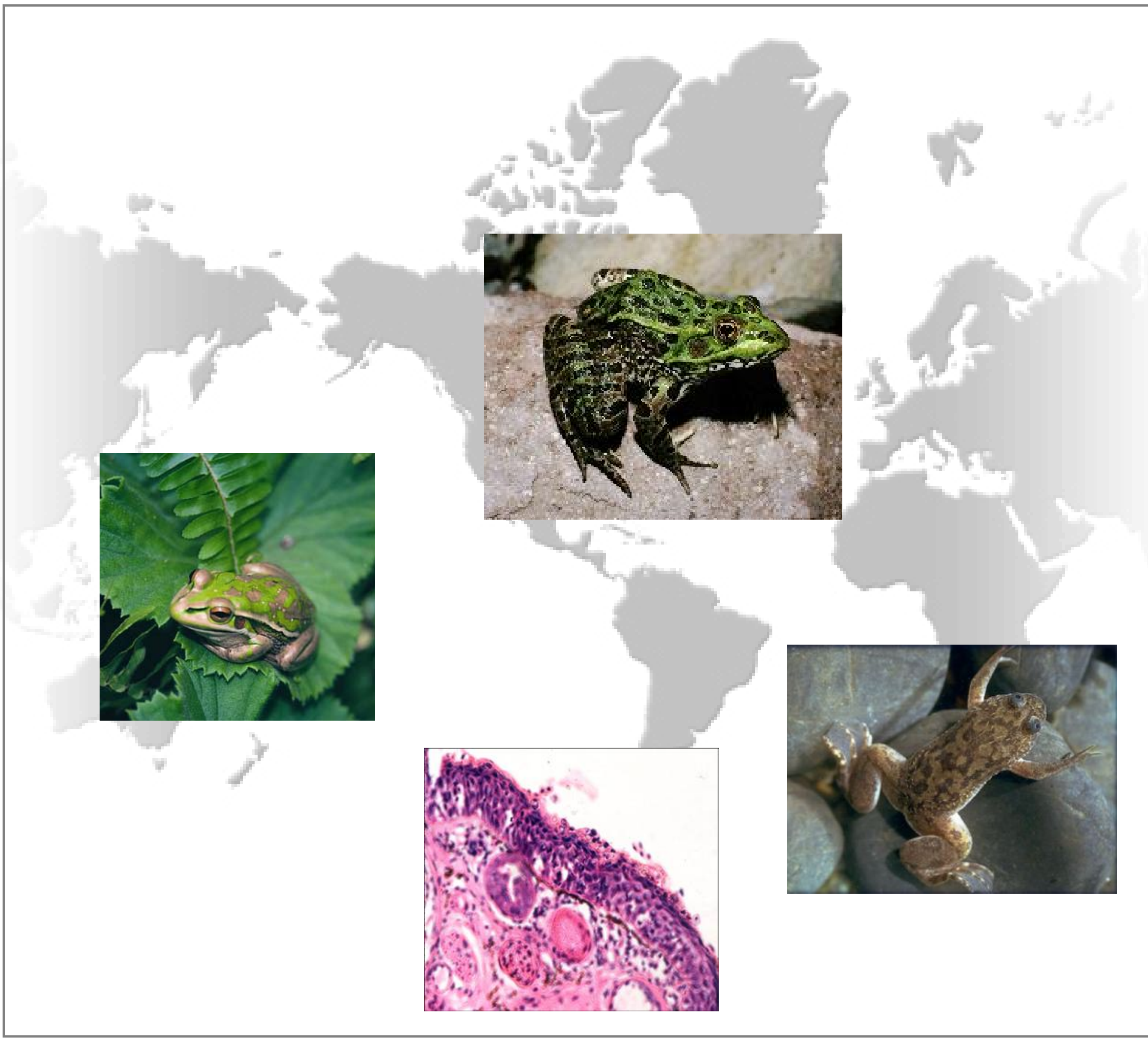
AMPHIBIAN DECLINES & CHYTRIDIOMYCOSIS:

TRANSLATING SCIENCE INTO URGENT ACTION



Tempe, Arizona, USA

5-7 November 2007

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Region 5

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Cover images, clockwise from left

- World map, http://www.remstar.com/content/WhyRemstar/KRIGroup.aspx
- Green and golden bell frog, *Litoria aurea*, http://www.environment.nsw.gov.au/sop04/summarysop04.htm
- Chiricahua leopard frog, *Rana chiricahuensis*, Courtesy US Fish and Wildlife Service
- African clawed frog, *Xenopus laevis*, http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/xenopus_laevis.htm
- Amphibian chytrid fungus, *Batrachochytrium dendrobatidis* in epidermal section, Courtesy Michael Garner, Northwest ZooPath

WELCOME to the
Amphibian Declines & Chytridiomycosis:
Translating Science into Urgent Action conference!

***"It's too much for a child to know: while we sit here on the back porch,
talking, the last glass frog on earth could be singing somewhere
in a cloud forest, lime-green jewel in the night, our own
grief entangled in its vanishing cadenza."***

from "The Silence of the Frogs"
Susan Rea Katz

As you may know, amphibians are one of the most threatened groups of animals worldwide. Since 1970, scientists have observed precipitous population declines and outright disappearances of numerous amphibian species. Many of these die-offs have been attributed to a newly-recognized fungal disease known as chytridiomycosis. Caused by the fungus *Batrachochytrium dendrobatidis* ("*Bd*"), chytridiomycosis is the worst infectious disease ever recorded among wildlife in terms of the number of species impacted and the severity of the impact.

The goals of this international, cutting-edge conference are to:

1) Examine the most current science on Bd and its impacts;
2) Review pressing prevention and management needs and current approaches; and
3) Identify means of limiting the further spread and impact of *Bd* in the US and elsewhere.

The conference organizers invite all of the participants to engage in pro-active, out-of-the-box discussions with a solution-oriented "what's best for the animals" perspective in mind.

Cross-sector conferences like this one are not common place and it may be years before participants will have the opportunity to come together again to address Bd in such a manner. Thus, the time to act is now! Please make the most of this opportunity.

Let's emerge as a unified front, ready to demonstrate not only that chytridiomycosis warrants our concern and high-priority attention, but also that we are prepared to work together to undertake the necessary measures to ensure that the silence of the frogs (and other amphibians) does not become a reality in the foreseeable future.

Thank you for your participation.

Sincerely,

The Amphibian Declines and Chytridiomycosis Conference Planning Team

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Keep checking the conference website for post-conference updates:
http://www.parcplace.org/Bd_conference.html

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Amphibian Conservation Summit

Washington DC, 17-19 September 2005

Declaration

Background

The amphibians – frogs, salamanders and caecilians – stem from an ancient lineage of organisms and they play essential roles, both as predators and prey, in the ecosystems of the world. Adult amphibians regulate populations of insects that are pests on crops, or which transmit diseases. The tadpoles of many amphibians, as herbivores or filter feeders, play a major role in aquatic ecosystems. Their well-being, or conversely their population declines and extinctions, signals that changes are occurring in the biosphere that have begun to negatively impact humans today.

Since 1970, scientists have observed precipitous population declines and outright disappearances of entire amphibian species. The extent of these declines and extinctions is without precedent among any other group of species over the last few millennia, and it has increasingly been the focus of scientific research. These declines have spread geographically and increasing numbers of species are involved. Recent research indicates that:

- Nearly one-third (32%) of the world's 5,743 amphibian species have been classified as threatened with extinction, representing 1,856 species.
- 122 species, perhaps many more, appear to have gone extinct since 1980. Further research may increase this number, since 23% of all species were classified as Data Deficient.
- At least 43% of all species have undergone population declines, but less than one percent is increasing in population size.
- As much as 50% of the amphibian fauna remains undescribed, and the possibility exists of discovering new groups that are widely divergent from any so far known.
- Habitat loss is the greatest threat to amphibians, impacting almost 90% of threatened species.
- A newly recognized fungal disease, chytridiomycosis, causes catastrophic mortality in amphibian populations, and subsequent extinctions.
- Many species are declining for reasons, such as disease, climate change, invasive species, and over-harvesting, that cannot be readily addressed through traditional conservation strategies.
- Other issues, such as the role of environmental pollutants in amphibian declines, need to be more thoroughly addressed.

Since 1990, scientists have referred to amphibians as canaries in the coal mine; the Global Amphibian Assessment (GAA) shows that the canaries are dying. This underscores a weakness in current strategies for biodiversity conservation: that habitat conservation is essential but not sufficient. Existing protected areas alone are not sufficient to protect amphibians from a growing array of threats.

The Amphibian Conservation Summit was called because it is morally irresponsible to document amphibian declines and extinctions without also designing and promoting a response to this global crisis. To this end, the Amphibian Conservation Summit has designed the Amphibian Conservation Action Plan (ACAP), and commends it to governments, the business sector, civil society and the scientific community for urgent and immediate adoption and implementation.

Amphibian Conservation Action Plan (ACAP)

Four kinds of intervention are needed to conserve amphibians, all of which need to be started immediately:

1. Expanded understanding of the causes of declines and extinctions
2. Ongoing documentation of amphibian diversity, and how it is changing
3. Development and implementation of long-term conservation programmes
4. Emergency responses to immediate crises

1. Expanded understanding of the causes of declines and extinctions

A. Emerging amphibian diseases. Emerging diseases are a major threat to the survival of human populations globally. Diseases like SARS, HIV/AIDS, Ebola, and avian influenza emerge because of changes to the environment (e.g., encroachment into wildlife habitat) and human behaviour (e.g., trade and travel). At the same time, a series of wildlife diseases have emerged, threatening many species. These are products of the same underlying causes – anthropogenic environmental changes – and highlight the growing link between conservation of biodiversity and the protection of human health.

