestage. For adult newts, we tested whether skin probiotics, mucosal nanoparticle vaccination and aquatic micropredator augmentation can increase survival and modulate infection dynamics; For larvae, we tested whether skin probiotics and mucosal nanoparticle vaccination at the aquatic larval stage would affect survival of juvenile eft that encounter Bsal. For both life stages, combination strategies were more effective at increasing survival than any single action. Continued development of integrative, multipronged approaches will be essential to combat significant biodiversity losses if Bsal emerges in the United States.

Probiotic application delays fatal Bsal chytridiomycosis in eastern newt metamorphs (*Notophthalmus viridescens*)

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The potential arrival of the pathogenic fungus *Batrachochytrium salamandrivorans* (Bsal) in North America and its consequences for native amphibians has prompted investigations into management strategies to mitigate species loss. The eastern newt (*Notophthalmus viridescens*) is a common salamander distributed over most of eastern North America. Its susceptibility to Bsal infection coupled with its dispersal ability as a terrestrial eft suggest it will feature prominently in Bsal disease dynamics. One area of research in combating Bsal is through bioaugmentation of amphibian skin microbiota via application of probiotic bacteria that possess antifungal properties. We investigated the efficacy of two probiotic isolates against Bsal infection in eastern newt efts.

Bacteria isolated from wild adult eastern newts were tested for Bsal inhibition *in vitro*. We selected two isolates – *Iodobacter fluviatilis* and *Bacillus pumilus* – that had strong antifungal properties and minimal non-target effects for *in vivo* experimentation. Individuals were treated with either one or both probiotics as recently metamorphosed efts, or just prior to emergence from the water (gills reduced by >50%). After all efts completed metamorphosis, we exposed them to an infectious dose of Bsal. Bsal infection intensity and skin microbiota was monitored through biweekly skin swabs. After eight weeks, efts treated with *Iodobacter fluviatilis* – either singly or in combination with *Bacillus pumilus* – showed higher survival than efts treated with *Bacillus pumilus* and pre-metamorphs treated with both probiotics. A single exposure to probiotics was insufficient to prevent Bsal infection, but repeated *Iodobacter* applications may increase likelihood of an individual clearing Bsal infection over time.

Coupling intra season disease dynamics and annual population demography with a hybrid model of *Batrachochytrium Salamandrivorans* in amphibian populations

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Infectious disease dynamics in amphibians with multiple routes of transmission are a complicated interwoven system because of the unique metamorphosis over their whole life cycle. The new infectious chytrid fungal pathogen (*Batrachochytrium Salamandrivorans*, Bsal) has caused dramatic declines in amphibian populations across Europe. Because Bsal has yet to make an appearance in the United States, current research has focused on empirical and theoretical approaches to predicting future outbreaks and identifying control strategies to prevent or mitigate the pandemic if it happens. In this study, we develop a mathematical model of Bsal-infected Eastern Newts by taking into account the population level and age stages (larvae, juveniles, and adults). This is a hybrid modeling approach that integrates disease with amphibian life history dynamics by coupling a system of continuous Ordinary Differential Equations (intra season disease dynamics) with a discrete system of difference equations (annual population demography). In order to incorporate annual breeding seasons, we use a birth pulse for the recruitment of larvae into the model. We compare model performance between purely continuous, annual discrete, and our hybrid approach.

Surveying for *Bsal* in Wild Salamander Populations of Tennessee: Lessons Learned

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The chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) continues to invade naïve regions of Europe, causing declines in salamander and newt populations. *Bsal*'s imminent spread into the United States threatens endemic salamander species. Here we report results of a survey for the presence of Bsal in wild populations of lungless salamanders (Family Plethodontidae) in Tennessee, a global hotspot for salamander biodiversity. Salamander skin swabs (N=137) and water samples were collected from 10 sites across three ecoregions of Tennessee between May 2016 and July 2018. All animals were examined for clinical signs consistent with chytridiomycosis. We photographed observed skin lesions and attempted fungal culture from swabs of lesions. All samples were screened in triplicate using a quantitative PCR (qPCR) assay targeting the Bsal 5.8s rRNA gene. Spurious positive results were detected in single replicates of six different swab samples. These ambiguous samples were screened again at an independent laboratory using the same assay but a different qPCR protocol and instrument. Additionally, we attempted to detect a second Bsal gene (28S rRNA) in ambiguous samples through conventional PCR. However, attempts to detect the 28S rRNA gene were unsuccessful, and the independent laboratory could not confirm positive qPCR results. Attempts to culture the fungus from lesions were also unsuccessful. Therefore, we concluded that no samples were positive for *Bsal*. False positive detections, which have been reported in other studies using the same assay, may explain the ambiguous results. We offer suggestions for developing a more reliable diagnostic protocol for future monitoring efforts.

Riding a swell: Is *Batrachochytrium dendrobatidis* still emerging in the Mediterranean?

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Batrachochytrium dendrobatidis (*Bd*) stands out as the worst infectious disease conservation threat recorded to date. A recent review of the global impacts documented the geographic and taxonomic scope of chytridiomycosis, which broadly supported the argument that *Bd* is the worst of the worst. However, this article also presented data that the global wave of chytridiomycosis has peaked and may be in decline. While this may be true for some tropical regions of the world, we argue that the peak has not yet crested in the Mediterranean region. Recent publications document previously undescribed and contemporarily occurring cases of lethal chytridiomycosis in species where it was not previously described. Even more compelling is a publication describing ongoing *Bd* range expansion in a susceptible species. Why the impact of *Bd* in the Mediterranean coincided with the global emergence and continues to increase is uncertain, but we postulate two possible mechanisms affecting this susceptible amphibian community existing in a permissive environment; 1) the Global Pandemic Lineage in Europe is a more aggressive form of the lineage, and; 2) interactions with endemic ranaviruses may impede the ability of Mediterranean species to limit and resolve *Bd* infections. We will present data to support both of these hypotheses.

Amphibian Surveillance Program of Catalonia (ASPrCAT): a risk-based approach for monitoring chytrid fungi in amphibian communities from Northeastern Spain

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Amphibian chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), is having an unprecedented impact on amphibian biodiversity. Despite chytridiomycosis has been sporadically detected in Catalonia (northeastern Spain), the distribution and potential impact of these pathogens on amphibian populations is limited. In order to contribute to amphibian conservation, in 2019 we launched the Amphibian Surveillance Program of Catalonia (ASPrCAT). From 2019 to 2021 we systematically surveyed nine areas selected to enhance the probability of pathogen detection. Overall, we obtained 1,175 skin swabs (2019 n=205; 2020 n=382; 2021 n=588) from 14 native amphibian species. Swabs were tested for the presence of Bd and Bsal DNA using a duplex qPCR. We detected Bd in 43 individuals from four areas and belonging to four amphibian species (*Hyla meridionalis*, *Pelophylax perezi*, *Alytes obstetricans* and *Triturus marmoratus*). Disease and mortality were only observed in *A. obstetricans*, which agrees with previous findings of this species being highly susceptible to chytridiomycosis. Conversely, *H. meridionalis* and *P. perezi* commonly harbored high Bd loads without clinical signs, suggesting that they may be important in pathogen maintenance. Bd was more frequently detected in areas with greater anthropogenic disturbance and at lower elevations. In positive areas, Bd was not consistently detected across years and when compared to previous reports, which could be associated with the abundance of maintenance hosts. Importantly, Bsal was not detected during our study period. The implementation of ASPrCAT improves our understanding of Bd epidemiology and is a strategic action to detect and effectively manage disease outbreaks.

Thermal shelters reduce the impacts of chytridiomycosis in an endangered frog

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Amphibians are experiencing worldwide declines exacerbated by the invasive fungal disease chytridiomycosis (primarily caused by *Batrachochytrium dendrobatidis – Bd*). Since *Bd* cannot be readily eliminated from ecosystems, the success of re-establishing chytridiomycosis-impacted amphibians across their former range have had limited success. High temperatures (> 30 °C) are known to limit *Bd* growth *in vitro*, and even moderate increases in temperature can limit the impacts of *Bd* in amphibian hosts. Seasonal outbreaks of chytridiomycosis have also been documented with pathogen burden and prevalence increasing through winter and early spring and declining in the summer. In such systems a major barrier to reintroduction success is seasonally cold temperatures that likely favor chytridiomycosis outbreaks. The deployment of artificial retreat sites that provide elevated temperatures in cooler months could swing the balance from “cool, and thus unable to survive *Bd* infection” to “warm, and thus able to clear the infection and survive”. We optimized and assessed the utility of thermal refuges for buffering against the impacts of *Bd* in semi-wild outdoor mesocosms. Frogs that had access to a higher range of operative temperatures provided by thermal refuges were caught at higher average body temperatures and with lower *Bd* infection over time. Our results illuminate a promising intervention that is inexpensive, easily deployable, and may have utility across several *Bd* impacted species.

Genomic approaches for increasing disease resilience in amphibians

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Amphibian genomics resources have increased dramatically in the last half decade, and the number of reference genomes has increased over fivefold. However, this remarkable resource has so far had limited application for conservation. I will discuss the current state of the amphibian genomics field, what can be learned from genomics approaches, and how this information can be used as a springboard for developing management strategies for threatened amphibian populations. Specifically, these resources can aid functional genetics research that informs genetic intervention when more targeted approaches are required such as increasing amphibian resilience to chytridiomycosis.

The other face of triazoles: How widespread use of fungicides in agricultural habitats could protect amphibians from chytridiomycosis

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The sixth mass extinction is a consequence of a complex interplay between multiple, anthropogenic stressors with a negative impact on biodiversity. We here examine the interaction between two widespread and global, anthropogenic drivers of amphibian declines: the fungal disease chytridiomycosis (caused by *Batrachochytrium dendrobatidis*) and antifungal pesticide use in agriculture. Field monitoring of 26 amphibian ponds in an agricultural landscape shows widespread occurrence of triazole pesticides throughout the amphibian breeding season and a negative correlation between the early season application of epoxiconazole and the prevalence of chytrid infections in aquatic newts. Although the water column levels of epoxiconazole remained below the minimal inhibitory concentrations (MIC) that inhibit growth of five *B. dendrobatidis* isolates tested in laboratory experiments, newt skin bio-accumulated epoxiconazole seven-fold, resulting in cutaneous growth suppressing concentrations. Early exposure to sub-MIC concentrations of epoxiconazole observed in the field indeed prevented infection of anuran tadpoles with *B. dendrobatidis* in a laboratory exposure setup. Despite the possible side-effects, the widespread application of triazole pesticides may thus temper fungal disease outbreaks in heavily anthropically modified agricultural landscapes.

Appalachian soil bacterial communities inhibit amphibian-killing fungal pathogen growth in experimental microcosms

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North American salamanders are under threat of possible intercontinental spread of chytridiomycosis, a deadly disease caused by the fungal pathogen *Batrachochytrium salamandrivorans* (Bsal). In order to predict potential routes that may facilitate long-distance dispersal to naive salamander habitats, we must evaluate environmental resistance to invasion by fungal pathogens. Here, we aim to determine the degree of habitat invasibility using soils collected from five locations throughout Great Smoky Mountains National Park, where there is high potential for spread to many species of susceptible hosts. Our experimental design consisted of replicate soil microcosms exposed to different propagule pressures of the non-native pathogen, Bsal, and an introduced but endemic pathogen, *B. dendrobatidis* (Bd). To compare the growth and competitive interactions, we used quantitative PCR to monitor each pathogen load, live/dead cell viability assays, and 16S rRNA amplicon sequencing to determine bacterial community response. We found that soil microcosms with intact bacterial communities inhibited Bsal and Bd growth. However, inhibition diminished with increased propagule pressure. Overall, Bsal showed greater persistence in soil than Bd. Soil microbial communities were dominated by phylum Proteobacteria followed by Acidobacteria, Planctomycetota, and Actinobacteriota. Linear discriminant analysis (LDA) identified bacteria in the family Burkolderiales increasing in relative abundance with decline of both pathogens. Although our findings provide evidence of environmental filtering in soils, such barriers weakened in response to pathogen alienness and propagule pressure. Our study serves as a first step to identify how habitats might vary in the level of invasion based on properties of their local microbial communities.

Accounting for bias in prevalence estimation: The case of the amphibian-killing fungus *Batrachochytrium dendrobatidis* in the southern Darwin's frog *Rhinoderma darwinii*

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Accurate estimation of infection parameters is essential to enable effective disease surveillance. However, wildlife hosts and pathogens are often imperfectly observed and key epidemiological parameters, such as infection prevalence, can be biased if this observational uncertainty is not considered, with potential negative consequences for disease mitigation and wildlife conservation. Here, we adjust for the combined effects of imperfect pathogen detection and host pseudoreplication (i.e. not knowing host identity) to provide a more reliable estimate of *Batrachochytrium dendrobatidis* (Bd) infection prevalence in the southern Darwin's frog (*Rhinoderma darwinii*). Between November 2018 and March 2019, we captured 1,085 individuals at two areas in Southern Chile. Captured frogs were individually identified to eliminate pseudoreplication, swabbed twice in sequence, and each swab analyzed in duplicate using a specific qPCR assay to detect Bd infection. Using a Bayesian model, we were able to correct prevalence estimates for false-negative error rates arising from both sampling and diagnostic testing. Our results showed that Bd prevalence could be underestimated by over 50% if false negatives and host pseudoreplication were not accounted for. The simulated host population trajectories showed that such a difference in prevalence estimates can change our interpretation of the impacts of Bd infection (causative agent of the amphibian chytridiomycosis) in our model species from a growing (uncorrected prevalence) to a declining (corrected prevalence) population.