Of the diseases known from amphibians, one, chytridiomycosis, is clearly linked to population declines and extinctions. This fungal disease is appearing in new regions, causing rapid population disappearances in many amphibian species. It is the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and its propensity to drive them to extinction.

A series of strategies to deal with disease in the field is needed. Research should focus on understanding why some species of amphibians become extinct in some regions and at certain times, while others do not. This will require studying the persistence of the pathogen, reservoir hosts, mechanisms of spread, interactions with climate change, and comparing disease dynamics between sites of declines and control sites where amphibians survive. Research is also urgently needed on the biology of this emerging pathogen, in particular on:

- how it causes death;
- how amphibians respond by developing immunity or changing behaviour;
- understanding the geographic distribution and dispersal of chytridiomycosis; and
- whether or not animals from decline and control sites differ in their responses to chytridiomycosis.

These research programmes should also consider possible interactions between disease and other factors involved in amphibian declines (such as climate change, habitat loss or contaminants) and mechanisms for dealing with them (such as captive breeding and reintroduction).

To implement this research on disease, Regional Centers for Disease Diagnostics will be set up in Latin America, North America, Europe, Australia, Asia and Africa. They will provide free testing to field research groups, and will manage the logistics for regionally based Rapid Response Teams. A seed funding system should be created to support imaginative approaches to stopping outbreaks from spreading and preventing extinction by infection.

B. Climate change. Evidence of a link between amphibian declines and climate change is growing. Changes in temperature or precipitation influence host-pathogen interactions, and short-term and seasonal patterns in amphibian behaviour. One consequence is an increase in the probability of outbreaks of lethal diseases such as chytridiomycosis. If efforts to address climate change remain inadequate, none of the other proposed conservation efforts can save amphibians in the long term. The current spate of extinctions might be the first wave in a more general, profound loss of biodiversity. Ultimately, preventing this requires greater political will to take all necessary measures to reduce human impact on the global climate.

Research is needed to understand how climate change affects amphibians, and why the impacts are greater today than they were historically. In particular, studies should focus on the impacts of climate change on disease dynamics, and should develop predictive models for future declines, thus enabling implementation of conservation measures. Research will also explore ways in which ecosystems could be made more resilient to climate change (such as measures to restore movement corridors that would ensure metapopulation functions or allow migration to new habitats), and whether or not there might be ways to manipulate local micro-scale climates.

C. Environmental contamination. Contaminants may have strong impacts on amphibian populations by negatively affecting immune function and causing infertility, developmental malformations, feminization, endocrine disruption, and alterations in food webs. There is evidence that environmental contaminants can cause local amphibian declines and extinctions. The effects of contaminants on broader geographic scales such as watersheds are not well understood. An ecotoxicology consortium should be formed in order to determine: how contaminant loads differ between stable and declining populations; the relationship between declines and contaminants in all regions; the effects of major chemical classes on both the aquatic and terrestrial life stages of amphibians; the effects of sub-lethal exposure in the presence of other threats such as disease; the role of contamination in amphibian population declines at the landscape scale; whether or not present regulatory screening is adequate; approaches to minimize the movement of chemicals through the environment; and how well the future impacts of contaminants can be predicted. The research should be integrated with the work of the regional centers recommended for disease research and management.

2. Ongoing documentation of amphibian diversity, and how it is changing

A. Exploration and biodiversity evaluation. Without an understanding of the amphibian fauna, its history, and its distributional patterns, conservation priorities cannot be set rationally. Therefore it is essential that basic exploration and species descriptions continue. The rate of species description among amphibians is higher than it has ever been. However, in many parts of the world, especially in the tropics, knowledge of amphibian species, their distributions, and their requirements for survival is still too poor to enable reliable conservation priorities to be identified. The ACAP will implement a greatly enhanced programme to: name at least 1,000 new species in five years, and 2,500 species in ten years; understand species limits and resolve species complexes; and carry out inventories of amphibian faunas. The implementation of this programme will require major building of taxonomic capabilities in a number of tropical countries, with priority being given to poorly known areas, and areas of high endemism and diversity. To assist in identification of species, new field guides and internet resources should be produced. Innovative mechanisms should be developed to enable taxonomists to devote more time to high priority work. Research should also focus on: Data Deficient species; identification of unique and ancient evolutionary lineages; understanding the extent to which similarity in vulnerability to threats is determined by degree of relatedness between species; and whether genetic diversity of species relates to their ability to persist in the face of an array of threats.

B. Updating the Global Amphibian Assessment continuously. An accelerated programme of assessment must underpin the ACAP. To build on its initial success, the GAA needs to be maintained continuously by: establishing a new full-time

GAA coordinating team; recording updates and corrections to the data; developing more efficient mechanisms within regions to update the data; making the data more widely available; maintaining and enhancing the GAA web site; and undertaking analyses and communicating findings. A complete update of the GAA should be finished by 2009. Particular emphasis should be given to improving discrimination between real and apparent declines.

3. Development and implementation of long-term conservation programmes

A. Protection of key sites for amphibian survival. Habitat loss and degradation are impacting nearly 90% of threatened amphibians. Most of these require habitat- or site-based conservation as the primary means to ensure their survival. Therefore, safeguarding key sites for threatened amphibians is the most urgent priority for the survival of many species. At least 940 amphibian species (422 of which are threatened with extinction) are not in any protected areas. An urgent priority of the ACAP is to identify the highest priority sites, using globally recognized, standardised, and quantified criteria, which are essential for the survival of threatened species that are currently receiving no effective conservation measures. These sites and their associated landscapes need urgent attention, such as protected area establishment, community level sustainable development, and local education and training. The ACAP will establish a site conservation programme with the following main elements: identifying the 120 highest priority sites; and applying appropriate conservation actions at each site, including the development and implementation of management plans, standardised monitoring and assessment protocols, and long-term sustainability plans for ongoing funding and management. Given that what goes on outside a key amphibian site will hugely impact the success of conserving that site, management plans should incorporate the need to protect ecosystem services at a broad ecological scale. This site conservation programme will involve governments, non-governmental organizations, community-based organizations and the business sector collaborating to bring about effective conservation in the highest priority sites, with the widest possible stakeholder support.

B. Reintroductions. The goal of reintroduction is to re-establish protected, viable amphibian populations in the wild where conventional habitat management and threat abatement alone are unlikely to result in population recovery. Many amphibian reintroductions will be needed once techniques for the management of chytridiomycosis and other threats become available. Experience and expertise in amphibian reintroductions need to be developed as a matter of urgency. The ACAP will determine which species will benefit from reintroduction programmes by developing and applying rigorous and objective criteria. Once the species have been selected, reintroduction programmes will be initiated. The animals used for reintroductions may either stem from captive breeding programmes or wild populations, depending on availability of stock and the nature of the circumstances. In the first instance, it is estimated that 20 species will be selected for reintroduction, but this may increase as funds and capacity are built.

C. Control of harvesting. In some parts of the world, especially in East and Southeast Asia, but also in some other tropical countries, unsustainable harvesting of amphibians, especially for food and medicines, has led to severe population declines. There are also instances of declines due to the international pet trade. The ACAP will establish a harvest management programme, concentrating on 15 countries that appear to be the focus of the heaviest levels of harvest. The programme will build management capacity in each of these countries to halt declines due to over-harvesting, with an emphasis on: the development of sustainable use projects (when the biology of the species permits this); the development and strict enforcement of appropriate legislation; monitoring the levels of amphibian harvests and trade; the implementation of recovery plans for the most threatened species; the certification and regulation of commercial captive breeding operations with a proportion of profits returning to conservation in the wild; and raising awareness of the impacts of unsustainable use of amphibians. Commercial captive breeding facilities should only use species native to their regions to reduce the risk of the spread of disease and alien frogs. Species that are threatened by international trade should be listed on the appropriate appendices of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES).

4. Emergency responses to immediate crises

A. Rapid response capacity. The short timescale of many amphibian declines requires the capacity for immediate response, as already mentioned. The regionally based Rapid Response Teams required to react to disease outbreaks should be established and implemented urgently.

B. Captive survival assurance programmes. The ACAP recommends prioritized (as outlined below) captive survival assurance programmes that are primarily in-country, coupled with an obligation to deliver in situ threat mitigation and conservation programs. This is both a stopgap to buy time for species that would otherwise become extinct, and an integral component of other approaches to tackling amphibian declines. Guidelines for including species in captive survival assurance programmes will be based on predictive models of threats so that species are targeted proactively and representative populations are collected. Decision processes will involve consultation with representatives across the ACAP consortium and the range country will be the ultimate arbiter.

Several hundred amphibian species, perhaps more, are facing threats such as disease and climate change that cannot be addressed in the wild with currently available conservation management strategies. Since solutions for the conservation of these species in the wild are not currently available, a short-term solution is to breed them in captive survival assurance colonies to maintain options for reintroduction. Capacity to implement a major captive programme for amphibians does not currently exist anywhere in the world. Therefore this should be achieved through the establishment of an Amphibian Survival Alliance to coordinate this effort globally, involving rapid-response teams to collect disappearing species, short- and long-term captive management, training and capacity building for captive conservation programs in range countries, research on captive breeding and reproductive science, disease management, and education and outreach. Captive programs will include a variety of operations from rapid-response, portable units, to large-scale permanent facilities. The goal is to maintain and breed in captivity species at risk of extinction, which should be collected from places where declines have not yet occurred, as well as from places where animals need to be rescued urgently before they disappear.

C. Saving sites about to be lost. The integrity of some of the top priority sites for amphibian survival is under immediate threat. In some cases, habitats are reduced to tiny fragments that will disappear very soon. An "amphibian emergency fund" should be established to implement immediate conservation measures in such sites before it is too late.

D. Saving harvested species about to disappear. Several species are close to extinction due to over-exploitation. The "amphibian emergency fund" should be used to address threats to these species.

Amphibian Action Fund

The implementation of the ACAP over the period 2006-2010 will cost approximately US$ 400 million. To help support the implementation of the ACAP, the Amphibian Conservation Summit announced the formation of the Amphibian Action Fund and received initial pledges from donors.

The Amphibian Action Fund will support:

1. Expanded understanding of the causes of declines and extinctions
2. Ongoing documentation of amphibian diversity, and how it is changing
3. Development and implementation of long-term conservation programmes
4. Emergency responses to immediate crises

Supporting a network of amphibian experts

The ACAP cannot be implemented without a global network of scientists and conservationists who work on amphibians. To date, the IUCN Species Survival Commission (IUCN/SSC) has focused on decline-related research through the Declining Amphibian Populations Task Force (DAPTF), on promoting conservation through the Global Amphibian Specialist Group (GASG), and on monitoring and assessments through an informal network of scientists contributing data to the GAA. All three of these programmes have made significant achievements, but all of them are also struggling for resources, and are based on broadly the same network of experts. In view of the extraordinary nature of the crisis facing amphibians, the IUCN/SSC should bring these three programmes together in a single Amphibian Specialist Group (ASG) focused on conservation, research and assessment. The ASG needs to have sufficient resources and finances to lead the implementation of the ACAP.

Conclusion

The Amphibian Conservation Action Plan is the most ambitious programme ever developed to combat the extinction of species. This response is necessary because the amphibian extinction crisis is unlike anything that the modern world has previously experienced, and a large proportion of amphibian diversity remains undocumented. The ACAP requires the international community to enter uncharted territory and to take great risks. But the risks of inaction are even greater. The Amphibian Conservation Summit calls on all governments, corporations, civil society and the scientific community to respond to this unprecedented crisis. There needs to be unprecedented commitment to implementing the Amphibian Conservation Action Plan with accompanying changes in international and local environmental policies that affect this class of vertebrate animals. They are indeed canaries in the global coalmine.

Amphibian Declines and Chytridiomycosis: Translating Science into Urgent Action

5-7 November 2007
Tempe, Arizona

Day 1 (Monday 5 November): The State of the Science

7:00 Registration opens

7:45 Continental breakfast served in conference room

Introduction [* = Speaker]

8:30 Welcome *(Stuart Leon, Chief of the Division of the National Fish Hatchery System of the U.S. Fish and Wildlife Service; Vern Herr/Brett Boston, Group Solutions)*

8:45 A Modern Extinction Event: The Science, Ethics, and Policy Implications of Global Amphibian Declines
James P. Collins

9:15 Biology and Implications of Amphibian Disease
Peter Daszak

Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis* = *Bd*)

9:45 Natural History of *Bd*
Lee F. Skerratt, Lee Berger, and Rick Speare*

10:05 Break

10:20 Overview of *Bd* Pathology
David E. Green

10:50 Detection of *Bd*: Methods and Recommendations
Alex Hyatt, Donna B. Boyle, and Veronica Olsen*

11:20 Risk of Exposure and Survival Following *Bd* Infection
Cindy Carey, Lauren Livo, Jeremy Ramsey, and Louise Rollins-Smith*

11:50 Q & A Panel Discussion

12:15 Lunch

1:15 Understanding Spatial and Temporal Patterns of a Pathogen in Wild Populations
Mike Adams

Case Studies

1:45 Amphibian Declines in Central & South America: Patterns and Causes
Karen R. Lips, Jay Diffendorfer, Joseph R. Mendelson, and Mike Sears*

02:15 The Biology and Ecology of *Bd* in Australia
Ross A. Alford

02:45 Break

03:00 Rapid Spread of *Bd* Causes Chytridiomycosis Outbreaks and Extinctions in Yellow-legged Frogs of the Sierra Nevada
Vance Vredenberg, Roland Knapp, and Cherie Briggs*

03:30 From Frogs in Demand to Frogs in Decline
Ché Weldon

04:00 Q & A Panel Discussion

05:00 Closing Remarks & Adjourn *(Rob Bakal; Vern Herr/Brett Boston)*

06:00 – 8:00 Poster Session and Mixer (At hotel)
Hors d'oeuvres and cash bar available

Amphibian Declines and Chytridiomycosis: Translating Science into Urgent Action

5-7 November 2007
Tempe, Arizona

Day 2 (Tuesday 6 November): Management Opportunities

Introduction

7:45 Continental breakfast served in conference room

8:30 Opening Remarks *(Rick Bennett; Vern Herr/Brett Boston)*

8:45 A Call for Risk-based Management of *Bd*
Charles R. Peterson, Jamie K. Reaser, Robert Bakal and Sophie St. Hilaire

9:05 The Australian Government Threat Abatement Plan (TAP) for Chytridiomycosis

Lee F. Skerratt, Rick Speare, and Lee Berger*

Management Needs/Options

9:35 Approaches to the Control and Treatment of *Bd* Infection in Captive Amphibian Collections
Allan Pessier

9:55 Field-based Protocols for Minimizing *Bd* Transmission and Monitoring for Disease Outbreaks
Purnima P. Govindarajulu

10:15 Q & A Panel Discussion

10:30 Break

10:50 Global Bd Mapping Project: A Glimpse of our Knowledge of Amphibian Chytridiomycosis
Deanna H. Olson and Kathryn L. Ronnenberg*

11:10 Linking *Bd* Spatial and Multilocus Genotype Data within a Global Mapping Tool: A Demonstration Using the European Union *Bd* Database
Matthew C. Fisher, Susan Walker, Trent Garner, Andrew Cunningham, Jaime Bosch, Gerardo Garcia, Ana Litvintseva, Rytas Vilgalys, Joyce Longcore, Lee Berger, Ché Weldon, Louis de Prez, Jess Morgan, Tim James*

11:40 Education, Policy, and Regulation: Opportunities for Risk-based Management of *Bd*
Jamie K. Reaser, Robert Bakal, and Robin Moore*

12:10 Lunch

1:00 Q & A Panel Discussion

1:30 Amphibian Population Rescue, Reintroductions, and Recoveries
Russell Poulter, Jason Busby, Margi Butler, Phil Bishop, and Rick Speare*

Case Studies

2:00 Pathways & Puzzles: Crossroads of Disease and Recovery for the Chiricahua Leopard Frog (*Rana chiricahuensis*)
Michael J. Sredl and James C. Rorabaugh*

2:30 Hellbender declines and recovery actions in Missouri, with emphasis on chytridiomycosis
Jeffrey T. Briggler, Mark D. Wanner, and Chawna D. Schuette*

3:00 Break

3:30 Boreal Toads and *Bd*: A Colorado Perspective
*Tina Jackson*and Erin Muths*

4:00 Amphibian Commerce and Pathogen Pollution: Chytrid Fungus, Ranaviruses, and the Tiger Salamander Bait Trade
Angela M. Picco

4:30 Q & A Panel Discussion

5:20 Closing Remarks & Adjourn *(Jim Collins; Vern Herr/Brett Boston)*

5:45 Meet in Hotel Lobby to board buses to the Banquet

6:30 Banquet and Zoo Tour (hosted by the Arizona Zoological Society and the Phoenix Zoo)

Amphibian Declines and Chytridiomycosis: Translating Science into Urgent Action

5-7 November 2007
Tempe, Arizona

Day 3 (Wednesday 7 November): Making Plans and Taking Action

7:45 Continental breakfast served in conference room

8:30 Opening Remarks *(Erin Muths; Vern Herr/Brett Boston)*

8:45 Facilitated Group Discussion: Determining the Scientific Priorities for Meeting *Bd* Management Information Needs
(Moderators: Cindy Carey and Joe Mendelson)

10:30 Break

11:00 Discussion Continued

11:45 Discussion Summary *(Cindy Carey and Joe Mendelson)* &
Orientation for Afternoon Sessions *(Priya Nanjappa Mitchell)*

12:00 Lunch

1:00 Session A: Developing Conservation Plans for Infected Populations
(Moderators: Mike Sredl and Jim Rorabaugh)

Session B: Identifying Opportunities and Initiating Plans for *Bd* Prevention & Early Detection/Rapid Response

Working Group 1: The Amphibian Field Activities Pathway
(Moderator: Purnima P. Govindarajulu)

Working Group 2: The *Bd*-Free 'Phib Campaign: Pet Industry and Others Interested in Ex-situ Conservation
(Moderator: Jamie K. Reaser)

Working Group 3: The Fisheries Management Pathway
(Moderator: Robert Bakal)

2:30 Break

3:00 Working Groups Continued

4:00 Working Group Reports to Plenary

5:00 Facilitated Group Discussion

5:30 Closing Remarks & Adjourn *(Jamie K. Reaser; Vern Herr/Brett Boston)*

Speaker Abstracts

Understanding spatial and temporal patterns of a pathogen in wild populations

Michael J. Adams
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Field surveys are an essential component of any effort to fully understand an ecological pattern. They are a source of hypotheses and a means to test predictions. In the case of a pathogen, field surveys can, among other things, be used to help understand variation over space and time in occurrence and prevalence (the proportion of animals infected by the pathogen) but the manner in which surveys are conducted has important implications for how the data can be used. There are two fundamental problems that must be addressed by the design of a field study: bias and spurious results. Bias is the tendency for a parameter estimate to be consistently greater or less than the true value. For example, counts of the number of animals in a wildlife population will usually have negative bias because some animals will be missed and the count will thus tend to be less than the true abundance. Spurious results arise when random chance causes a given sample to exhibit a pattern that is not present in the population of inference or that is simply not important. Both of these issues are as important to the study of pathogens as they are to any other topic. Both should be considered when designing field studies or interpreting field survey data.