Evaluating environmental DNA-based detection of *Batrachochytrium salamandrivorans* in trade and captive settings

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The trade of live animals has contributed to the emergence and spread of pathogens, such as *Batrachochytrium dendrobatidis* and *B. salamandrivorans* (*Bsal*). Comprehensive, routine surveillance for pathogens could be used to minimize their spread, but most strategies involve individual samples (e.g., screening tissue samples or swabs), which become prohibitive at scale. We therefore tested an alternative approach, using environmental DNA (eDNA) to detect *Bsal* in whole groups of animals (e.g., shipments, captive populations). In a series of experiments with experimentally-infected newts (*Taricha granulosa*) we estimated limits of detection, compared *Bsal* DNA recovery among substrates and water, evaluated diagnostic performance of eDNA relative to traditional swabs, and tested whether clumped eDNA or environmental inhibitors might limit performance. We find that eDNA-based detection performs well relative to individual swabs for population-level detection under a wide range of conditions. There are, however, important considerations when designing a surveillance program, and eDNA is not universally appropriate. Overall, eDNA-based detection can facilitate large-scale, routine surveillance for important emerging pathogens such as *Bsal*.

Lovesick? The effect of *Batrachochytrium dendrobatidis* infection on amphibian breeding display

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The devastating fungal pathogen, *Batrachochytrium dendrobatidis*, (*Bd*), has caused widespread amphibian declines and extirpations. Although its pathogenesis has been examined in a range of species, little is known about its effect on reproduction. Here, we investigated how *Bd* affects male mating display across Australian tree frog species that have different patterns of decline and infection susceptibilities. We collected call recordings of wild frogs in the field and used a spectrophotometer to analyse male breeding colouration, while swabbing all individuals for infection. We then analysed the call characteristics and colour profiles of infected and uninfected frogs to determine whether infection influenced calling performance and breeding colouration. We found that colouration was affected by *Bd*, with UV chroma increasing with infection status and load. These are the first results to show that *Bd* infection influences male breeding colouration. Calling performance was also affected by infection status, and was closely linked to temperature variations within different amphibian microhabitats. The results that we present here are important but often overlooked aspects of disease ecology. Sublethal effects of disease can impact breeding behaviour and display. Changes in reproduction and breeding success in response to disease might have dramatic consequences on population trajectories and substantially influence population decline or recovery potential. It is therefore crucial that we investigate sublethal effects of infection and their influence on reproduction and recruitment, so that we can understand the impact of disease on populations.

Assessment of physiological and behavioral responses of *Osteopilus septentrionalis* to infection with *Batrachochytrium dendrobatidis*

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The lethal skin disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been a major player in amphibian declines. Though the costs of infection remain unclear in hosts with the ability to clear the pathogen. Disease-induced changes in immune function and stress physiology can significantly impact amphibian survival and fitness, yet are often ignored in tandem with disease. Glucocorticoid hormones, including corticosterone, help individuals maintain homeostasis during stressful events and thus may mediate infection intensity. Immune parameters, including white blood cell (WBC) profiles, are also likely to respond to infection to protect a host against disease. Here, using a species that can be susceptible to Bd, *Osteopilus septentrionalis* (Cuban treefrog), we examined the relationship between repeatedly-measured corticosterone release rates and WBC profiles to track responses in the endocrine system and the activation of the immune system before and after experimental infections. We also measured exploratory activity in these individuals to test if physiological processes were coupled with changes in frog behavior. Our results indicate that frogs with high infection loads showed increased corticosterone release rates, but no relationship with lymphocyte counts. Both experimentally-infected and sham-infected (water) frogs became less active and more exploratory after treatments. This experimental approach addresses the complex relationships among disease, physiology, and behavior, underscoring the role of experimental design in shaping behavioral responses. Furthermore, these results indicate potential mechanistic explanations for Bd-induced decreases in survival and fitness via non-lethal effects on key physiological and behavioral processes.

Developing indicators of poor welfare for assessing non-model amphibians used infectious disease research

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Infectious disease research poses as an excellent facilitator to understand and develop indicators of poor welfare for non-model amphibians. This is due to ongoing *in vivo* testing, designed to answer key questions on the highly infectious diseases chytridiomycosis and ranavirosis. In the UK alone, these experiments involve thousands of amphibians, with patterns repeated globally. At present, how individuals are assessed within the confines of such work lacks definition, but by using the biological costs of disease, we can begin to identify indicators that depict poor welfare. Published experiments and surveyed experts in the field of amphibian research highlighted disease effects that might be intuitive to indicators of poor welfare. Yet literature showed that *in vivo* testing of ranavirosis required refinement, as infected animals were dying before clinical signs could be identified and humane euthanasia elicited. Importantly, if welfare assessments are to be incorporated into experimental designs, the animals used need to be monitored non-intrusively, and the methods to do this should not interfere with scientific questioning. This emphasizes behavior or behavioural changes over time as key factors for assessment. Therefore, working with a planned experiment, we monitored ranavirus exposed *Rana temporaria* via daily welfare checks and recorded video footage. This study identified specific postural changes and new clinical signs associated with advancing disease that can be used in future research to improve animal care and scientific reproducibility.

Near-infrared spectroscopy (NIRS) as a screening tool for chytrid fungus (*Batrachochytrium dendrobatidis*) in Fowler's toads (*Anaxyrus fowleri*) and leopard frogs (*Rana pipiens*)

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The emergence of chytridiomycosis three decades ago has driven hundreds of amphibian species towards extinction. Despite the wide-spread loss of amphibian diversity due to chytridiomycosis, techniques for effectively detecting *Batrachochytrium dendrobatidis* (Bd) in anurans and *B. salamandrivorans* in caudates remain limited in application. Although histological examination and quantitative polymerase chain reaction (qPCR) have proven useful for Bd diagnosis, they are costly and time-consuming, limiting their use in conservation programs where rapid diagnostics are required to guide on-the-ground decisions. Near-infrared spectroscopy (NIRS) provides a solution as a rapid, non-invasive biophotonic technique for screening various diseases in wildlife (e.g., cancer, chlamydia). Once spectra are collected and a reliable prediction model has been established, the disease status of unknown individuals can be assessed in real-time. The objective of this pilot study was to develop NIRS as a screening tool to rapidly and reliably detect the presence of Bd using two model species, *Anaxyrus fowleri* and *Rana pipiens* (*N*=50). Individuals were scanned using a spectroscopic probe, then swabbed for qPCR analysis (used as a reference). Distinct biochemical patterns were observed between Bd(+) and Bd(-) individuals, and chemometrics were applied to calibrate then test the prediction model. Preliminary results indicated that Bd(-) and strongly Bd(+) individuals were classified with a promisingly high level of accuracy (>90%), while weakly Bd(+) individuals with low fungal loads had a propensity to be misclassified as Bd(-). Increased sampling with experimental controls will improve the capacity of NIRS as an early Bd-screening tool for guiding effective treatment and quarantine regimens.

Translocation does not influence prevalence of amphibian chytrid fungus among translocated wild Eastern Hellbenders (*Cryptobranchus alleganiensis*)

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Disease monitoring is an essential step in translocation projects, specifically in amphibians where emerging pathogens such as the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) are linked to population declines. The Eastern Hellbender (Cryptobranchus alleganiensis) is a large, fully aquatic salamander experiencing precipitous range-wide population declines, however the role *Bd* plays in these declines is unclear. To augment declining hellbender populations and determine effects of translocation on *Bd* prevalence, we conducted a translocation study of wild adult hellbenders from two source streams with abundant hellbender populations to two streams with declining populations in east Tennessee, USA. In 2018, we implanted radio transmitters into 30 hellbenders and sampled them periodically for *Bd* until 17 of the 30 hellbenders were translocated in 2019. We attempted to recapture translocated hellbenders approximately every 45 days for three months to determine *Bd* prevalence post-release. We used qPCR to detect *Bd* and quantify zoospore loads on positive samples. Hellbenders had a pre-translocation Bd prevalence of 50% (15/30), which decreased to 10% (1/10) post-translocation. The average zoospore load for positive samples was 73.63 ± 30.82, and no hellbenders showed signs of chytridiomycosis throughout the study. Although we detected no significant effect of translocation on *Bd* prevalence, we observed a reduction in *Bd* prevalence post-release. Our results indicate that translocation did not lead to an increase in disease susceptibility in translocated wild adult hellbenders, suggesting that chytrid did not impact the success of short-term translocations of Eastern Hellbenders in the Blue-Ridge ecoregion.

High mortality due to *Bd* chytridiomycosis in transported Broadfoot Mushroomtongue Salamanders, *Bolitoglossa platydactyla*

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Chytrid fungus (*Batrachochytrium dendrobatidis* or *Bd*) is a globally important pathogen implicated in several amphibian declines over the past few decades; but it remains unclear how *Bd* has impacted salamander populations. Zoos and researchers have reported high mortality in transferring several salamander species from the wild into captivity, and *Bd*-chytridiomycosis is implicated in a majority of these. Currently in Mexico, there are 90 lungless salamander (Family: Plethodontidae) species classified by IUCN as threatened or endangered. It is important to document these mortalities to aid in future conservation plans that may require captive breeding colonies. We present a case report of chytridiomycosis in the Mexican plethodontid salamanders, *Bolitoglossa platydactyla*. Thirty-three apparently healthy animals were collected from sites in Veracruz, Mexico for transport to the United States for use in an ongoing disease study. All animals were collected and stored separately in individual containers. We changed gloves and used new materials for each individual to prevent any subsequent pathogen transfer. However, 13 individuals died within the two-week transfer and acclimatization period. Clinical signs were acute lethargy followed by complete tail drop and death within 12-24 hours. We performed histopathological analysis of seven individuals. All individuals had marked erosion and ulceration affecting 75 % of the body. Remaining keratinized skin contained numerous round organisms consistent with *Bd* zoosporangia. Several areas had large bacterial colonies covering the skin surface and, in some cases, were invading the dermal layer. All but one animal were positive for *Bd* via qPCR assay, however this one individual had visible *Bd* zoosporangia in histopathological analysis. This report brings to light that *B. platydactyla* likely carry subclinical levels of *Bd* in the wild. It also follows that under some likely stressful conditions, these infected animals can develop clinical disease and succumb to chytridiomycosis.

Chytridiomycosis outbreak in a captive breeding program of the Chilean giant frog (*Calyptocephalella gayi*): Genomic characterization and pathological findings

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Emerging infectious diseases in wildlife are increasingly associated with animal mortality and species declines, but their source and genetic characterization often remains elusive. Amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been associated with catastrophic and well-documented amphibian population declines and extinctions at the global scale. We used histology and whole-genome sequencing to describe the lesions caused by, and the genetic variability of, two *Bd* isolates obtained from a mass mortality event in a captive population of the threatened Chilean giant frog (*Calyptocephalella gayi*). This was the first time an association between *Bd* and high mortality had been detected in this charismatic and declining frog species. Pathological examinations revealed that 30 dead metamorphosed frogs presented agnathia or brachygnathia, a condition that is reported for the first time in association with chytridiomycosis. Phylogenomic analyses revealed that *Bd* isolates (PA1 and PA2) from captive *C. gayi* group with other *Bd* isolates (AVS2, AVS4, and AVS7) forming a single highly supported Chilean *Bd* clade within the global panzootic lineage of *Bd* (*Bd*GPL). These findings are important to inform the strengthening of biosecurity measures to prevent the impacts of chytridiomycosis in captive breeding programs elsewhere.

Application of disinfectants for environmental control of a lethal amphibian pathogen

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Chytridiomycosis is an emerging infectious disease threatening amphibian populations worldwide. While environmental disinfection is important in mitigating the disease, successful elimination of Batrachochytrium dendrobatidis (Bd) without excessively harming ecosystems is challenging. We selected peracetic acid (PAA) as the most potent of six commercially available products regarding their ability to inhibit growth of a highly virulent Bd strain. PAA killed Bd after 5 min of exposure to approximately 94.7 mg/L. We examined the toxicity of PAA against three invertebrate species and Discoglossus pictus tadpoles. 93% of invertebrates, but none of the tadpoles survived 5 min of exposure to 94.7 mg/L. Tadpoles showed no adverse effects after 5 min exposure to concentrations of approximately 37.9 mg/L or lower. Addition of PAA to aquatic microcosms decreased pH, while dissolved oxygen (DO) initially increased. Degradation of PAA reversed the pH drop, but caused a massive drop in DO, which could be remedied by aeration. As proof of concept, microcosms that were aerated and treated with 94.7 mg/L PAA sustained survival of tadpoles starting 48 h after treatment. Disinfecting aquatic environments using PAA could contribute to mitigating chytridiomycosis, but requires temporary removal of resident amphibians.

Genetic evidence for recovery of the endangered Fleay's barred frog (*Mixophyes fleayi*) throughout its range after declines associated with amphibian chytridiomycosis

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The amphibian skin disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been a driver of unprecedented amphibian extinctions and mass mortalities around the world. Many affected species continue to decline after initial epidemics, but the endangered Fleay's barred frog (*Mixophyes fleayi*) has demonstrated strong resurgence in a few localities despite persistence of Bd in the environment. While these isolated recoveries are promising, little is known about the status of remaining populations in the narrow *M. fleayi* distribution of eastern Australia. Here, we conduct a range-wide population genetic assessment of *M. fleayi* to characterize (1) genomic signatures of its Bd-associated decline in the late 20th century and (2) evaluate the extent of recent genetic recoveries throughout its distribution. To achieve this aim, we collected 574 *M. fleayi* genetic samples across 27 sites and used genotyping-by-sequencing to identify thousands of biallelic single nucleotide polymorphisms in each individual. With genomic tools to evaluate the plausibility of competing demographic histories, we then characterized range-wide trends in Bd-associated decline and subsequent recovery of this endangered amphibian. Exploring these patterns of recovery may be integral in our continuing fight against amphibian chytridiomycosis, especially if *M. fleayi* has evolved to become resistant or tolerant to Bd across its range.

Recovery of the Neotropical stream-breeding hylid *Duellmanohyla rufioculis* following chytrid related declines

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The spread of *Batrachochytrium dendrobatidis* and the subsequent wave of declines in Central America has been well studied. These declines caused a clear loss of amphibian biodiversity throughout the region and many species were feared extinct. Despite the clear impacts of *Bd* on Neotropical amphibian communities, particularly those from riparian habitats, there have been increasing reports of population and species recovery. *Duellmanohyla rufioculis*, the rufous-eyed stream frog, declined precipitously in portions of its range following the arrival of *Bd* in Costa Rica. Over the last 20 years some of these populations have apparently begun to recover, despite the continued presence of *Bd*. We used population genetics and mark-recapture to evaluate the historic and current trends of a population of *D. rufioculis* on the Caribbean slope of Costa Rica. The Rara Avis population is consistently increasing in size while maintaining enzootic *Bd* infections. While community monitoring has occurred throughout the last 20 years, ddRAD-Seq analyses were able to fill in gaps about historic bottlenecks and the longer-term relationship with *Bd* that occurred prior to survey programs. We were unable to identify any clear patterns in host microbiome that may be contributing to recovery. Our results suggest that this population, along with those of other species, are able to recover despite the continued presence of *Bd*.

Comparison of swab and tissue samples for detection of *Ophidiomyces ophidiicola* in Eastern Wormsnakes (*Carphophis amoenus amoenus*)

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Snake fungal disease (SFD) is an emerging threat to North American snakes, which is caused by the fungus *Ophidiomyces ophidiicola* and can cause lesions, abnormal molting, clouding in the eyes and facial disfiguration. Efforts are underway to establish monitoring systems for snake populations to understand the distribution and impacts of SFD. Skin swabs are the current standard for surveillance. However, our ongoing collection of tail tips from snakes to test for the presence of ranavirus provides an opportunity to compare this method to swab sampling for *O. ophidiicola*. Since June 2021, we have captured Eastern Wormsnakes (Carphophis amoenus amoenus) using artificial cover objects in a survey network in central Virginia, USA. We have weighed, measured, taken tissue samples, and PIT tagged snakes. Since August 2021, we have taken two samples to test for *O. ophidiicola* using swabs moistened with water or RNAlater which are run across the body in a standardized manner; if lesions are present, a third swab is used to sample lesions exclusively. Swab and tissue samples are tested for the presence of *O. ophidiicola* using quantitative PCR. At the time of submission, we have collected 28 Wormsnakes, some exhibiting lesions, and some testing positive for O. ophidiicola. By the conference, we will have a large enough sample (>40 individuals) to estimate prevalence of *O. ophidiicola* and compare sensitivity between sampling methods. This study will yield additional valuable information by presenting the largest survey of Wormsnakes to date for this pathogen and indicating the abundance of asymptomatic carriers.

August 7, Session Five (AM)
Ranaviruses and Other Amphibian Pathogens

Ranaviruses: Four things we (mostly) know and three we (largely) do not

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The genus *Ranavirus* (family: *Iridoviridae*) includes seven species of viruses with icosahedral capsids and double-stranded DNA genomes. They are collectively capable of infecting and causing systemic disease in reptiles, amphibians, and bony-fish; at least one, the type virus *Frog virus 3* can infect animals in all three host classes. They have been detected in free-living and captive animals around the world, most often during die-offs, but also in the apparent absence of mortality or even morbidity. In this overview, I will briefly describe the history of ranavirus research and then provide an overview of several aspects of ranavirus ecology I think we largely understand, with important caveats, namely: 1) broad-scale patterns of phylogeny and evolution, 2) routes of transmission, 3) the range of possible outcomes of infections, and 4) the capacity and means of viral persistence. I will then review several topics I think are quite important, but still poorly understood: 1) why, at the individual and population levels, the outcomes of ranavirus infections vary so enormously, 2) the extent to which ranaviruses threaten host populations and communities, and 3) what ranaviruses are doing between epizootics, especially in highly seasonal environments. My hope is that this overview, as opinionated as it may be, helps organize our knowledge and ignorance, and spark discussions about what we still *need* to learn.

Environmental factors and individual susceptibility shape *Ranavirus* epidemics in experimental *Lithobates sylvaticus* populations

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Epidemic frequency has increased in amphibians over the last century, potentially exacerbated by global environmental changes. We tested whether mechanisms known to alter susceptibility of individuals translate into population-level outcomes by exposing *Lithobates sylvaticus* tadpole populations to ranavirus in outdoor mesocosms. We manipulated temperature and salinity in 144 populations, introduced ranavirus-infected tadpoles into 96 populations early in development (Gosner stage 25), and observed 94 epidemics with an average mortality rate greater than 95%. In 12 populations, we introduced ranavirus-infected tadpoles late in development (Gosner stage 35) and tracked tadpoles by size cohort. We hypothesized that elevated temperatures would accelerate epidemic timing, and high salinity would increase epidemic magnitude. Surprisingly, we found no differences in the frequency or magnitude of epidemics among environmental treatments when populations were exposed early in development. Epidemics at Elevated Temperatures occurred 3–4 days before epidemics at Ambient Temperatures ($p < 0.001$) and had more individuals die on a single day than Ambient populations ($p < 0.001$). Low Salinity epidemics occurred 1–2 days after High Salinity epidemics in both temperature treatments ($p = 0.014$). Epidemic timing of late exposure populations was similar, with decreased epidemic magnitude in Elevated populations, as some large tadpoles accelerated development, metamorphosing prior to succumbing to disease. Our results contribute to our understanding of how shifting global environmental conditions may alter local epidemics, potentially limiting detection of die-off events by shortening the window of mortality, and creating conditions where tadpoles could metamorphose with sub-lethal infections and spread ranavirus to naïve systems.

Impacts of asynchronous emergence of *Batrachochytrium dendrobatidis* and Ranavirus in Florida amphibian assemblages

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As emerging pathogens expand and overlap in geographic ranges, novel interactions can potentially exacerbate declines in already fragile host populations. Alternatively, heightened immune responses of infected hosts may suppress additional infections. Here we focus on characterizing the interactions between two emerging pathogens impacting North American amphibians: the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) and Frog-Virus 3-related Ranavirus. We investigated the co-occurrence of these pathogens in amphibian assemblages in Florida over two years, identified factors associated with their emergence using linear models, and quantified the impacts of subsequent disease outbreaks. The emergence of Bd and Ranavirus was asynchronous but overlapping, and patterns of infection and disease varied among sites and species. Ranavirus infections were more prevalent and caused lethal episodes of ranavirosis in susceptible life stages, resulting in severe population declines of striped newts (*Notophthalmus perstriatus*). In contrast, Bd often emerged after Ranavirus, and infections were milder and limited to fewer host species. Co-infections of Bd and Ranavirus were common at a single site where Bd was the dominant pathogen and Ranavirus showed consistently lower prevalence and infection intensity. Interestingly, striped newt populations persisted at this site and did not experience severe ranavirosis. Our findings provide strong evidence that Ranavirus has driven declines of threatened species in Florida, permanently changing host community composition of sites post-outbreaks. Overall, our results highlight that immune-mediated competition between Bd and Ranavirus may be associated with dampened ranavirosis and population stability. Quantifying pathogen interactions can help us design management strategies to change the course in natural outbreaks.

Non-lethal sampling: Detecting ranaviruses in UK native amphibian species (*Rana temporaria* and *Bufo bufo*)

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Whilst ranavirus infections have been involved in multiple amphibian mass mortality events across the globe, they have also produced subclinical infections in wild hosts resulting in poor detectability due to lack of disease. With recent studies highlighting the presence of ranaviruses in the international amphibian trade, it is clear that current screening tools are not suitable for the detection of early-stage infections or those resulting in no ill health and hence there is an urgent need for non-lethal screening of hosts with these types of infections in order to prevent further spread of ranavirus. My PhD research aims to investigate the reliability of non-lethal sampling methods (swabbing, clipping and environmental) at detecting (1) varying levels of ranavirus (*Frog virus 3*) at different stages of infection in common frogs (*Rana temporaria*), and (2) multiple ranaviruses (*Frog virus 3* and *Common midwife toad virus*) in common toads (*Bufo bufo*). Across both host species, non-lethal detection of ranavirus was observed in the absence of clinical signs, buccal swabbing performed the best of the non-lethal samples and in common toads, there was no difference in detectability observed between ranaviruses. The results of these studies suggest non-lethal sampling techniques have potential as a standard practice for ranavirus screening in the international trade as well as in the field.

Widespread amphibian Perkinsea infections associated with ranid hosts, cooler months, and *Ranavirus* co-infection

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Amphibians suffer from large-scale population declines globally, and emerging infectious diseases contribute heavily to these declines. Amphibian Perkinsea (Pr) causes mass mortality events in anuran species throughout the United States, but little is known about epidemiological patterns for individuals infected with Pr outside of these events. Here we sought to establish Pr infection patterns and their covariates including seasonality, attributes of the host, and coinfection with Ranavirus (Rv) of wild anuran populations in Florida. We sampled anurans using standardized methods from wetlands throughout central Florida from February 2017 to August 2019. We used quantitative (q)PCR to determine the presence and infection intensity of Pr and Rv across 1232 sampled individuals. We then implemented random forest ensemble learning models to predict the presence of both pathogens in an individual based on physiological and environmental characteristics. Perkinsea infections significantly outnumbered Rv infections across month, region, life stage and taxonomic family with only one location where Rv infections outpaced Pr infections. Perkinsea prevalence differed significantly based on host family, host species, co-infection status, location, and month, and was higher in ranid frogs, cooler months, metamorphosed individuals, and those with Rv infection. Perkinsea infection intensity differed significantly based on host species, location, life stage, and survival, and was higher in gopher frogs, the ranid frog family, and individuals collected dead. Ultimately, by understanding the epidemiological patterns of Pr in Florida, we can ensure our ability to adequately respond to the challenges this pathogen pose to anurans both in Florida, and across the continent.

Susceptibility of U.S. Pacific Northwest native amphibians to fish rhabdoviruses

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Spring viremia of carp virus (SVCV), infectious hematopoietic necrosis virus (IHNV), and viral hemorrhagic septicemia virus (VHSV) are aquatic rhabdoviruses that infect fishes. They can cause severe outbreaks in naïve fish stocks and are notifiable fish diseases listed by the World Organization for Animal Health. In 2015, SVCV was detected in distressed ornamental Chinese firebelly newts (*Cynops orientalis*) imported into the U.S. and appeared to be responsible for the observed morbidity. This discovery represented the first isolation of a rhabdovirus in an amphibian species. Susceptibility testing was initiated to better understand the potential host range of foreign (SVCV) and endemic (IHNV, VHSV) rhabdovirus strains in amphibians native to the Pacific Northwest. Pacific tree frog (*Pseudacris regilla*) tadpoles and/or larval long-toed salamander (*Ambystoma macrodactylum*) were exposed to the viruses by either intra-peritoneal injection, immersion, or co-habitation with rhabdovirus-infected fish. The highest mortality occurred in amphibians exposed to specific SVCV strains (100%). Lower mortality was observed in amphibians challenged with VHSV (43%) or IHNV (38%) strains. SVCV was detected by plaque assay and RT-qPCR assay in both amphibian species regardless of the virus exposure/transmission method, and amphibian hosts displayed measurable levels of viable virus 28 days following exposure. Comparable sample analysis of IHNV or VHSV -exposed amphibian specimens are ongoing. Results thus far indicate that these aquatic rhabdoviruses can be transmitted and cause lethal disease in amphibian species. As such, amphibians may serve as virus carriers and pose a risk for sympatric fish and amphibian populations vulnerable to IHNV, VHSV, or SVCV.