There are three basic components in the design of a survey-based field study: (1) identifying the response variable(s); (2) selecting sites to sample; and (3) specifying explanatory models. If the response variable cannot be quantified without error, then it is important that it be unbiased relative to the true value. In the case of *Batrachochytrium dendrobatidis* (*Bd*), a response we are often interested in is the proportion of amphibian populations that it infects or in environmental conditions that are associated with the presence of this pathogen. The problem is that when we fail to detect *Bd*, we do not know if it is absent or if we just missed it. The proportion of animals infected in a population is also of interest, and variation in this proportion causes variation in the probability of detecting *Bd* when it is present. This greatly complicates the study of occurrence patterns for *Bd*. In addition to these issues with the response variable, it is generally desirable to use random or some other probabilistic form of site selection so that any parameter estimates generated can be applied to a population of inference beyond the sites that were specifically sampled. However, in some cases, it may be better to deliberately choose sites with certain characteristics (e.g., sites representing a hydroperiod or temperature gradient) so that hypotheses about those characteristics can be tested. Finally, it is always preferable to specify a set of a priori statistical models that reflect hypotheses about the response. Exploration of models other than those specified in advance can be valuable but increases the potential for spurious results.

In the early stages of studying an ecological pattern, almost any field observations are important as long as their interpretation reflects the potential for bias and spurious correlations. However, to make progress, it is essential that increasingly well-designed field surveys be conducted to test and to generate hypotheses. We are beginning to see such studies that address the occurrence and prevalence of *Bd*, and we can expect that our understanding of field patterns will shift as more such studies are reported.

The biology and ecology of *Batrachochytrium dendrobatidis* in Australia

Ross A. Alford
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The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) has been detected in at least 50 species of amphibians in Australia. It is widely distributed over the more populated near-coastal regions of the continent, and on the island of Tasmania, but may be absent from the Cape York Peninsula and Top End of the Northern Territory. Preliminary environmental modeling suggests that it could occur in some areas in which it has not yet been detected. Epidemic outbreaks of chytridiomycosis caused major declines and local and global extinctions of stream-associated frogs living at higher elevations in tropical and subtropical rainforests in Queensland, commencing in the 1970s. There were four major regional outbreaks, which followed a south-to-north temporo-spatial pattern. Several species that had populations at lower elevations that did not suffer declines have recolonized some upland sites from which they were extirpated in the initial die-offs. The amphibian chytrid is now endemic in frog populations in all areas where die-offs occurred. More localized epidemics of chytridiomycosis appear to have caused local declines in many populations of some species of frogs in other areas of the continent. Several captive populations are being maintained in an attempt to supplement natural populations and avoid global extinction. Experiments and field studies have demonstrated that the prevalence and virulence of the disease is highly sensitive to environmental factors. In the laboratory, environmental temperatures strongly affect the rate of disease development, as do moisture conditions. In the field, prevalence changes seasonally, and tracking studies have demonstrated that individual frogs that periodically elevate their body temperatures are substantially less likely to be infected by *Bd*. Field data suggest that much of the variation among species in susceptibility to *Bd*-associated declines could be due to differences in patterns of microenvironment use. Work on antimicrobial peptide secretions has also demonstrated variation within and among species, which could account for additional variability. Australian data suggest that with detailed information on species' habitat use and AMPs, it should be possible to make very accurate predictions of how they will respond to *Bd* outbreaks.

Hellbender declines and recovery actions in Missouri, with emphasis on chytridiomycosis

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Both eastern (*Cryptobranchus alleganiensis alleganiensis*) and Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) have experienced marked population decline with an average of 77% since the 1970s. Data reveal a shift in age structure of hellbender populations, with larger, mature individuals being most prevalent and young age classes being virtually absent. Due to the decline of hellbender populations in Missouri, research and survey efforts have been greatly increased over the past several years

The Ozark Hellbender Working Group, which consists of scientists from universities, public zoos, fish hatcheries, along with herpetologists and state and federal agency representatives, have led these efforts. Conservation work undertaken by this group for hellbenders includes examining population genetics, conducting comprehensive health analyses, creating captive propagation programs, and evaluating potential and existing threats.

One emerging threat that has received increased attention in the past few years is the prevalence of amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). In March 2006, captive-reared hellbenders, which were raised from eggs collected from two rivers in Missouri in 2002, tested positive for *Bd*. Tests for *Bd* on hellbenders in the wild began and immediately yielded positive confirmation of *Bd* on hellbenders. With this new discovery, additional information has been collected over the past two field seasons to document the frequency and distribution of this fungus on hellbenders in Missouri. This fungus was found on hellbenders in five of the eight rivers surveyed in 2006 and 2007. Positive *Bd* results were found in three Ozark hellbender rivers and two eastern hellbender rivers with the majority of the infected animals occurring in Ozark hellbender rivers. Positive animals tended to be isolated to a few locations on each river, and frequency of infection was between 2% and 25% of hellbenders tested. Infected animals' total length ranged from 26.5 cm to 51.5 cm with longer hellbenders having higher average infection rates. Other aquatic species (amphibians as well as turtles, fish, etc., as possible vectors) were tested for *Bd*, but all results were negative.

With future plans to propagate hellbenders for release back into the wild, Saint Louis Zoo staff are investigating treatment options for this aquatic salamander which lives in the cool water in which *Bd* thrives. To date, standard treatment of itraconazole soaking has not been effective, but heat treatment has yielded some successful results.

In this talk, we will provide details on the recovery actions of hellbenders especially with respect to infectious rates of animals with *Bd*, treatment of infected animals, captive propagation and other research efforts that to continue to address the conservation actions necessary to ensure full recovery of this species.

Risk of exposure and survival following *Bd* infection

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A number of factors are known to influence transmission of *Bd* within a population and, if infected, how long an individual could live following infection.

Isolates of *Bd* have been obtained from numerous amphibian species from around the world. When groups of a susceptible species are exposed under controlled conditions to a variety of isolates, the groups will survive varying lengths of time but usually succumb to the infection. Therefore, transmission of *Bd* from one reservoir or susceptible species to another susceptible species is not difficult.

Ten or fewer zoospores can cause an infection in susceptible species. A lethal infection is caused by multiple reinfections by released zoospores. Death does not occur until a threshold of infection is achieved. The likelihood of becoming infected in a breeding population depends on a number of factors: number of infected individuals releasing zoospores, the number of zoospores released per day, the amount of time spent by the amphibian in the breeding pond, the removal of zoospores from the water by other individuals, rate of water flow, or other factors, sex, body mass, mating behavior, and other factors.

Once infected, a number of factors determine how long an infected individual might live. Temperature affects almost all rate processes of living organisms, including growth rates. *Bd* can cause death of susceptible individuals from 4°C to at least 23°C, although the length of survival is longer at 4°C than 23°C. Exposure of infected animals to 30°C for 12 hrs/day does not clear the infection, but controls

Bd growth, with the result that infected individuals die only when prohibited from reaching 30°C each day. Arguments that global warming might be fostering deaths of infected amphibians have failed to receive experimental support.

Some amphibians can "clear" the infection by thermoregulation at very high body temperatures or by remaining dry.

Not all species are susceptible to *Bd*. Although multiple reasons probably exist why some animals are susceptible and others are not, a good correlation exists between the effectiveness of antimicrobial peptides against *Bd* secreted by various species and their relative risk to infection

Since *Bd* appears to infect only the outer epidermis, the adaptive immune system (B and T cells) does not appear to be activated. No evidence exists that antibodies are produced by chytridiomycosis or that, should an animal be able to "shed" the infection, immunity to subsequent infection occurs. When inactivated *Bd* are injected into the body cavity, serum collected several weeks later can block *Bd* growth in vitro, but antibodies do not prevent death upon subsequent exposure to *Bd*. Therefore, efforts to produce a vaccine against *Bd* will prove fruitless.

A modern extinction event: the science, ethics, and policy implications of global amphibian declines

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Extinction is inevitable—more than 99.9% of Earth's species are extinct. Although extinction is inevitable, determining the causes is difficult. Modern amphibians appear to be an unfortunate exception in offering an opportunity to study the biology of extinction. Increasing evidence suggests that we are in the midst of a modern extinction event that is affecting an entire vertebrate class. According to the first global assessment of amphibians, almost half of all populations are declining and nearly 10% of species are close to extinction or presumed extinct. I will review six suspected causes of declines with a special focus on the role of infectious disease.

Paleontologists discuss various causes for mass extinctions but rarely disease. Late in the 20th century as herpetologists reported amphibians had gone missing within protected parks and reserves, they attributed these "enigmatic" losses to emerging infectious diseases (EIDs) among other causes. I will address the question: When is emerging infectious disease a force in extinction? I will review the evidence supporting the conclusion that an EID attributable to a chytrid fungus is causing amphibian declines and extinctions. Throughout the presentation, I will review the ecological and socio-economic implications of amphibian declines.

But once research yields the capacity to predict the amphibian communities where the next declines and perhaps extinctions might occur, it raises a series of ethical and policy questions; for example, should species be removed before an infection emerges? If so, where should they be housed and for how long? Who makes such a decision? And what policies should be changed in response to what appears to be a global amphibian pathogen? I will end with a discussion of how the research and conservation communities are answering these questions and in doing so responding to the challenge of global amphibian declines.