History and taxonomy of the family *Iridoviridae*

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Although most research involving viruses in the family *Iridoviridae* (generically termed iridovirids) has been conducted within the last 20 years, disease attributed to iridovirids has been known since the beginning of the 20th century when fish displaying external tumor-like growths were identified. However, for a variety of reasons, definitive identification of a viral etiology for these “tumors” was not made until lymphocystis disease virus (LCDV), genus *Lymphocystivirus*, was visualized in the 1960s by electron microscopy. In the 1950s, invertebrate iridoviruses (IIV), genera *Iridovirus* and *Chloriridovirus*, were serendipitously identified in various insect species, but subsequent molecular studies were limited. The discovery of frog virus 3 (FV3), genus *Ranavirus*, from a tumor-bearing frog in 1965, and the ability to study viral replication in defined *in vitro* cell culture systems, led to a rapid expansion of our understanding of iridovirid biology and the realization that LCDV, FV3 and the IIVs comprised a new family of dsDNA-containing viruses possessing unique characteristics. Because additional FV3-like viruses were isolated from ostensibly disease-free frogs, ranaviruses were initially thought to be relatively non-pathogenic. However, in the 1980s, it became apparent that ranaviruses triggered severe, life-threatening diseases in multiple amphibian, reptilian, and fish species. Furthermore, representatives of additional iridovirid genera targeting fish (megalocytiviruses), crustaceans (decapodiridoviruses), and daphnia (daphniairidovirus) were identified and linked with the potential to cause life-threatening diseases and adverse economic and ecological impacts. Moreover, study of iridovirus gene function identified viral-encoded immune evasion proteins, elucidated key aspects of anti-viral immunity in lower vertebrates, and suggested methods for protecting susceptible species through vaccination.

Frog and toad herpesvirus-associated proliferative skin disease: A paradigmatic example of host-pathogen-environment interaction

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The recent discovery of two novel amphibian herpesviruses has provided a unique opportunity to carry out a comparative study on two different amphibian species (*Rana temporaria* and *Bufo bufo*) affected by distinct herpesviruses (*Ranid herpesvirus* 3-RaHV3, and *Bufonid herpesvirus* 1-BfHV1, respectively) showing a remarkably overlapping pathology phenotype. Both affected species show a characteristic similar, but distinct proliferative skin disease associated with an inconspicuous cellular immune response. A thorough investigation evaluating the morphological, ultrastructural and molecular features of the disease revealed a remarkable compartmentalization of the lesions, with the viruses being confined during their lytic stage, within the upper and dispensable layers of the thickened epidermis. Strikingly, the lower layers, containing the epidermal germinal layer, would be spared, suggesting the potential for a recurrent, seasonal disease. The post-hybernation time, characterized by low temperature and the physiologically decreased host immune response, would be likely functional to the viral replication. The epidermal proliferation, might be primed by a concerted dysregulation of a set of genes conserved both in frogs and toads, part of the signaling and cell remodeling pathways. A number of putative viral immunomodulatory genes, present both in RaHV3 and BfHV1 appear to be actively transcribed during the disease. Initially identified in Switzerland, we have now evidence that these viruses and their associated disease are widsperad in Europe. Long-term investigation is necessary to clarify if these herpesviral-associated diseases might pose a risk to the survival of these species in the wide areal of distribution of the hosts and of the pathogens.

‘Suckers for amphibians’: Investigating the occurrence of leech predation on amphibians in Southern England and The Netherlands

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Whilst leech predation on amphibians has been known to occur in Europe for decades, observations of an unusually severe leech infestation affecting a common toad (*Bufo bufo*) in Southern England in 2020 led to a campaign on social media and a citizen science survey, organised by the Hampshire and Isle of Wight Amphibian and Reptile Group (HIWARG) with the Isle of Wight Reptilarium. The campaign appealed for further sightings, which revealed clusters of reports from Southern England, dating from around 2018 onwards, affecting both common toad and common frog (*Rana temporaria*). Sightings were also passed to specialists at the Garden Wildlife Health project (www.gardenwildlifehealth.org) and Buglife for further analysis. Affected amphibians were parasitized by multiple leeches adhering mostly to the ventral body and notably covering the eyes. All reports were investigated and, where possible, leeches were collected from affected animals for identification using morphological and genetic techniques. Where leeches were carefully removed, the animals appeared to make a good recovery. Results indicate that, although a number of different species of native leech may parasitize amphibians, the leeches involved in the majority of these recent incidents are likely to be a species not previously described in the UK. In 2022, the citizen science survey was extended to The Netherlands by Reptile, Amphibian and Fish Conservation Netherlands (RAVON), and leech specimens collected from three urodelan species and the Edible frog (*Pelophylax klepton esculentus*). These are pending species identification. The impact of this leech predation on amphibian population health remains uncertain.

Hepatocellular Toxicity of the metabolite emodin produced by the common buckthorn (*Rhamnus cathartica*) in green frog (*Lithobates clamitans*) tadpoles

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The secondary metabolite emodin produced by the widely distributed invasive shrub known as the common buckthorn (*Rhamnus cathartica*) has been shown to produce deformities and mortality in invertebrates, fish, and amphibian larvae. Here, we describe the effects on the liver of green frog (*Lithobates clamitans*) tadpoles after 21 d of exposure to high concentrations of emodin in a controlled environment. Histopathological analysis showed fibrosis, bile duct proliferation, hepatocellular swelling, and accumulations of flocculent material consistent with emodin within the gallbladder and bile ducts of exposed individuals. The extensive fibrosis produced probably impeded the blood flow within the portal triads, limiting the detoxification function of the liver and resulting in hepatocellular necrosis and premature death for the individuals exposed. Exposure to emodin in the environment could represent a significant threat to developing amphibian larvae and contribute to local declines of populations.

Parasitic infections of amphibians in the Pendjari Biosphere Reserve, Benin

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Habitat alteration arising from anthropogenic activities such as logging, agricultural land use and urbanization are known to have profound effects on amphibian populations. Landscape alteration for agricultural purposes increased the transmission of certain parasites in amphibians. There is a possible link between parasitic infections and amphibian population decline but for Africa, studies are lacking. The purpose of this study was to investigate the link between land-use and host and/or parasite-specific in three land-use types in Northern Benin. The amphibians were collected and post mortem examinations were carried out for nematode parasites, monogeneans, trematodes and cestodes. Results obtained show the possible influence of land-use pattern on parasite distribution. Infection with trematodes occurred predominantly in hosts collected in the Agricultural Zone, indicating that landscape alteration, pesticide use and nutrient enrichment from fertilizers in this zone may be enhancing intermediate host populations and hence parasite prevalence. Furthermore, this infection pattern may be indicative of an immunosuppressive effect of pesticides on the frogs of the Agricultural Zone. It is possible that the pesticide-contaminated environment of the Agricultural Zone inhibits the development of the free-living stages of these nematodes.

Investigating phylogenetic relationships between intradermal mites infesting amphibians in Texas

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Amphibian populations have been declining worldwide due to habitat destruction, climate change, and the introduction of invasive species. Chytridiomycosis, a fungal disease of the skin, has also had serious impacts on anuran populations globally. However, these are not the only organisms that thrive in amphibian skin. In North America, the endoparasitic larvae of the mite genus *Hannemania* also infest amphibians. In Texas, subdermal mites have previously been misidentified because their biodiversity in the region is not well described. Thus, we investigated the taxonomic diversity of *Hannemania* spp. present in different ecoregions of Texas. We collected mites at four different sites from three anuran host species (*Acris crepitans*, *Lithobates berlandieri*, and *Eleutherodactylus marnockii*). Thereafter, we sequenced the mite rDNA genes for 18S and 28S to infer their phylogenetic affinity to sequence data available in GenBank. Our phylogenetic analyses showed that some Texas mites clustered closely with sequences from *H. hepatica*, while others displayed more divergence from known sequences. Unraveling the phylogenies of these ubiquitous parasites will help us characterize their regional biodiversity and thus better understand their host-parasite dynamics.

First evidence of Ranavirus in native and invasive amphibians in Colombia

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Ranaviruses can cause mass mortality events in amphibian populations. They affect all life stages and persist in multiple hosts. We surveyed for ranaviruses using 274 liver samples collected between 2014 and 2019 in 41 localities in Colombia. These localities range from lowlands to mountaintop páramo habitats, between 114 and 3678 m. We sampled 59 native frog species and one invasive species, the American bullfrog *Lithobates catesbeianus*. Using two diagnostic techniques, quantitative PCR and End-Point PCR, we detected *Ranavirus* in 13 individuals representing six species from seven localities. This constitutes the first report of *Ranavirus* in Colombia and should set off alarms about another emerging threat to amphibian populations in Colombia.

Algae-supplemented diet enhances tolerance to ranavirus infection but also augments viral replication in wood frog larvae

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Salinization of freshwater habitats by road de-icing salt run-off, habitat disturbance or industrial waste deposition poses a growing threat to amphibians. Exposure to high salinity conditions increases ranavirus (RV) infection intensity; however, elevated salinity may also result in increased nutrient availability, which could increase infection tolerance. In this study, we explored the effects of these combined effects on outcomes of ranavirus infection in larval wood frogs (*Rana sylvatica*), a species susceptible to ranavirus infections and highly sensitive to elevations in salinity. We reared larvae in outdoor semi-natural mesocosms with two salinities (300 and 1200 mSi/cm) crossed with two diets: baseline diet (leaf litter/alfalfa pellets), baseline + *Chorella* algae. When larvae reached Gosner 35-37, they were exposed to either culture media or ranavirus in individual containers for 6 d. Prior to infection, high salinity and algae supplementation increased weight in an additive manner. Elevated salinity, and to a lesser degree algae supplementation, also increased RV titers (qPCR assay). Relative to mock exposure, RV-exposed larvae lost weight and accelerated development in the baseline diet, but these effects were not exhibited in the algae-supplemented diet. As expected, salinity and nutrition augmented body size, although likely through different mechanisms, salinity increased RV titers, and algae supplementation enhanced tolerance to high RV loads; but counter to expectations, virus replication increased in larvae fed higher quality diet. Future research is needed to understand the mechanisms underlying diet-dependent responses to infections, and whether this algae-mediated increase in tolerance results in a higher likelihood of surviving RV infection.

Anti-*Batrachochytrium* immunity and chytrid immune evasion

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The *Batrachochytrium* fungi, *B. dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*), cause the skin disease chytridiomycosis linked to global amphibian population declines and some species extinctions. Amphibians have complex immune defenses including a protective skin microbiome, antimicrobial peptides released from the granular glands in the skin, and lymphocyte-mediated cellular and antibody responses. In spite of these immune defense capabilities, chytridiomycosis is still a frequently lethal disease. Research in the last two decades will be summarized to show our current understanding of mechanisms of immune protection but also evidence for immune evasion by the pathogen. The fungal immune evasion strategy appears to include the release of immunomodulatory metabolites as well as a factor associated with the fungal cell wall that inhibit lymphocytes and macrophages.

Inhibition of amphibian lymphocytes by cells wall components of *Batrachochytrium dendrobatidis*

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Amphibians continue to decline in many parts of the world due to the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). While amphibians have an excellent set of immune defenses, *Bd* is able to evade those defenses to cause a fatal disease. Previous studies have revealed the presence of three small metabolites released by *Bd* that alone or together inhibit lymphocyte responses. We hypothesized that another molecule or molecules associated with the fungal cell wall is also inhibitory for lymphocytes. In a series of experiments, mature *Bd* cells were fragmented and washed to remove small molecules leaving fragments enriched in cell wall material. These fragmented cell walls strongly inhibited proliferation of both amphibian lymphocytes and a human lymphocyte cell line. Cell wall preparations from a second chytrid fungus, *Homolaphlyctis polyrhiza* (*Hp*), were also strongly inhibitory, suggesting that the inhibitory factors are shared by more than one chytrid fungus. Treatment of mature *Bd* and *Hp* sporangia with a mixture of carbohydrase enzymes resulted in reduced lymphocyte inhibition by the cell wall fragments from both *Bd* and *Hp* suggesting that the active factor is a carbohydrate or a protein-carbohydrate complex in the cell wall. Ongoing studies aim to further understand the nature of the lymphocyte inhibitory factors associated with the chytrid cell wall. Support: NSF IOS 1557634 and IOS-2011291.