Biology and implications of amphibian disease

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During the 1990s, two amphibian diseases (ranaviral disease and chytridiomycosis) have become recognized as significant threats to amphibian conservation in the USA and globally. These two diseases are caused by different pathogens, with different life cycles, ecology, and impact on host populations. In this talk, I will give a brief overview of both diseases, their biology and impact. I will focus on what I believe is key to raising awareness about these diseases, to developing a research strategy, and for turning science into action—treating them as emerging infectious diseases (EIDs). I will review the evidence for the drivers of *Bd* emergence, and whether it can be considered as simply another invasive species; and for the underlying causes of ranavirus outbreaks. I will also discuss strategies to control similar EIDs of humans and wildlife that have or have not succeeded. Finally, I will go through some of the key issues in chytridiomycosis research that remain to be answered.

Linking *Batrachochytrium dendrobatidis* spatial and multilocus genotype data within a global mapping tool: a demonstration using the European Union *B. dendrobatidis* database

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Detailed multilocus molecular typing is now regularly used to describe the population genetic structure of mycoses, leading to insights into their principle modes of transmission, their environmental reservoirs, and their longer-term evolution. In parallel, there have been extensive developments in bioinformatic, databasing, spatial analyses, and the worldwide web that allow multilocus typing projects to be universally available and merged with powerful spatial mapping tools in realtime. Here, we describe the development of a mapping tool, *www.spatialepidemiology.net*, that allows the spatial population genetic structure of *B. dendrobatidis* to be analysed within the species global occurrence. We show, using the European Union *B. dendrobatidis* surveillance database, that the organism is widespread across Europe. We demonstrate, using multilocus genotype data, that the amphibian trade and amphibian re-introduction programs have likely lead to the recent introduction of *B. dendrobatidis* in several regions. Bayesian statistical analysis of the European Union database shows that there is significant heterogeneity in the risk of infected species developing chytridiomycosis, and we show that environmental variables are associated with risk of developing disease.

Field based protocols for minimizing *Bd* transmission and monitoring for disease outbreaks

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The mounting evidence of *Bd* impacts has necessitated government agencies to draft protocols to manage various aspects of disease control. In Emerging Infectious Disease situations such as *Bd*, these protocols have to be drafted with incomplete knowledge of disease dynamics. The talk will summarize information on (1) hygiene protocols to minimize disease transmission among sites and animals, (2) monitoring protocols for early detection of disease outbreaks, and (3) protocols for containment and/or eradication of the *Bd*. Field researchers could act as potential vectors for the transmission and introduction of *Bd* and other diseases into novel sites with the potential for disastrous consequences. Although the probability of this mode of transmission is not fully understood, invoking the precautionary principle, it is essential that all field researchers incorporate a hygiene protocol as Standard Operating Procedure. While most amphibian researchers incorporate a hygiene protocol in their research plans, the challenge is to expand the compliant group to other wildlife researchers and recreational users of wetlands. In the absence of wider compliance, hygiene protocols will be ineffective in preventing the spread of *Bd*. The protocols for early detection of disease outbreaks and those for disease containment under field conditions remain in the early stages. The goal of this talk is to serve as a platform to launch the development standard protocols at the regional, national, and international levels for the management of *Bd*.

Overview of *Bd* pathology

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Infection by *Batrachochytrium dendrobatidis* (*Bd*) is strictly limited to keratinized epithelial cells. In amphibians, keratinized cells are found in anuran larvae in the oral disc (jaw sheaths and toothrows) and palate, in caudate larvae at the tips of digits, and only after metamorphosis in the skin of the body, head, and appendages.

Clinical signs: Postmetamorphic frogs and toads may show lethargy, poor righting reflex, fearlessness, anorexia, dehydration, and emaciation (wasting). Increased frequency of molting and abnormal molts (dysecdysis) may occur in captive anurans. Careful examination of the skin may show subtle flakes, rolls, or wads of tannish-brown abnormal molt on the skin of the ventral body, limbs, toewebs, and digits. Infrequently, adult frogs or toads may present with reddening of the skin of the legs and ventrum. Infected larvae are asymptomatic.

Gross findings: In anuran larvae, *Bd* infection is restricted to the oral disc and presents as loss of black color in the jaw sheaths and toothrows. In advanced (long-standing) infections, jaw sheaths may appear completely white and have distinctly rounded cutting edges. Recent oral infections may not show any loss of black color or multifocal (spotty) loss of blackness. In caudate larvae, *Bd* infection occasionally presents as strands of molted cells and mucus dangling from the toe tips. Adult salamanders with *Bd* infection are not well documented, but occasionally red-spotted newts show generalized necrosis of body skin resembling a molt with rolls or wads of tannish-brown necrotic epidermis resembling on the body and limbs; unshed molt may dangle from tips of digits.

Histological findings: Mild *Bd* infections show little or no change in the host's epidermis, only scattered thalli in superficial keratinized cells. Subacute and advanced infections may present as (1) thinning, necrosis, micro-vesicles, erosions and ulcers of the epidermis or (2) marked thickening of the epidermis called acanthosis and hyperkeratosis. The acanthosis and hyperkeratosis may be subtle or severe. Normally, the amphibian epidermis is 3 to 5 cells thick, but in moderate and advanced *Bd* infection, the skin may be 8 to >20 cells thick. Oral *Bd* infection of anuran larvae shows chytrid thalli in the black keratinized cells of the jaw sheaths, toothrows, and adjacent nonblack cells (i.e., sides of jaws and palate). Jaws and toothrows in advanced infections may lack black surface cells and have distinctly rounded edges. Skin necrosis, ulcers, and thinning are observed in postmetamorphic Australian anurans and American red-spotted newts. Host inflammatory cell response in the underlying dermis usually is absent or negligible, but when secondary bacterial infections occur, inflammatory cells may be prominent in the dermis and around dermal vessels.

Cause of mortality: Mechanisms of death in *Bd* infections has not been determined. Three leading theories are (1) production of toxins or enzymes, (2) disruption of skin functions (e.g., respiration and water absorption, or (3) secondary or opportunistic bacterial infections.

Detection of *Batrachochytrium dendrobatidis* (*Bd*): methods and recommendations

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Bd is the highly infectious aetiological agent responsible for a potentially fatal amphibian disease chytridiomycosis which is currently decimating many of the world's amphibian populations. The fungus infects two amphibian orders (Anura and Caudata), 14 families, and at least 200 species and is responsible for at least one species extinction. Whilst the origin of the agent and routes of transmission are being debated, it has been recognised that successful management of the disease will require effective sampling regimes and detection assays. We have developed a range of unique sampling protocols together with diagnostic assays for the detection of Bd in both living and deceased tadpoles and adults.

Histochemistry. *Bd* is found only in the keratinised tissues, which includes the mouthparts of healthy tadpoles. The epidermis of infected post-metamorphic frogs is thickened (hyperkeratosis), and the superficial layer can sometimes slough. Diagnosis is most commonly performed on stained sections of toe clips or ventral skin. Accurate interpretation can be difficult and requires a high level of expertise, particularly in infected animals exhibiting hyperkeratosis with sloughing. Misdiagnosis can occur when zoosporangia of *Bd* are shed with the superficial keratin layers. A new staining and immunoperoxidase protocol based on previously described methods has been developed to detect both *Bd* and keratin. This simple technique makes identification easier for the inexperienced diagnostician.

Sampling techniques and real time TaqMan PCR. We have also developed and trialed a range of noninvasive and nondestructive sampling protocols for the collection of Bd zoospores and zoosporangia. The techniques have been validated as per O.I.E. protocols and distributed internationally. The real-time and other diagnostic assays together with the sampling protocols have been assessed in terms of sensitivity, specificity, repeatability, and reproducibility. Quantitation of these performance criteria has been undertaken to enable the assays and sampling protocols to be used in a range of functions including (1) identification of *Bd* in adults and tadpoles from both captive and "free-ranging" animals; (2) estimation of prevalence of infection of "free-ranging" and captive populations; (3) identification of infected animals or groups towards implementing disease control measures; (4) demonstration of disease-free zones via surveying; and (5) demonstration of eradication of infection from individuals undergoing treatment.

Recommendation. We suggest usage of the protocols as described will avoid generation of spurious results thereby providing the international scientific and regulatory community with a set of validated procedures that will assist in the future successful management of chytridiomycosis.

Boreal toads and *Bd*: a Colorado perspective

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The Southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) was historically found in South-Central Wyoming, North-Central New Mexico, and the mountainous region of Colorado. The toad has experienced drastic declines throughout this area, which prompted designations by the U.S. Fish and Wildlife Service as a candidate for federal listing in 1989 and by the Colorado Division of Wildlife as endangered in 1993. This state designation led to the creation of a recovery plan and multi-agency Boreal Toad Recovery Team to address the threats facing boreal toads, including *Batrachochytrium dendrobatidis* (*Bd*). I will provide an overview of boreal toad recovery in Colorado and how the Colorado Division of Wildlife and the Boreal Toad Recovery Team have provided vital leadership in regards to *Bd* research, monitoring, and education.

Amphibian declines in Central and South America: patterns and causes

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Amphibians are declining across the globe for a variety of reasons. We describe patterns and causes of population declines in Central America and then critically examine similar evidence from South America. We review the evidence for the role of chytridiomycosis, an emerging infectious disease of amphibians, as an invasive pathogen causing population declines and the potential role of climate change in triggering disease outbreaks. The primary question for any site is whether Bd is an epidemic pathogen causing die-offs and extirpations or whether it is endemic and the severity of infection is determined by micro-environmental conditions. We critically examined reports of amphibian declines from lower Central America and Andean South America and determined that available data support multiple invasions of this invasive pathogen in both regions. Climate change may affect amphibians directly or may interact with emerging infectious diseases where they are endemic, but we found no evidence to support the hypothesis that climate change has been driving outbreaks of amphibian chytridiomycosis, as has been posited in the climate-linked epidemic hypothesis. Both climatic anomalies and disease-related extirpations are recent phenomena, and both are especially prevalent at high elevations in tropical areas; thus, it is difficult to separate their confounding effects. Effective conservation actions require knowledge of the direct threats to biodiversity and possible synergistic interactions.