Antimicrobial peptide defenses of the iconic coqui frogs of Puerto Rico against *Batrachochytrium dendrobatidis*

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The pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), is widespread in Puerto Rico and has been linked to population declines of the common coqui frog, *Eleutherodactylus coqui*. To better understand how this species persists with the continuing presence of *Bd* in the environment, we collected skin secretions from juvenile and adult frogs during both wet and dry seasons in upland forests at El Yunque. The skin secretions were enriched for hydrophobic peptides, quantified, tested for their capacity to inhibit growth of *Bd* in culture, and examined by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry. Peptides recovered from adult males and females averaged from about 15-20 µg/g, and there were no significant differences between the amounts of peptides recovered from males versus females or from all adults by season during both wet and dry seasons. Juvenile frogs, however tended to produce significantly greater amounts of recovered peptides per gram body weight than adults in the months designated as wet months, averaging about 109 µg/g, with less produced in the dry months at about 67 µg/g. Although the amounts of peptides are quite low, preliminary results suggest that the peptides can inhibit growth of *Bd* zoospores at concentrations greater than 25 µg/ml in culture. MALDI-TOF mass spectrometry revealed two peptides of mass-to-charge ratio (m/z) of 1250 and 1477 that were correlated with inhibitory activity suggesting that this species may have at least two identifiable antimicrobial peptides with activity against *Bd*.
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Relationship of chytrid infection and environmental microbes with a pathogen-protective trait from Appalachian salamanders: A view from a microbiome network perspective

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A growing focus in amphibian disease ecology is understanding the role of environmental microbiomes on host-pathogen dynamics. The amphibian skin microbiome is an integral component of the immune system, serving as a barrier to pathogen infection and disease progression. We studied the relationship of the chytrid pathogen *Batrachochytrium dendrobatidis* (Bd) and bacteria from three environments (pond, stream and forest) with host skin-associated bacteria in three salamander species (*Nothophtalmus viridescens* [n = 77], *Eurycea bislineata* [n = 53] and *Plethodon cinereus* [n = 57]) from a 300 km range in the Central Appalachians, USA. Bd infection was quantified using qPCR, and skin and environmental bacterial communities using 16S rRNA gene amplicon sequencing. We incorporated null-based assembly models to identify the strength of environment on structuring skin-bacterial communities. We used co-occurrence networks to investigate bacterial associations within salamander species and between Bd- uninfected and infected individuals, and the importance of potentially Bd-protective and environmental bacteria in microbiome networks (i.e., the most interactive bacteria, "hub taxa"). Bd-infected *N. viridescens* had the highest proportion of negative associations in the bacterial networks (40%; 946/1554), while Bd- uninfected *N. viridescens* and Bd- uninfected *P. cinereus* had more positive associations (82% [995/1209] and 70% [951/1354], respectively). We observed putative Bd-inhibitory bacteria in the three environments. Putative Bd-inhibitory bacteria were shared between environment and salamander species, ranging from 27 to 29 bacteria. We detected both putative Bd-inhibitory and environment bacteria as hub taxa across salamanders. These results suggest environments serve as reservoirs of potential protective bacteria. Interestingly, our study suggests that Bd infection may lead to competition and dysbiosis in the microbiome or vice versa, and that environmental reservoirs of functional bacteria are likely important to maintaining microbiome structure. Our findings improve understanding of complex host-microbiome-environment-pathogen dynamics on salamanders.

Host-defense peptides and skin microbiota in frogs and salamanders

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Animal immune systems establish intimate relationships with host microbiota. Host-defense peptides are major players in this relationship, in which they can kill pathogenic microbes, while allowing mutualistic or commensal microbes to persist. Our aim was to quantify skin microbial diversity, peptide diversity and their interactions in wild red-backed salamanders (*Plethodon cinereus*), two-lined salamanders (*Eurycea bislineata*), and eastern newts (*Notophthalmus viridiscens*) and one laboratory frog species, African clawed-frog (*Xenopus laevis*). Using 16S rRNA gene amplicon sequencing, we found distinct skin microbiomes among amphibian species. For peptides, it is unclear how to stimulate peptide release in salamanders. Therefore, we quantified peptides from skin soaks of acetylcholine-stimulated and control salamanders, and used mass spectrometry coupled with transcriptomics to identify candidate host-defense peptides. Acetylcholine injections led to higher peptide yields and greater diversity compared to control salamanders. Each salamander species had 10 – 20 transcriptionally active candidate host-defense genes, each potentially encoding multiple peptides; one Ascaphin-like antimicrobial peptide was detected in all three salamander species. In *X. laevis* (n = 7), we found that 31 prevalent bacteria showed correlations with peptides in eight host-defense peptide classes, with each bacterial taxa generally exhibiting the same responses to all peptides. Our findings show that salamanders, like frogs, have diverse repertoires of defense peptides that interact with diverse bacterial communities. While these peptides are often believed to be antimicrobial, our findings indicate they may also be promicrobial. Insight into the skin microbiome-peptide interface is critical to understanding how balanced or sometimes imbalanced interactions impact disease outcomes.

Three to tango: Linking pathogen-microbiome-host interactions to explain amphibian population dynamics

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Symbiotic interactions can determine the evolution of host species, influencing genetic variation through, for instance, selection processes and changes in demography. In the context of strong selective pressures such as those imposed by infectious diseases, symbionts providing host defenses could contribute to increasing host fitness upon pathogen emergence. We generated genome-wide data of an amphibian species to explore evolutionary pressures of two skin symbionts: the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and an antifungal bacterial symbiont. We study an *Eleutherodactylus coqui* frog population, for which *Bd* detection dates back to 1976. Despite population persistence, we found evidence of decreased effective population size using demographic modeling with coalescent simulations. Likewise, we explored host genetic associations with infection status, beneficial bacteria abundance, and overall microbiome diversity through structural equation models. We uncovered relatively lower nucleotide diversity in the infected frogs and potential heterozygote advantage to recruit beneficial symbiont and fight infections. Also, we discovered a potential offsetting effect of host heterozygosity-fitness correlations, pointing to different eco-evolutionary processes among the three species. We showed that evolutionary pressures not only arise from the pathogen but also from the beneficial symbiont, and both processes shaped host genetics. Our results advanced the knowledge about multipartite symbiotic relationships providing a framework to model these eco-evolutionary dynamics. To further characterize this interaction, we are applying an integrative molecular approach, analyzing pathogen-microbiome-host functional genomics through triple RNA-Seq, host telomere length attrition as a disease biomarker, and immunogenetic data from coqui frog populations under different environmental conditions.

Xenopus laevis Research Resource for Immunobiology (*XLRRI*): tools, reagents, cell lines, genetically modified animals and pathogens, resources, assistance, and training for studying amphibian immunity

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For almost 20 years the XLRRI has served the scientific community by developing and safeguarding *X. laevis* strains, clones, genetically modified transgenic (Tg) lines, cell lines and reagents, as well as by assisting and training established and future researchers as well as students. The XLRRI in collaboration with Xenbase curators, NCBI Refseq staff and several labs is working on improving the annotation of immune genes in the two Xenopus genomes. Of particular interest for infectious diseases plaguing amphibians, the facility is developing reverse genetic technology such as RNA silencing and CRISPR/Cas9 genome editing to establish various immune deficient *X. laevis* lines. It is noteworthy that these reverse genetic methods can be applied to other amphibian species. These reverse genetic approaches are complemented by implementing and improving methodologies to investigate immune responses against pathogens such as immune cell proliferation responses with EDU or CFSE, antibody response by ELISA, pathogen loads determination (colony and plaque assays, TCID50), and primary immune cell cultures. To further monitoring immune responses, a wide panel of validated primers specific for selected immunologically-relevant genes is maintained and regularly updated. Tools developed and made available by the XLRRI include wild type and recombinant pathogens such as ranavirus and mycobacteria. Furthermore, to contribute minimizing animal use and suffering, we are engineering and producing transfected and deficient cell lines, expression vectors, recombinant tagged immune molecules and MHC tetramers. Information about the resource, animals, tools, and protocols is available at https://www.urmc.rochester.edu/microbiology-immunology/research/xenopus-laevis.aspx.

Potential role of bacterial and fungal co-infections on ranaviral persistence and reactivation

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Ranaviruses such as Frog virus 3 (FV3) are causing emerging infectious diseases leading to extensive morbidity and mortality of amphibians and other aquatic vertebrates worldwide. Notably, ranaviruses can persist in asymptomatic hosts. We have established *Xenopus laevis* as a reliable experimental organism for studying host interactions with FV3. Despite rapid viral clearance by the *X. laevis* adult robust immune system, FV3 persists quiescent in macrophages of otherwise healthy asymptomatic frogs. We have shown that inflammation induced by intraperitoneal injection of heat-killed (HK) *E. coli* reactivates FV3 in infected asymptomatic frogs, leading to lethal systemic infection. Similar FV3 reactivation was obtained with HK-mycobacteria (*M. marinum*), another bacterial pathogen. Since Toll-like receptors (TLRs) are critical for recognizing microbial molecular patterns, we investigated their involvement in inflammation-induced FV3 reactivation. Among the 10 different TLR genes tested, only TLR5 and TLR22, both recognizing bacterial products, showed significant differential expression following FV3 infection and HK-bacteria stimulation. However, only the TLR5 ligand flagellin induced FV3 reactivation in macrophages *in vitro* and *in vivo*. Thus, TLR5 is critical to trigger FV3 reactivation. We also investigated whether chytrid fungus (Bd), which is often co-infect hosts with ranaviruses affects ranaviral persistence. While stimulation of with HK Bd flagellated zoospores did not reactivate FV3 in asymptmatic infected frogs, FV3/macrophage interaction was altered by pre-exposure with HK Bd. Collectively, these data suggests a role for secondary bacterial and possibly fungy infections and/or microbiome alterations (stress, pollution) in initiating sudden deadly disease outbreaks in amphibian populations with detectable persistent asymptomatic ranavirus.

Water pollution and toxicity increase the risk of Chytridiomycosis in Mexican amphibians

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Chytridiomycosis is causing the decline and extinction of several amphibian populations worldwide. The disease is caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), a multihost pathogen living in freshwater habitats. While several environmental factors including water availability, have been associated with the prevalence of *Bd* and its virulence, the effects of water quality on chytridiomycosis are not clear yet. Here, we analyzed the relationship between water quality and the presence of *Bd* using a spatial data mining framework with published water quality data from 4,202 distinct lentic and lotic water bodies and *Bd* records of aquatic amphibians from 2012 to 2021 in Mexico. Our Bayesian model was successful in identifying sites where *Bd* has been previously reported and *Bd* presence was positively associated with low water quality, i.e., polluted water with urban and industrial waste and we extended these findings to infer possible suitable areas for *Bd* in Mexico. We also recommend that actions to reduce water pollution become an integral part of public policies to prevent the further spread of chytridiomycosis and protect amphibians from this deadly pathogen.

Surveillance and assessment of skin keratin abundance associated with *Batrachochytrium dendrobatidis* prevalence in red-spotted newts (*Notophthalmus viridescens viridescens*) and mole salamanders (*Ambystoma talpoideum*)

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Surveillance for *Batrachochytrium dendrobatidis* (*Bd*) in amphibians and understanding risk factors associated with detection and infection are necessary for mitigating the potential detrimental impacts of *Bd* on amphibian populations. The prevalence of *Bd* at two sites in Georgia, USA, Whitehall Experimental Forest in the Piedmont region and Sandhills Wildlife Management Area in the Upper Coastal Plain region, was investigated from September 2020 – April 2021. The potential association between *Bd* detection prevalence and skin keratin abundance between the red-spotted newt (*Notophthalmus viridescens viridescens*) and mole salamander (*Ambystoma talpoideum*) was also investigated. The prevalence of *Bd* detection varied by season, location, male sexual characteristics, and species sampled. The overall prevalence of *Bd* detection was 25.1% (n=553) with the highest prevalence in adult red-spotted newts (39.1%, n=220) and paedomorphic mole salamanders (27.7%, n=94). Marked seasonality was noted from a low in September 2020 (n=21, 0%) to a peak in December 2020 (n=84, 44.0%). The skin keratin abundance was scored (scale 0-3) based on histologic examination of skin samples from the caudal abdominal cavity and hindlimbs. Keratin abundance was higher in adult red-spotted newts (score=1.69) compared to paedomorphic mole salamanders (score=0). These data support the purported role of red-spotted newts as a reservoir of *Bd*, along with seasonality of Bd detections, and higher association between skin keratin abundance and *Bd* detection prevalence. The novel finding of relatively high *Bd* prevalence in mole salamanders not only suggests that they may be reservoirs but warrants additional investigation into their susceptibility to associated morbidity and mortality.