Chloramphenicol cures frogs with chytridomycosis

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Chloramphenicol is an antibiotic produced by *Streptomyces venezuela*e, an organism first isolated in 1947 from a soil sample collected in Venezuela. Chloramphenicol is an antibacterial antibiotic and therefore would not be expected to inhibit fungi (at least at low concentrations). We have demonstrated that chloramphenicol inhibits the growth of the chytrid *Batrachochytrium dendrobaytidis* (*Bd* chytrid). Initially chloramphenicol was added to plates of TGhL agar media inoculated with a lawn of the *Bd* chytrid. This produced a zone of inhibition. TGhL agar plates were then made with defined amounts of chloramphenicol. Five micrograms/ml (5 parts per million or ppm) produced almost complete inhibition of growth. Ten micrograms/ml and higher produced complete inhibition. In a modification of this protocol *Bd* chytrid was added to liquid TGhL+chloramphenicol and samples taken each day and plated on TGhL agar plates without chloramphenicol. This demonstrated that the *Bd* chytrid rapidly died in the presence of 10ppm or higher concentrations of the drug. We do not know why the chloramphenicol is inhibiting the *Bd* chytrid.

Frogs and tadpoles were tested to determine if chloramphenicol at 20ppm was toxic. In one experiment tadpoles were grown in 20ppm of the drug for about 4 weeks before metamorphosis and 2 weeks after metamorphosis. The survival (>95%) and weight of tadpoles was the same in both the tadpoles exposed to the antibiotic and a control group of tadpoles. There was no apparent toxicity at 20ppm in these tests with either *Litoria ewingii* or *Litoria raniformis*.

To determine if chloramphenicol could cure chytridomycosis frogs of the two *Litoria* species were infected with the *Bd* chytrid and then treated by continuous baths (approximately x10 their volume, a 10g frog would be in a 100ml 20ppm bath). *Bd* chytrid presence was assayed by qPCR using a Roche Lightcycler and the PCR protocol of Boyle, Hyatt et al. Results were independently validated by sending 100 samples to the Hyatt laboratory to check accuracy.

The chloramphenicol frogs showed considerable reduction in chytrids at two weeks, most were free of chytrids at 4 weeks and all were free at 6 weeks. In a small experiment a group of these cured frogs were tested to see if they could be re-infected. QPCR at two weeks post-infection indicated most *L. raniformis* failed to re-infect while all *L. ewingii* became re-infected. Almost all re-infected frogs spontaneously cleared the infection by 6 weeks. We are repeating this experiment with larger numbers to see if resistance can be produced using this infection/chloramphenicol treatment protocol.

Global Bd mapping project: a glimpse of our knowledge of amphibian chytridiomycosis

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Although *Batrachochytrium dendrobatidis* (*Bd*) is identified as an emerging infectious disease related to amphibian population declines, we have only a limited understanding of its overall scope and impact. As this threat gains recognition as a worldwide issue, *Bd* sampling efforts are burgeoning. It is timely to conduct a synthesis of our current understanding of the geographic and taxonomic extent of *Bd* occurrence and particularly a review to assess patterns of geographic and species-specific risks to persistence. Such a global synthesis of current findings will aid in development of effective science and management strategies to further understand the amphibian-*Bd* interaction, where inventory and monitoring may be needed, and how or where mitigation strategies might need to be implemented. Our overarching objective was to compile as much of the existing *Bd* data as possible to gain a snapshot look at the status of our global knowledge. Specifically, we aimed to produce a map of the global distribution of *Bd*, where it was linked to mortality, and where it had been sampled but not detected. We gleaned data from the published literature, internet resources, and researchers. This task was facilitated by over 25 regional coordinators who compiled locality records for their area. While we successfully collated a huge dataset, we acknowledge our glimpse of the current situation is biased by what we have been able to collect; it is likely that our understanding of global *Bd* patterns will change as new data are evaluated. We found sampling has occurred or is ongoing in over 70 countries. *Bd* is not ubiquitous; it has not been detected in 45% (30 of 66) of the countries for which we have data. Similarly, over 400 amphibian species of 25 anuran families and 5 caudate families have been sampled for *Bd*, and it has not been detected in 43% of these taxa (over 170 species with no detections, with over 230 species testing positive for *Bd*). *Bd* was absent from much of western Africa (11 countries) and Europe (9 of 18 countries) and from native wild animals in addition to imported food-trade and pet-trade animals in Hong Kong. *Bd* was detected in all 16 countries in the Americas for which we compiled data, occurring from the Kenai National Wildlife Refuge, Alaska, USA, to Buenos Aires, Argentina. Countries or regions with *Bd* detections usually also had many samples and species without *Bd* detection; *Bd* prevalence rates in regions with *Bd* ranged from about 2-70%, and case studies show prevalence varies with numerous factors including life stage, season, aquatic or terrestrial life history, elevation, anatomical area sampled, and method of laboratory detection (e.g., histology or PCR). *Bd* detection without amphibian mortality is a common theme for many locations and species worldwide.

Approaches to the control and treatment of *Batrachochytrium* infection in captive amphibian collections

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Infections with *Batrachochytrium* (*Bd*) are best known in relation to the amphibian extinction crisis but are also important in the management of captive amphibians. *Bd* infections are found in zoo collections, farmed amphibians (food and for use in the pet trade), laboratory animals, and animals distributed for use in education. Infected captive animals are a source for dissemination of *Bd* to new locations and impact conservation programs such as captive breeding for re-introduction or survival assurance colonies of threatened species. Institutions housing and distributing amphibians should develop standard operating procedures for disease surveillance and control to reduce or eliminate the risk of translocating amphibian pathogens such as *Bd*.

Disease surveillance programs should include necropsy and histopathology of animals that die. In addition to detecting animals with chytridiomycosis, this practice can help to detect other infectious diseases of concern. In relation to *Bd*, it is important to distinguish between infection and disease caused by *Bd* (chytridiomycosis). Animals with chytridiomycosis often die during quarantine periods and are easily diagnosed by histopathology. Subclinically infected animals (SCAs) carry and shed *Bd*; appear healthy and can remain this way indefinitely; and are major risks for dissemination of *Bd*. PCR screening (TaqMan) is the only reliable method to detect SCAs. Routine testing prior to shipment and/or during quarantine at the receiving institution is proposed.

Development of *Bd*-free amphibian collections begins with PCR testing of collection animals. If positive animals are identified, the entire group or collection is treated with antifungal drugs, and each treatment is combined with transfer of animals to *Bd*-free enclosures. Successful treatment using disinfectants (benzalkonium, malachite green, povidone-iodine); specific antifungal drugs (itraconazole); or elevated environmental temperatures have all been reported, but treatment failures do occur. Multiple treatment cycles are sometimes required to completely eliminate *Bd* infection. Use of PCR surveillance to monitor treatment efficacy is a major advance. Experimental refinement and validation of treatment protocols including combination approaches (e.g., antifungal drug and elevated temperature) are necessary.

Implementation of good husbandry and quarantine practices that reduce the possibility of *Bd* transmission is also warranted. The extent of these practices depends on the intended purpose of the animals (e.g., strict isolation or "permanent quarantine" for re-introduction to the wild). At a minimum, facilities should avoid mixing species; routinely and properly disinfect enclosures between occupants; and use dedicated (or properly disinfected) equipment for each enclosure. *Bd* is primarily spread by animal to animal contact, water, and wet equipment or substrates and measures that eliminate these risk factors should be clearly understood and consistently practiced by animal care staff.

A Call for Risk-based Management of *Bd*

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Science alone cannot achieve conservation aims. Rather, science needs to be applied through public education, wildlife management, policy development, and other relevant actions. Risk management is the process of evaluating alternative regulatory and non-regulatory responses to risk, and strategically selecting among them. The selection process necessarily requires the consideration of not only the best available science-based risk assessment, but also legal, economic, social, and technical factors.

Considerable advances have been made on the science of amphibian declines, and on *Bd* in particular, over the last decade. In addition, several amphibian monitoring programs conducted by herpetologists and citizen volunteers have been put in place. However, comparatively little on-the-ground progress has been made in our efforts to arrest amphibian population declines and extinctions in the US and elsewhere. While limiting factors do include less-than-perfect knowledge of *Bd* demographics and dynamics, the greater barriers are a lack of public and policy-maker awareness, functional divisions between herpetologists and resource managers, limited technical feasibility to address Bd in the wild, and, of course, financial resources.

Partners in Amphibian and Reptile Conservation (PARC), has joined with the U.S. Fish and Wildlife Service and other conference sponsors in the hope this meeting will help translate the science of *Bd* into urgent action, into risk management strategies and action plans. In order to achieve these goals, we challenge meeting participants to have the flexibility to transcend disciplinary paradigms, listen constructively to new ideas, borrow models and approaches from other fields, and set the bar for success high enough to be strongly motivating and yet not so high as to be insurmountable. We hope that the dialogue and programs of work conceived at this meeting will significantly increase our capacity to minimize the spread and impact of *Bd* on amphibian populations in the US and other countries. Ultimately, the outcome will depend not on *Bd* and not on amphibians, but on the people determined to make a difference.