The use of probiotic applications in early life stages to mitigate *Batrachochytrium dendrobatidis* infections in *Rana luteiventris* (Columbia spotted frogs)

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Chytridiomycosis, an amphibian skin disease caused by a chytrid fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), has been linked to global amphibian declines, although some amphibian populations are resistant to infection due to symbiotic antifungal skin bacteria. Attempts to develop probiotics have been inconsistently successful because bacteria fail to persist on the skin, and studies have largely been conducted on adult and juvenile amphibians, despite amphibians having fewer known mechanisms for microbiome regulation prior to metamorphosis. We hypothesize that probiotics will persist longer and thus be more effective against pathogen infection if they are applied at an early life stage. First, to identify potential probiotics and to examine the correlation between skin microbiomes and Bd infection status, we conducted a field study of *Rana luteiventris* in eastern Washington and northern Idaho. We found a higher abundance of *Pseudomonas* species on uninfected frogs than infected frogs, and *Pseudomonas* bacteria have been shown to be anti-Bd, suggesting some *Pseudomonas* bacteria may limit infection. We conducted a laboratory study to test the effectiveness of Pseudomonas sp. probiotic applications to *Rana luteiventris* eggs, tadpoles immediately after hatching, tadpoles one week after hatching, and newly metamorphosed frogs at two temperature regimes based on current and modeled future temperatures. We exposed all treatment groups to Bd and swabbed to collect skin microbiome samples. To evaluate the host-microbiome-pathogen dynamics, we will analyze 16S rRNA gene amplicon sequencing and Bd qPCR data. This research will inform future probiotic strategies to combat a pathogen contributing to significant amphibian declines.

Developing gnotobiotic tadpoles to investigate the influence of the microbiome on the amphibian immune system

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The equilibrium model of immunity suggests that early life conditions, especially exposure to microbes, prime the immune system for secondary exposures later in life. Indeed, studies show that there are critical time periods in development where exposure to a sufficiently diverse microbiome is key for optimal immune system efficacy. As such, pinpointing the specific mechanisms underpinning host-pathogen-microbiome interactions is an essential step in understanding responses to severe infectious diseases. We are developing a gnotobiotic system using *Xenopus laevis* tadpoles to investigate the microbiome's role in amphibian immune development and later susceptibility to infectious disease. We first reduced the microbiome in *X. laevis* embryos with a mixture of antimicrobials and subsequently reared the tadpoles in sterile conditions until metamorphosis. We verified the reduction in the richness of the microbiome using 16s microbiome sequencing methods and challenged froglets with *Batrachochytrium dendrobatidis* to determine the relative susceptibility to disease as a measure of immunity. Understanding the mechanisms of amphibian immune system development will ultimately help develop effective conservation efforts for at-risk species as well as create effective therapeutic strategies.

The microbiomes of adult Eastern Newts (*Notophthalmus viridescens*) are distinct and dynamic after two exposures to *Batrachochytrium salamandrivorans*

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The repertoire of defenses a host may use against a pathogen is often thought of in the context of innate and adaptive immunity, with all factors produced by the host. However, new perspectives are beginning to examine host symbionts, such as the microbiome, as part of an individual's defense against disease. Chytridiomycosis, caused by the fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) is characterized by an infection of the skin, and has resulted in losses in global amphibian biodiversity. Thus, understanding the factors that may influence the development of resistance to or tolerance of disease, such as interactions with microbial symbionts, is critical to developing prevention measures and understanding risk to a population. Adult *Notophthalmus viridescens* were exposed twice to Bsal and were swabbed weekly to examine the influence of disease on the skin microbiome. Upon more than one exposure, individuals had distinct microbial communities compared to those only exposed once or naïve to disease, including higher predicted antifungal function in re-exposed individuals. Metrics of diversity were dynamic through time, and also followed distinctive trajectories through time depending on exposure history and survival outcome. These data point towards a microbiome that is responsive to disease and may be adapting to interact with the pathogen and host in this new context. Understanding the nature of this response may be critical for developing preventative, probiotic treatments to protect existing biodiversity. Support: NSF 1814520.

The impacts of peptide secretions and environment on the skin microbiome of the Northern leopard frog, *Rana pipiens*

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To predict disease outcomes and devise effective treatments for chytridiomycosis, it is essential to understand how amphibians interact with defensive microbes in their skin mucosa. Many amphibians produce an array of skin defense peptides (antimicrobial peptides; AMPs) which play an important role in constitutive defense against invading pathogens. We hypothesized that these compounds function as regulators of symbiotic microbial communities on amphibian skin. To study AMP-microbe interactions in-vivo, we housed three groups of Northern leopard frogs (*Rana pipiens*) in each of two microbially distinct habitats characterized by minimal or enriched substrate. One group had AMPs depleted by two doses of norepinephrine that elicited secretion from skin granular glands, while another group remained with peptides intact through sham injections. A third group was exposed to sterilized peptides from the AMP-depleted group to control for effects of AMP release during norepinephrine injection. In the nine weeks following treatment, we tracked responses of the host microbiome, associated antifungal function, and dissimilarity from environmental microbiota. Preliminary results suggest that host microbiomes are strongly influenced by habitat, and robust to disturbance by peptide secretions. Further analysis is underway to examine differential peptide expression in different environments as a mechanism for microbiome resilience, and to test the microbiome antifungal function following treatments. These results will inform our understanding of host-microbiome regulation and clarify the significance of external sources of microbiota and host defense peptides in regulating amphibian skin microbiota including pathogens.

A Mucosal Medium to Refine Assessment of Growth Inhibition of *Batrachochytrium dendrobatidis* by Skin-Associated Microbiota

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Numerous amphibian-associated skin bacteria have been shown to produce secondary metabolites inhibitory to the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Bd transitions from its motile life stage to a sessile reproductive phase within minutes of zoospores arrival at the host. Mucins present on amphibian skin induce rapid encystation of Bd and synchronize development upon arrival at the amphibian host. Rapid encystment of a Bd zoospore may serve to reduce time spent vulnerable to bacterial metabolites. Currently, anti-fungal function of amphibian skin bacteria is assessed in vitro by measuring growth rate of live Bd zoospores exposed to sterile metabolites collected from bacterial cultures. As a refinement, mucins added to media may create testing conditions more representative of those found on amphibian skin, and may result in greater accuracy in estimates of anti-fungal function of skin microbiota. Results of Bd inhibition with and without mucin when challenged against known anti-Bd metabolites will be reported. Bioaugmentation candidates from the anti-Bd database may require further challenge trials against Bd to show that metabolites remain inhibitory when zoospore encystation rate is increased due to mucin exposure.

Insulin goes viral: The role of *Iridoviridae* viral insulin/IGF-1 like peptides in a host context infection

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Viruses have developed several mimicry mechanisms to manipulate the host's biology including immune responses and metabolism. We recently showed for the first time that six viruses in *Iridoviridae* family encode genes mimicking human insulin. We previously showed that these viral insulin/IGF-1 like peptides (***VILPs***) can bind to human insulin and IGF-1 receptors and further stimulate post-receptor signaling. VILPs also stimulate glucose uptake and proliferation in mammalian cells and lower the blood glucose in mice. In this project, we hypothesize that Grouper Iridovirus (GIV, one of the VILP carrying viruses) VILPs will manipulate host insulin/IGF signaling and thereby, the downstream functions. GIV is one of the most serious pathogens in mariculture that causes high mortality rates in finfish, specifically in grouper species farms. To this end, we first investigated the GIV VILP properties compared to insulin and IGF-1 on grouper kidney (GK) cells and AB9 zebrafish cells. We showed that VILPs are as potent as insulin in its activity on insulin/IGF signaling. When we examined the viral kinetics, we showed that GIV32 gene, encoding VILP, is an early gene in both cell types. VILPs are not a part of the viral particle, however, they are secreted during the viral cycle and elicit an autocrine and paracrine effect stimulating insulin/IGF signaling.Future directions will lead to understanding if VILPs are essential for viral replication. Infection on in vivo fish models will provide clues about VILPs role in the pathogenesis of VILP-carrying viruses.

Could genomic approaches unlock the key to saving the iconic Southern Corroboree frog

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Functionally extinct in the wild, the Southern Corroboree frog (*Pseudophryne corroboree*) relies solely on captive management and breeding to maintain their existence. Their main threat, like so many other amphibians, is the deadly amphibian chytrid fungus. With limited successful management strategies to control chytrid in wild amphibians, hope for the survival of this species outside of captivity is grim. There is one management strategy which holds hope, that is improving host resistance through genetic intervention. One method to achieve this is to breed for targeted genetic improvements. This has been successfully achieved in companion animals and livestock but has yet to be applied to wildlife. We are in a unique position to evaluate the efficacy of this approach in *P. corroboree*, as the entire extent of the genetic diversity of this species is held in captivity and a successful breeding program is already in place. To do this, traits correlated with resistance/susceptibility first need to be identified, to do this, we are conducting an exposure experiment. Once traits have been identified, we will then perform a genome wide association study, to correlate phenotype to genotype. Using the phenotype-genotype information gathered, we hope to implement a breeding strategy across the captive colonies to increase beneficial alleles, and decrease deleterious alleles, so that released animals will be more tolerant in the presence of chytrid. Alternatively, genetic information identified could be used to develop synthetic biology approaches to improve disease resilience. While this project is currently in the pilot stage, I will present initial phenotypic data, and use previously published genomic information on this species to give an overview of the planned experimental methodology.

Modeling the amphibian chytrid fungus growth dynamics using optical density, MTT assays, and zoospore count data

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Collecting enough data to parameterize mathematical or statistical models used to explore biological processes can be difficult. Data can be time-consuming and/or expensive to collect, and therefore, a balance between resources (i.e., time and money) and collecting enough data to fit the model is needed. Using multiple data sources could help parameterize models with limited data or reduce the need to collect more expensive data. In this study, we modeled the growth of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), a pathogenic fungus that infects amphibians, by combining three types of data: zoospore counts, MTT assays (a viability assay), and optical density (OD) measurements. Models of Bd growth dynamics commonly incorporate zoospore counts, which can be time-intensive to collect, and uncommonly include data about the zoosporangia life stage. We aimed to limit the zoospore count data needed to fit the model and better understand Bd growth dynamics by using OD and MTT assay data. We simulated all three types of data based on experimental data and then fitted the Bd growth model with a range of zoospore count data scenarios. Unsurprising, we found that the more data used to fit the model, the better. However, using MTT assay and OD data to supplement zoospore count data improved model accuracy, especially dynamics related to the sessile zoosporangia stage of the fungus. This study shows that using multiple types of data in a model can improve our ability to estimate model parameters and biological processes.

Amphibian disease immunogenetics: MHC, Bd, and beyond

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Acquired immune systems are encoded by the most polymorphic genes in the vertebrate genome, likely due to ongoing selective pressure for novel immune variants capable of eliminating a wide array of pathogen threats. While specific coevolutionary relationships between immune variants, pathogens, and disease phenotypes are well-characterized in a variety of mammalian taxa, we know far less about immunity in ectothermic vertebrates. Relating disease susceptibility to immune variation in amphibians and reptiles therefore often relies on targeted sequencing of candidate immune genes identified from mammalian systems. Additionally, novel features of ectotherm immunity can be identified using genomic approaches to explore immune gene expression or to identify regions of the genome under selection across disease phenotypes. Characterizing these immunogenetic features is not only critical to more broadly understanding vertebrate immune system evolution, but also to evaluating the potential for adaptation in disease-threatened taxa. Here, I illustrate how these approaches have been used to understand immunogenetic adaptation to the fungal disease chytridiomycosis in *Rana yavapaiensis*, a frog species with varying susceptibility at both the population and individual level. I discuss experimental and field studies that find relationships between susceptibility to chytridiomycosis and Major Histocompatibility Complex immunogenetic variation, immune gene expression, and genome-wide outlier loci. I also touch on other ectothermic vertebrate diseases and immunogenetic relationships to address whether general patterns are becoming evident. While many questions are outstanding and methodological limitations persist, these studies highlight how functional genetic datasets are critical for understanding immunogenetic adaptation to pathogens in natural populations of non-model organisms.

Amphibian infection tolerance and resistance in the context of chytridiomycosis

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Animal defenses against infection involve two distinct but complementary mechanisms: tolerance and resistance. Tolerance measures the animal host's ability to limit detrimental effects from a given infection, whereas resistance is the ability to limit the intensity of that infection. Tolerance is a valuable defense for highly prevalent, chronic, persistent or endemic infections where mitigation strategies based on traditional resistance mechanisms are less likely to be effective, or less evolutionarily stable. Selective breeding of amphibians for enhanced tolerance to *Batrachochytrium dendrobatidis* (hereafter Bd) has been suggested as a valuable alternative approach in the context of mitigating the impacts of the devastating fungal disease, chytridiomycosis, longer term. Here, we (1) define infection tolerance and resistance in the context of chytridiomycosis, (2) synthesize current research on tolerance and resistance to chytridiomycosis including across different scales and study types, study limitations and challenges, and avenues for future research, (3) evaluate current evidence for variation in tolerance versus resistance to Bd, (4) explore evidence for mechanisms underlying tolerance versus resistance to Bd, (5) outline ecological, epidemiological and evolutionary implications of tolerance versus resistance to chytridiomycosis across life stages, species, and between populations and individuals, and (6) explore outstanding questions including whether a negative correlation between resistance and tolerance should occur, the relative importance of tolerance versus resistance in defense against Bd, and potential costs of both tolerance and resistance. Improving our understanding of infection tolerance and resistance greatly broadens our repertoire of strategies for mitigating the ongoing impacts of emerging infectious diseases such as chytridiomycosis.

Understanding *Xenopus laevis* mast cells: Sentinels of antifungal immunity

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The global decline of over 500 amphibian species across six continents is driven largely by emerging pathogens like the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). These massive die-offs also threaten the thousands of other species in their interdependent ecosystems. Unlike other fungal infections that progress to distal organs, *Bd* is confined to the skin and causes death by interfering with essential ion regulation. Yet almost nothing is known about the contribution of skin-resident immune cells to frog immune responses against *Bd*. We recently reported that amphibians possess novel mast cell-like granulocytes in their skin, and that these cells are involved in the *Xenopus laevis* African clawed frog antimicrobial responses. Although conventional mast cells are known to be critically important to barrier defenses across taxa, their roles have yet to be explored in the amphibian skin. Presently, we produced the mast cell growth factor, Kit ligand (Kitl, SCF), in recombinant form and validated its utility in generating *Xenopus*-specific mast cells *in vitro* and enriching them *in vivo*. Using these approaches, we define the immune roles of amphibian mast cells and disentangle their complex interactions with the deadly *Bd* fungus in the context of the skin microenvironment. Thus, this work not only provides critical insight into amphibian skin immunity, precise mast cell antimicrobial actions, and mast cell interactions with fungi, it also offers abundant opportunity to further examine the roles of these versatile cells in many other contexts of amphibian cell biology, physiology, and immunity.

Enhanced survival in Eastern Newts after a second exposure to *Batrachochytrium salamandrivorans*

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Amphibian health and survival in many parts of the world are affected by the presence of two pathogenic chytrid fungi in the genus *Batrachochytrium*. *Batrachochytrium salamandrivorans* (*Bsal*) has been linked to devastating losses of fire salamanders in the Netherlands and Belgium. The southeastern region of the United States of America is especially rich in salamander diversity, and there is great concern for survival of these endemic species if *Bsal* is accidentally introduced. The Eastern Newt (*Notophthalmus viridescens*) is a widely abundant species in the Eastern United States and has previously been shown to be susceptible to *Bsal*. Little is known about the capacity of Eastern Newts to develop an immune defense against *Bsal*. Here we compared survival and pathogen burden in Eastern Newts after a first and second exposure to low numbers of *Bsal* zoospores at 17.5ºC, a temperature suitable for survival of both pathogen and host. 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 to newts that were exposed only once. These results suggest that prior exposure and clearance provides some protection of survivors from a second exposure. Ongoing studies will examine possible immune defense mechanisms in the skin mucus, including defensive peptides and a possible adaptive microbiome, that would explain the improved survival. Support: NSF 1814520.

Endogenous retroviruses augment amphibian (*Xenopus laevis*) tadpole antiviral protection

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The global amphibian declines are compounded by infections with members of the *Ranavirus* genus such as Frog Virus 3 (FV3). Pre-metamorphic anuran amphibians are believed to be significantly more susceptible to FV3 while this pathogen targets the kidneys of both pre- and post-metamorphic animals. Paradoxically, FV3-challenged *Xenopus laevis* tadpoles exhibit lower kidney viral loads than adult frogs. Presently, we demonstrate that *X. laevis* tadpoles are intrinsically more resistant to FV3 kidney infections than cohort-matched metamorphic and post-metamorphic froglets and that this resistance is epigenetically conferred by endogenous retroviruses (ERVs). Using a *X. laevis* kidney-derived cell line, we show that enhancing ERV gene expression activates cellular double-stranded RNA-sensing pathways, resulting in elevated mRNA levels of antiviral interferon (IFN) cytokines and thus greater anti-FV3 protection. Finally, our results indicate that large esterase-positive myeloid-lineage cells are responsible for the elevated ERV/IFN axis seen in the tadpole kidneys, wherein CRISPR-Cas9-mediated ablation of colony-stimulating factor 3-mediated homing to this tissue significantly abolished the expression of both ERVs and IFNs. We believe that this manuscript marks an important step forward in understanding the mechanisms controlling amphibian antiviral defenses and thus susceptibility and resistance to pathogens like FV3.

A snapshot of *Batrachochytrium dendrobatidis* (Bd) genetic diversity across the continental United States

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The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) has had variable impacts on amphibian populations in the continental United States (US). Several species have suffered catastrophic population declines, while others coexist with Bd in an enzootic state. Additionally, some amphibian populations that once declined are now persisting or recovering, transitioning from epizootic to enzootic Bd dynamics. The underlying mechanisms that allow for such heterogeneity in disease outcomes are largely unknown. One hypothesis is that some amphibian populations have coexisted with Bd (or with certain Bd lineages) for longer periods of time and have therefore developed resistance or tolerance, while other populations were previously naïve (either to Bd in general or to specific Bd lineages) and were therefore highly susceptible to a novel pathogen introduction. Here, we compile over 500 published and unpublished Bd genotypes collected from 2011-2019 spanning the continental US. These samples represent regions that have had vastly different disease outcomes. Using this robust dataset, we 1) map Bd lineages across the US, 2) use phylodynamic methods to investigate historical Bd dynamics in each region, and 3) investigate potential drivers of Bd genetic diversity (i.e., host species, geography, environment). We show a remarkable amount of genetic diversity with the global panzootic lineage of Bd (Bd-GPL) and highlight unique and shared patterns of Bd genetic diversity across the region. Our study provides a comprehensive snapshot of Bd genetic diversity in the US and offers new hypotheses for how these patterns have been shaped over the last century.

Unexpected effects of tropical seasonal environmental factors in the response of *Eleutherodactylus coqui* to *Bd* infections

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Previous studies (2005-2009) on the dynamics of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) on *Eleutherodactylus coqui* in Puerto Rico revealed that these terrestrial direct-developing frogs were more vulnerable to chytridiomycosis during the dry season, which coincides with the cooler temperatures. Although the association of *Bd* with lower temperatures has been reported for many species of amphibians, the mechanism that explains how a synergy with drought may exacerbate disease risk has only been documented for *E. coqui*. Ten years later under the effect of global climate change, and after hurricane Maria devastated the island in 2017, we re-examined the effect of seasonality on this host´s response to *Bd* infection. We found that despite a significant increase in *Bd* infection loads one-year post-hurricane, coqui populations prevailed. At present, the forest canopy has recuperated and microhabitat temperatures are comparable to pre-hurricane times. *Bd*-monitoring over 32 months (2020-2022) revealed that infection loads increase during the cool-dry season and decrease when it is warmer and wetter. In contrast, *Bd* prevalence is lower in the cool-dry season probably because during these harsh times *Bd*-infected frogs may be too sick with chytridiomycosis to be active at night. A trade-off between being active (feeding and reproduction) and remaining in retreat sites may contribute to host survivorship until the next favorable season. Although these findings reiterate a cyclic seasonal pattern in chytridiomycosis that warrants persistence of coqui frogs, this may occur at a cost to fitness that may lead to population declines under circumstances of extreme drought.

A perspective into the relationships between amphibian (*Xenopus laevis*) myeloid cell subsets

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Macrophage (Mϕs)-lineage cells are integral to the immune defenses of all vertebrates, including amphibians. Across vertebrates, Mf differentiation and functionality depend on activation of the colony-stimulating factor-1 (CSF-1) receptor by CSF-1 and intreluekin-34 (IL-34) cytokines. Our findings to date indicate that amphibian (*Xenopus laevis*) Mfs differentiated with recombinant (r)CSF-1 and rIL-34 are morphologically, transcriptionally, and functionally distinct. CSF-1-Mϕs confer susceptibility to intracellular pathogens such as *Mycobacterium marinum* and the Frog Virus 3 (FV3) ranavirus whereas rIL-34-Mϕs offer protection against both infectious agents. Mammalian Mϕs share common progenitor population(s) with dendritic cells (DCs), which rely on fms-like receptor tyrosine kinase 3 (FLT3L) for differentiation and *X. laevis* IL-34-Mϕs exhibit many features attributed to mammalian DCs. As such, presently we compared *X. laevis* CSF-1- and IL-34-Mϕs with FLT3L-derived *X. laevis* DCs. Our transcriptional and functional analyses indicated that indeed the frog IL-34-Mϕs and FLT3L-DCs possessed many commonalities over CSF-1-Mϕs. Vertebrate Mϕs and DCs play important roles in antigen presentation and as such, are also involved during allogenic responses. In this context, our findings indicate that IL-34-Mϕs and FLT3L-DCs are better at eliciting mixed leukocyte responses *in vitro* and generating *in vivo* memory immune responses against both *M. marinum* and FV3 than CSF-1-Mϕs. Further analyses of non-vertebrate myelopoiesis will grant unique new perspectives into the evolutionarily converging and diverged pathways of Mϕ and DC functional differentiation.

Interorgan dynamics during the amphibian inflammation: Roles for the liver and the spleen in immune proteins gene expression

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Despite the recent efforts to characterize inflammation in ectotherms, the liver and its role in immunity are still neglected in amphibians. At the same time, the spleen has been the organ in the spotlight. We believe that exploring interorgan crosstalk during inflammatory assemblage will provide an integrative framework of the inflammatory response. Therefore, we investigate organ-related immune proteins gene expression in an anuran model using endotoxin to mimic a bacterial infection. *Rhinella diptycha* adult male toads were injected with saline or lipopolysaccharide (LPS, 2mg/kg). Pro- (C1s, IFN-γ, IL-1β, IL-6) and anti-inflammatory (IL-10) proteins mRNA levels were tracked in four time-points post-injection (1h, 3h, 6h, and 18h) in the spleens and livers. We found acute C1s up-regulation in the liver and down-regulation in the spleen. Liver leads IFN-γ expression in early (1h) and late (18h) time-points, but spleen dominance (6h) was also observed. Early hepatic dominance was found for IL-1β, but spleens take over the late expression. A similar pattern was observed for IL-6, with mRNA levels increasing over time in both tissues. Both organs showed late up-regulation of IL-10, but early detection only occurred in the liver. Our results point to a pivotal role for hepatic tissue during the beginning of the inflammatory response, especially in complement system protein gene expression, while splenic tissue takes over the late cytokine transcription.

The impact of temperature on the within-host dynamics of Ambystoma tigrinum virus (ATV) epizootics in larval salamanders (*Ambystoma tigrinum*)

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Climate change has a complex effect on disease, potentially leading to expanding pathogen spatial distribution, accelerating transmission cycles, shifts host life cycles, and the emergence of disease in naïve populations. Quantitative frameworks like disease modeling are needed to improve our ability to predict the effects of climate and disease on host population dynamics. Especially vulnerable to these changes, amphibian populations are uniquely at risk of decline due to climate change and disease. Infecting salamander populations across North America and Arizona, the effects of temperature on Ambystoma tigrinum virus (ATV) and the pathogen's interaction with its host are not well quantified making risk prediction difficult. We hypothesize seasonal variation in temperature and the resulting fluctuations to the host's immune system and the virus' replication rates likely play significant roles in ATV epizootics. Using mechanistic models accounting for temperature and host susceptibility, we will first evaluate the effects of temperature on ATV disease dynamics within each host. Through a viral transmission experiment using larval salamanders, we evaluated the effects of dose and temperature within a host. Our results reveal a clear non-linear effect of temperature on mortality and shedding rates that is likely mediated by temperature-influenced pathogen replication and host immune response, where cumulative mortality and shedding rate peak at 20°C. Future research will combine these findings with a full model considering the relationship between hosts. This study demonstrates the utility of combining data and modeling techniques to better understand and forecast the effects of climate and disease on threatened host populations.

Stimulation with heat-killed bacteria (*Aeromonas hydrophila*) promotes immunological and endocrine alterations in toads

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The bacteria *Aeromonas hydrophila* is the pathogen responsible for the red-leg syndrome in amphibians, accounting for the mortality of wild and captive populations. However, little is known regarding the inflammatory assemblage in anurans infected with this pathogen. This study evaluated the immune and endocrine effects induced in *Rhinella diptycha* toads by stimulation with heat-killed *A. hydrophila*. We evaluated: bacterial killing ability (BKA), neutrophil: lymphocyte ratio (NLR), corticosterone (CORT), melatonin (MEL), and testosterone (T) plasma levels. Control animals were not manipulated, and the others received an intraperitoneal injection of 300 µl of saline (APBS) or heat-killed bacteria A1 (3 x 107 cells), A2 (3 x 108 cells), and A3 (3 x 109 cells). Toads were euthanized six hours post-injection. All three concentrations of bacteria induced changes in the CORT levels and NLR, peaking at A3 and A2, respectively, while only the two highest concentrations decreased the MEL levels. Alternatively, there was no treatment effect on BKA and T levels, possibly due to the time post-injection being too short for any observable changes in these parameters. These results indicate the onset of inflammatory assemblage, especially at the two highest concentrations of bacteria. In the future, we plan also to evaluate the gene expression of cytokines, receptors, and complement proteins in the spleen and liver collected from these animals. The association between physiological and molecular data will help elucidate more about the inflammatory assemblage in animals challenged with this bacteria.