Amphibian commerce and pathogen pollution: chytrid fungus, ranaviruses, and the tiger salamander bait trade

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The spread of nonindigenous species is exacerbated by the global wildlife trade. Pathogens move with target wildlife species through trade and are often released into naïve populations with outcomes that are often unpredictable. Amphibians are moved commercially for pets, food, bait, and biomedical research or clinical applications and are an excellent model for studying how trade in wildlife relates to pathogen pollution. The chytrid fungus *Bd* and ranaviruses are amphibian pathogens increasingly moved through the commercial trade of amphibians. Both pathogens are of concern because of their association with amphibian die-offs. There is no evidence of *Bd* in the tiger salamander bait trade. However, multiple strains of tiger salamander ranaviruses are moved through the bait trade in the western United States. Several ranaviruses infect amphibians and fish and are of special concern because of their potential ability to switch hosts. Since tiger salamanders are used as live fishing bait for recreational and sport fishing, they are a potential source for host switching. I experimentally injected largemouth bass with a bait trade tiger salamander ranavirus. Preliminary results indicate that largemouth bass were infected but did not exhibit any signs of disease. These results suggest that amphibian bait ranaviruses have the potential to switch hosts and that the fish may act as carriers, potentially spreading infection as a result of trade.

Education, policy, and regulation: opportunities for risk-based management of *Bd*

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Education, policy, and regulatory measures are important components of a comprehensive approach to minimizing the spread and impact of *Bd*. This presentation will largely focus on existing measures and opportunities at the international level, as well as within the United States (US). The World Organization for Animal Health (OIE) offers a potential international policy mechanism for the prevention and control of chytridiomycosis and has recently agreed to include amphibian diseases in its remit. The Convention on Biological Diversity (CBD) and Ramsar Convention on Wetlands (Ramsar), however, provide opportunities for raising awareness of *Bd* and its consequences and inspiring national-level efforts to minimize its spread. These bodies also have an influence on funding priorities set by the Global Environment Facility (GEF). Within the US, gaps in regulatory authority regarding disease impacts on wildlife make it difficult to use legal measures to prevent its spread. Under specific circumstances, the Lacey Act might to be applied to certain amphibian species that transmit *Bd*, and the Endangered Species Act might be applied to heavily impacted species. However, the processes through which the applications of these Acts are determined can take years. Codes of conduct could be applied by specific sectors to prevent the spread of *Bd*. For example, the pet industry is considering a code of conduct coupled with best management practices for application by amphibian distributors and retailers in order to minimize *Bd* transmission. Fisheries managers and field biologists might consider developing a code of conduct or HACCP plan for biosecurity measures that could minimize the transmission of *Bd* between aquatic sites. At this conference, the Pet Industry Joint Advisory Council (PIJAC) is hereby launching the first large-scale education/outreach campaign on *Bd*, specifically targeting amphibian distributors, retailers, and hobbyists. There are other campaigns being directed by PIJAC and cooperating federal and state agencies, including the U.S. Fish and Wildlife Service, that could be employed for *Bd* education and outreach. These include HabitattitudeTM and Stop Aquatic HitchhikersTM. Ideally, education, policy, and regulatory measures should be targeted to the sectors responsible for the most high-risk pathways of *Bd* spread. Thus, further research detailing these pathways as well as associated risk management opportunities is urgently needed.

Natural history of *Bd*

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Chytridiomycosis is a potentially lethal amphibian disease caused by infection of the skin by the fungus *Batrachochytrium dendrobatidis* (*Bd*). *Bd* is in the Phylum Chytridiomycota (chytrids) and its ancestors diverged early from most other fungi. *Bd* is a newly emerged pathogen based on the novel discovery of chytridiomycosis in 1998 and the description of *Bd* in 1999. Zoosporangia of *Bd* live within cells of the superficial epidermis of adult and larval amphibians. Transmission and reinfection occur via release of asexually produced flagellated zoospores into the environment. *Bd* can be grown in culture where its life cycle takes between 4-5 days at 23°C. Cultures do not survive desiccation and die above 29°C. Natural spread is thought to occur within water bodies by movement of zoospores by currents and within and between amphibian populations by movement of amphibians. Human assisted movement could occur by movement of infected amphibians or substrates contaminated with *Bd*. There is strong evidence that anthropogenic spread to naïve populations is the reason for *Bd*'s global emergence. Retrospective studies suggest chytridiomycosis emerged in the 1970s after apparently being introduced to Australia and the Americas where it is thought to have caused the decline or extinction of about 200 species of amphibians. The earliest record of *Bd* in museum specimens is from southern Africa in 1938, indicating Africa as the possible origin. Climatic modeling and surveillance suggest there are potentially vulnerable populations of amphibians that are currently free of *Bd*. It is likely that ecosystems are substantially affected by loss of amphibians.

The Australian government Threat Abatement Plan (TAP) for chytridiomycosis

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Chytridiomycosis was nominated as a "Key Threatening Process" (KTP) to Australian biodiversity in 2001 by Rick Speare and the steering committee of the Getting the Jump on Amphibian Disease Conference held in Cairns, Australia in 2000. The purpose of the Cairns conference and workshop was to build consensus among researchers, wildlife managers, industry, and the public about the importance of chytridiomycosis and to develop recommendations for action. This nomination was based on knowledge of the disease and legislation that provides for recognition of KTPs and action to protect biodiversity from KTPs under the Australian Commonwealth Environmental Protection and Biodiversity Conservation Act 1999. This nomination was accepted by the Australian federal government in 2002, and a draft Threat Abatement Plan (TAP) against "Infection of amphibians with chytrid fungus resulting in chytridiomycosis" was produced in 2004, subjected to public comment, and accepted in 2006 (Australian Government Department of the Environment and Heritage 2006 *http://www.deh.gov.au/biodiversity/threatened/publications/tap/chytrid/*). Recommendations in this plan cover disease control strategies and hygiene protocols, endangered species recovery projects, and future research. The TAP proposes four general strategies to limit the introduction of *Batrachochytrium dendrobatidis* (*Bd*) into chytrid-free regions: (1) reduce risk of importation, (2) reduce risk of release of *Bd*, (3) reduce risk of release of infected amphibians, and (4) limit spread at point of introduction. It also makes a series of recommendations for research where data is deficient to enable informed management strategies. The quality of evidence to guide intervention was particularly inadequate for populations were *Bd* is already endemic. It has led to major funding (several million dollars) from the Australian Government Department of the Environment and Heritage (now the Department of the Environment and Water Resources) and the Australian Research Council for research on priority issues with knowledge gaps such as determining the distribution, preventing the spread, and understanding the epidemiology and pathogenesis of chytridiomycosis. This work is ongoing, but some of the research has been published in scientific journals, as government reports, and on the Amphibian Diseases Homepage *http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm*. It is important for other nations to develop their own control plans. On the international scene, currently the Aquatic Animal Commission of the World Organisation for Animal Health is considering a proposal to make chytridiomycosis an internationally notifiable disease. Acceptance of this proposal may result in countries having to demonstrate knowledge of their *Bd* status.

It is important for conservation agencies to provide long-term research funding in order that chytridiomycosis can be adequately understood and controlled. Lastly, chytridiomycosis provides us with an opportunity to be better prepared for recognizing and dealing with the next pandemic to affect biodiversity.

Pathways and puzzles: crossroads of disease and recovery for the Chiricahua leopard frog (*Rana chiricahuensis*)

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Chiricahua leopard frogs (*Rana chiricahuensis*) were historically found in aquatic systems in central and southeastern Arizona, west-central and southwestern New Mexico, and the sky islands and Sierra Madre Occidental of northeastern Sonora and western Chihuahua, Mexico. The range of the species is split into two disjunct parts—(1) the northern populations along the Mogollon Rim in Arizona east into the mountains of west-central New Mexico and (2) the southern populations in southeastern Arizona, southwestern New Mexico, and northern Mexico. Chiricahua leopard frogs require permanent or semi-permanent aquatic sites at elevations from 1000 to 2710 m. Historically, these sites included a variety of aquatic habitats, including montane and river valley cienegas, springs, pools, cattle tanks, lakes, reservoirs, streams, and rivers. The species is currently limited to a subset of these aquatic systems in which nonnative predators (e.g., American bullfrogs, fishes, and crayfish) are rare or absent.

During the last 30 years, populations of Chiricahua leopard frogs declined dramatically. Implicated in these declines are predation by nonnatives, disease, drought, floods, degradation and loss of habitat (e.g., drought, poor livestock management, and catastrophic fires), disruption of metapopulation dynamics, small population biology, and environmental contaminants.

To prevent its extinction, the Chiricahua leopard frog was federally listed as threatened in 2002. The Chiricahua Leopard Frog Recovery Team completed a recovery plan in 2007, which, if implemented, should mitigate threats and result in recovery and delisting of this species. Key recovery actions include protecting existing populations and habitats, habitat restoration, population augmentation and reestablishment, monitoring, research, and adaptive management. One of the more severe threats identified by the recovery team was the pathogen *Batrachochytrium dendrobatidis* (*Bd*). Mitigating this threat will be particularly challenging, but to be successful, the plan is intended to reduce or eliminate the potential risk of introducing *Bd* into pathways of transmission. Recommended recovery actions include measures to minimize or prevent the likelihood of transmission during monitoring, reestablishments, and other recovery actions; but also include research to explore and develop tools for eliminating *Bd* from aquatic sites and populations, and to investigate whether *Bd*-resistant populations of frogs can be developed and used in the recovery program.

In this talk, we elaborate on recovery actions designed to reverse the spread of *Bd*, potentially treat *Bd*-positive habitats and populations, and ultimately manage the pathogen, its hosts, and aquatic habitats in ways that will allow recovery of the threatened Chiricahua leopard frog within a context of multiple and often interacting threats.