Recapture history of *Eleutherodactylus coqui* indicates that it can clear *Bd* infections but does not develop resistance

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Long term disease monitoring in Puerto Rico has revealed substantial information about factors affecting the incidence of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) in *Eleutherodactylus coqui*. These terrestrial direct-developing frogs have persisted in spite of being susceptible to chytridiomycosis. However, data on the progression of *Bd* infection loads and the potential of frogs to clear *Bd* is not available. Using Passive Internal Transponders (PIT), we tracked adult frogs for 32 months and monitored the change in *Bd* infection through time. The purpose was to determine if *E. coqui* could clear *Bd* infections, and also to assess how seasonal environmental factors (temperature and precipitation) influenced active infections. Results revealed a high recapture rate of 23% (90/392 marked animals), with 35 individuals captured at least three times, and some individuals captured up to eight times. We found that frogs were able to clear *Bd* infections as soon as in 21 days. Recapture history revealed that infection loads increased and/or decreased during both the cool-dry and warm-wet seasons, highlighting complex trade-offs among environmental factors, host susceptibility, and virulence traits of *Bd*. Finally, we found multiple cases of similar infection loads after having cleared *Bd*, suggesting that *E. coqui* does not develop complete resistance. Our work expands our understanding of the long-term interactions of a deadly pathogen in persistent hosts that represent our best hope of maintaining the ecosystem functions offered by nocturnal amphibians in tropical forests.

Maintaining Resistance to *Batrachochytrium salamandrivorans* Infection Despite Depletion of Skin Defense Peptides

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Amphibian mucosal secretions contain defense compounds that can contribute to host defenses against infections and disease mitigation. Interactions between these skin defenses, such as peptides and microbes, play a vital role in the host health, development, and immunological adaptations. Disease-induced population declines in amphibians are thought to be modulated by physiological and immunological responses, since some species seem to show host-level resilience toward infections. Amphibian populations worldwide have been declining due to the devastating impacts of *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). For this experiment, we tested the influence of peptide depletion on vulnerability towards *Bsal* in Northern leopard frogs (*Lithobates pipiens*). We also examined skin microbiome and peptide composition resilience towards infection. Peptides were depleted immediately before exposure and collected again 4 weeks after exposure to compare changes in peptide quantity and composition using micro-BCA for peptide quantification and MALDI-MS for composition. Skin swabs were collected before and after exposure of frogs to *Bsal*, and the DNA extracted for infection diagnostics by qPCR. Data was analyzed using QIIME2 and R studio. Peptide depletion was predicted to increase *Bsal* susceptibility, but this was not demonstrated. Our results suggest that *L. pipiens* did not become infected with *Bsal*, even after depletion of skin peptides. Instead, peptide composition and microbiome were altered by exposure to *Bsal*. Microbiome shift seemed to be more infection focus rather than treatment dependent. Discovering mechanisms of resilience to disease emergence in amphibian populations is critical to conservation and disease mitigation.

August 8, Session Eight (PM)
One Health and Wildlife Trade

One Health needs Herp Health – we must learn to learn from each other

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One Health means different things to different people, usually coloured by professional perspectives. To help overcome these silos and develop a common understanding of One Health, the WHO/OIE/FAO/UNEP One Health High Level Expert Panel recently published an holistic definition. This rejects an anthropocentric view of One Health; identifying that the health of all living beings on planet Earth is interconnected and that restoring and maintaining the health of ecosystems is core to ensuring the health of domestic and wild animals and the health and wellbeing of people. By definition, this includes ensuring the health and conservation of amphibians and reptiles in the wild at both population and species levels. With a common understanding, there can be common goals and direction. The only way to ensure that One Health is put into practice and does not fall on the wayside of history is to promote and teach interdisciplinary and transdisciplinary approaches to research and practice, involving – amongst others – the ecological, conservation, veterinary, medical, and social sciences. Additionally, cross-learnings between disciplines need to be identified and promoted. There is no doubt, for example, that the herp-health world can inform research and mitigation on the origins and spread of pandemics, such as how *Bd*GPL arose and spread globally. Also, that two of the most devastating emergent diseases of wildlife in recent years (amphibian chytridiomycosis and white nose syndrome of bats) have been caused by fungi should challenge the almost exclusive virology focus of those working on public health pandemic prevention, preparedness and response.

Utilising citizen science to investigate pond creation across the British Isles during COVID-19 and explore impacts on amphibian health and human wellbeing

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Survival of the common frog (*Rana temporaria*) in the British Isles is threatened by habitat loss, disease, and pollution. Understanding the factors that shape the distribution of common frog habitat, and how those habitats vary in quality and/or disease risk, are crucial in developing conservation strategies for the species. During COVID-19 lockdowns, restriction of the public to their homes led to a dramatic rise in the creation of new garden ponds, but despite the potential importance of such resources for both human wellbeing and wildlife health, we lack a comprehensive understanding of such changes in habitat availability at the landscape scale. Here we present the results of a citizen science survey with over 2000 respondents designed to investigate the motivations for pond creation, use of newly created ponds by key amphibian species, and to record spatial coverage of garden chemical usage and active cases of amphibian disease. We discuss the impact of pond creation on human and wildlife health, and implications for our understanding of the spread of pathogens. We also explore the utility of citizen science for monitoring the real-time incidence and distribution of amphibian diseases by comparing survey-based estimates to data from dedicated field surveys of common frog populations and associated Ranid herpesvirus infection in the United Kingdom.

Reversing the low social capital of US herpetofauna to increase disease-threat investments

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Amphibian and reptile conservation is at a pivotal moment due to increasing threats and population losses. We reframe herpetofaunal conservation from the perspective of interwoven human value and decision systems, with a focus on the United States. At the crux is the need to reverse the pattern of the relatively low social capital of amphibians and reptiles. Capital is a term used to describe material goods of value, or assets. Social capital extends to shared values of people in a society, which include applications to natural resources. Mobilization of social capital for at-risk taxa and emerging threats including diseases is an urgent priority, especially relative to the need for increased incentives for stewardship. We expand upon this framework for US herpetofauna, where social capital for species (and their conservation) has a dynamic set of dimensions with multiple feedback loops connecting different societal sectors, such as: 1) specific human communities; 2) people in defined geographies and jurisdictions; 3) specialists and advocates of myriad species or taxa; 4) associated researchers; and 5) related managers and policy makers. Each of these five sectors has a constituency for species conservation, within which herpetofauna warrants elevation. For emerging herpetofaunal diseases in the US, this framework is drawing renewed attention to key partnerships among sectors that is elevating herpetofaunal conservation urgency and investments. Examples from the North American Bsal Task Force and Partners in Amphibian and Reptile Conservation's Disease Task Team support heightened attention to US herpetofaunal health. Sustainability of healthy herpetofauna is within reach.

The spread of amphibian pathogens through international wildlife trade

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The global spread of amphibian pathogens through international wildlife trade contributes towards species population declines and extinctions. Despite this biodiversity crisis, the trade in infectious material carrying ranavirus and amphibian chytrid fungi continues without consistent government surveillance and regulation. This presentation summarizes three case studies where both chytrid and ranavirus were detected among shipments of live amphibians imported to the United States from Madagascar, Hong Kong, and the Dominican Republic. In addition to the presence of pathogens on live animals, contaminated shipping containers and substrates were also frequently detected, demonstrating the extended potential for trade to spread disease through the disposal of waste items even in the absence of amphibians. Improved trade policies are urgently needed to facilitate a "cleaner" wildlife trade that is less likely to introduce and spread emerging infectious diseases.

Awareness, attitudes and perceptions of US pet amphibian businesses and owners regarding pathogen threats, biosecurity and acquisition of certified disease-free amphibians

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Precipitous declines in global amphibian populations necessitate a better understanding of the US pet amphibian trade and potential for pathogen transfer and spillover at various stages of the supply chain. Using an online survey of US pet amphibian businesses and owners, this study aimed to: 1) characterize the size and composition of businesses engaged in the pet amphibian trade; 2) understand the awareness and attitudes of amphibian businesses and owners regarding harmful pathogens and beneficial microbes; 3) estimate the value businesses and owners place on amphibians certified as pathogen-free; and 4) characterize the husbandry practices of businesses and owners and their willingness to adopt measures to promote beneficial microbes and reduce harmful pathogens in their amphibians. Pet amphibian owners are aware of harmful pathogens and recognize the threat of spillover from captive to native populations. They also expressed a responsibility to engage in preventative biosecurity measures and a strong preference, and willingness to pay a premium, for animals that are certified pathogen-free. Most businesses are also aware of harmful pathogens, recognize the threat of spillover from captive to native amphibian populations and believe using biosecurity practices to control pathogens in trade is their responsibility, within their control, and will benefit their operations. Businesses also expressed strong interest in acquiring amphibians certified as pathogen-free and strongly supported the concept of clean-trade. These findings suggest the feasibility of an industry-led clean-trade program aimed at improving animal well-being and customer satisfaction while mitigating disease-related financial losses for businesses and threats to native amphibian populations.

Amphibian pet trade stakeholders' biosecurity practices, relationships, and connection to the spread of novel chytrid fungus *Batrachochytrium salamandrivorans*

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Amphibians face a variety of threats causing population decline including the recently identified chytrid fungus, *Batrachochytrium salamandrivorans* (*Bsal*). This pathogen caused several massive salamander mortality events in Europe. Though currently not found in North America, the most likely pathway of intercontinental spread is through the amphibian pet trade. The lack of documentation and regulation in this industry may allow infected amphibians to unknowingly be transported across large geographic areas. Existing social science research on this topic is limited, resulting in amphibian pet trade stakeholders having little to no input in management actions. Here, we conducted semi-structured interviews with 22 amphibian pet trade stakeholders (eg. amphibian pet owners, breeders, and pet store owners) from across the United States. Interview questions addressed their identities, knowledge, opinions, and behaviors surrounding biosecurity, husbandry, and *Bsal* management actions. Interviews were analyzed using conventional qualitative content analysis. This form of text analysis categorizes interview text to identify themes and patterns of the target group. We found this group of stakeholders to rely heavily on online forums and interpersonal relationships for information sharing and idea exchange. Their support for management actions varied depending upon the proposed action, but differences were often associated with their identity. These results are crucial for creation and implementation of effective management to combat amphibian population declines through the human-driven spread of *Bsal*.

Reptile and amphibian diseases in EU's policy: Theory versus practice

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Infectious threats to native amphibian and reptile diversity are theoretically covered by several European and national legislations. Focus is currently on amphibian diseases, three of which are OIE listed (ranaviruses, *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* [Bsal]). EU countries have the obligation to mitigate threats to native, threatened herpetofauna according to the Habitats directive, which covers the conservation of natural habitats and of wild fauna and flora. A single disease (Bsal) is included in the European Animal Health law, which covers specific animal diseases and includes a.o. trade regulations to mitigate the Bsal risk. A "clean trade" has been advocated widely by several stakeholders but currently lacks the legal instruments for implementation. Overall, enforcement of legal obligations to mitigate the impact of the three major amphibian infections on European wildlife is hampered by limited resources and lack of coordination of efforts between member states. This situation sharply contrasts with massive efforts to mitigate wildlife diseases with potential impact on the livestock industry.

Commonly traded amphibians are susceptible to the emerging fungal pathogen *Batrachochytrium salmandrivorans*

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Batrachochytrium salamandrivorans (*Bsal*), is a pathogenic chytrid fungus affecting amphibians, and particularly threatens North American salamanders. While many salamanders experience *Bsal* chytridiomycosis, few frogs are known to be carriers. We are working to understand if globally traded frog species can contribute to spreading *Bsal* across borders. *Litoria caerulea*, *Hymenochirus spp.*, *Bombina orientalis*, and *Kaloula pulchra* are among the top 10 traded amphibians in the US. We conducted controlled exposure experiments to evaluate whether or not these highly-traded amphibians carry *Bsal* infection and develop chytridiomycosis. We collected weekly skin swabs and bi-weekly weights throughout a 7-12 week experiment for each species. Susceptibility to *Bsal* varied across species. We detected *Bsal* via qPCR on exposed *Litoria* and *Hymenochirus spp*. across multiple time points with prevalence ranging from 20-70%. No *Bsal* infection was detected on *Kaloula* or *Bombina* individuals throughout the experiments. While no mortality occurred, exposed *Litoria* individuals gained significantly less weight than control individuals. Our results suggest that *Litoria* and *Hymenochirus spp*. are susceptible to *Bsal* infection, but do not experience severe disease. Therefore, they could be hidden carriers spreading *Bsal* across borders and posing a threat to North American salamander diversity. These results highlight the need for regular monitoring of captive amphibians and potentially implementing clean trade initiatives.