Rapid spread of *Batrachochytrium dendrobatidis* causes chytridiomycosis outbreaks and extinctions in yellow-legged frogs of the Sierra Nevada

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Chytridiomycosis, an emerging infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is found in collapsing amphibian populations and has been implicated as a major cause of worldwide amphibian declines. The effect of this pathogen on amphibians has been called the worst case of disease-caused biodiversity loss in recorded history. However, because many amphibian species appear to not be susceptible, some question whether it is the causative factor in many declines. Our 11-year study tracks rapid spread of *Bd* through >500 populations of two closely related frog species (the Sierra Nevada mountain yellow-legged frog, *Rana sierrae*, and the southern mountain yellow-legged frog, *Rana muscosa*) in California, USA. Repeated surveys show a marked easterly spread of *Bd* across the Sierran range causing a wave of chytridiomycosis outbreaks. These epidemics resulted in >100 population extinctions since 1997. To better understand the dynamics of the disease, we used quantitative PCR to trace the spread and infection load of infected frogs through three widely separated metapopulations of frogs consisting of 73 subpopulations. The disease swept through all three areas at approximately 1km/year. At all three sites, frog metapopulations were relatively stable prior to Bd arrival but declined precipitously following the arrival of *Bd*. Within weeks of first detection, infection prevalence reached 100%, and a exponential growth rate of infection on individual hosts resulted in mass mortality, rapid population crash, and extinction. In individual frog populations, infection intensity predicted the onset of population collapse.

From frogs in demand to frogs in decline

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The sudden appearance of chytridiomycosis as the cause of amphibian deaths and population declines in several continents suggests that its aetiological agent, *Bd*, was introduced into many of the affected regions. Harvesting wild amphibians for animal trade and consequent introductions of exotic species are considered threats to biodiversity. The global demand by the scientific trade for the subclinically infected African clawed frogs, *Xenopus laevis*, makes this species a model vector for *Bd*. Uncontrolled harvesting of *X. laevis*, feral populations, and the spread of disease agents, highlight the relevance of this trade to the conservation of amphibian biodiversity. Chytridiomycosis has been a stable endemic infection in southern Africa before any positive specimen was found outside this region. Current knowledge of the geographical distribution of *Bd* in Africa remains incomplete. Positive cases have been reported from west, east, and southern Africa with very little evidence of associated population decline. The gaps in the distribution data do not imply a disjunct distribution but rather reflect the intensity of research effort into amphibian diseases on the continent.

Poster Abstracts

www.spatialepidemiology.net: A global tool for managing disease surveillance and tracking routes of infection using genotypic data demonstrated by the EU *Bd* database

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We describe the development of a community-focused mapping tool, www.spatialepidemiology.net, that allows realtime surveillance and identification of sources/sinks of infection within a pathogens global occurrence. The Spatialepidemiology.net framework is described briefly as follows:

Data Visualisation—The *Bd* database is viewable as an interactive web-based map using the Google Maps and Google Earth interfaces. All isolate positions within the database are highlighted and allow drilling down via the map to obtain further epidemiological data, including amphibian distribution, genotype (if known), epidemiology, etc. For those isolates where genotypes have been obtained, the ability to investigate further is provided through the eBURST algorithm which attempts to define founding genotypes within a multilocus dataset. All data is also available to interrogate through standardized database search methods.

Data Surveillance—Monitoring occurs at various levels of spatial resolution (region, country, globally) for a number of parameters (amphibian species, genotype(s)) allowing summary statistics available online and in real-time to track levels of incidence and to highlight unusual increases of chytridiomycosis to the *Bd* community as a whole.

Data Storage and deposition—All data is stored centrally. Private databases are maintained and updated by users via the internet and allow analysis of private-user data combined with publicly available data prior to publication and subsequent data release. Users can enter data retrospectively, and the provision of tools to upload widely recognized GPS data formats provides the potential for field investigators to upload data directly and in a timely fashion.

We show using the European Union *Bd* surveillance database, that the organism is widespread across Europe however is spatially heterogeneous at smaller scales. We demonstrate, using multilocus genotype data, that the amphibian trade and amphibian re-introduction programs have likely lead to the recent introduction of *Bd* in several regions.

Occurrence of *Batrachochytrium dendrobatidis* in pond waters in the western United States—initial findings from a 2007 pilot study

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Using recently published methods, we sampled for *Batrachochytrium dendrobatidis* (*Bd*) in ponds and on resident amphibians (where possible) around the USA. Field protocols to facilitate sampling in remote locations were also tested. *Bd* occurrence in the environment (water) was patchy, including within individual ponds. Where detected, *Bd* densities ranged from 0.7 – 56.4 zoospores/L, which are in the same range as densities reported in recent literature. Even accounting for incomplete recovery, *Bd* zoospore densities in water appear to be orders of magnitude less than those used in published laboratory exposure studies, raising questions about the duration and mode of exposure needed to cause chytridiomycosis in nature. Swab results from individual amphibians are not yet available; so, comparisons between detection of *Bd* in the environment and on local animals could not be made. Results from this pilot study will guide the design of a more comprehensive study of *Bd* occurrence in water and resident amphibians within the United States.

Over-wintering larvae of bullfrogs (*Rana catesbeiana*) as biological reservoirs of the pathogens causing chytridiomycosis (*Batrachochytrium dendrobatidis*) in Trinity Co., California

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Invasive species have been recognized as one of four future challenges in the next 20 years by the USFWS and USGS. Threats posed by invasive species to native species include predation, competition, and spread of pathogens. The term “pathogen pollution” has been put forth for the spread of new or novel pathogens from invasive species to native species as a result of anthropogenic introduction of diseased animals into new geographical locations. Bullfrogs were brought into California in the late 1800s for a potential food crop and have since been established throughout the state. Adult bullfrogs have been recognized as potential carriers of the fungal pathogen, *Batrachochytrium dendrobatidis*, which has been associated with die-offs and population declines of numerous amphibian species. From October 2004 to April 2005, we conducted monthly collections and surveys of bullfrog larvae from a pond in Trinity Co., California, a site where native amphibians (*Bufo boreas* and *Hyla regilla*) are known to overlap with bullfrogs during the breeding season. We collected and tested bullfrog larvae to determine if they were infected with *B. dendrobatidis*, using mouthpart examinations and real-time PCR. Bullfrog larvae from the hatch year of 2003 were found to be infected with *B. dendrobatidis* throughout the winter, although younger larvae from hatch year 2004 were not infected. Therefore, bullfrogs may act as biological reservoirs of the fungal pathogen, *B. dendrobatidis*, to the native amphibians during the spring/summer breeding season.

Batrachochytrium dendrobatidis inhibition conveyed by cutaneous, amphibian bacterial metabolites

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Microbial ecology plays an important role in many biotic and abiotic systems. Complex relationships between the host and the host's microbes drive natural processes like nitrogen fixation in soils, the digestion of cellulose in termites, and fungal control agents in leaf cutter ant colonies. We have also discovered that some amphibian species may use the microbiota on their skin to protect themselves and/or their eggs from opportunistic pathogens. Many of the bacterial species isolated have displayed an inhibitory response to the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), a leading cause of amphibian decline worldwide. Our studies in vivo have shown that we can apply antifungal bacteria to amphibian skins and that this ameliorates the symptoms of chytridiomycosis. Often microbial inhibition is due to resource competition, phagocytosis, or metabolite secretion. The production of inhibitory metabolites has a practical application in the treatment of Bd-infected amphibians and is likely to be an important main contributor in the host amphibian's innate immune system. Using several analytical techniques, such as 1H NMR, HPLC/UV-Vis, GC/MS, HRMS, and IR, in conjunction with biological assays, inhibitory Bd metabolites can be identified. Once the metabolites are identified, they or the bacteria producing them can be employed in treating *Bd*-infected amphibians thereby providing a means to manage chytridiomycosis in amphibian populations.

Evidence supporting the climate-disease hypothesis from direct-developing frogs in Puerto Rico

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Long-term field data on population dynamics of *Eleutherodactylus* at El Yunque, Puerto Rico revealed declines in several species. These declines were correlated with periods of extended drought in the island. Diagnosis of *Batrachochytrium dendrobatidis* (*Bd*) suggested a potential synergistic interaction between local climatic patterns and disease prevalence. Ex-situ experiments were performed to test the effect of drought and *Bd* in highland *E. coqui*. While frogs exposed to water deprivation treatments died after 5 days of inoculation with *Bd*, infected frogs with plenty of water survived. Direct developing frogs limited by water, clump at humid retreat sites. When infected with *Bd*, these frogs are more likely to die from chytridiomycosis. Data from 2 years of field monitoring reveal that *Bd* prevalence is higher during the wet-hot months when frogs can tolerate the disease. During the dry-cool season (December-April), population abundance declines; however, prevalence of *Bd* among the few active frogs is lower. Results form our ex-situ experiments suggest that during the dry season, sick frogs remain in their retreat sites and die from chytridiomycosis. This explains the cyclic population dynamics observed in the coqui frogs where populations crash during extended periods of drought but recover during the wet season. Thus, direct-developing *Eleutherodactylus* may have an intrinsic host-pathogen mechanism that interacts with local climate allowing *Bd* to persist in tropical ecosystems, while wiping out other,

more vulnerable species. This may explain amphibian community responses to *Bd* in other tropical areas where direct-developers survive while aquatic species succumb.

Chytridiomycosis survey in wild and captive Mexican amphibians

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Mexico, a rich country in terms of amphibian diversity, hosts about 363 described species. Population declines have been documented for several species where it is evident that their habitat is being destroyed or modified. However, other species which inhabit pristine areas are declining as well. It has been suggested that the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) may be one of the causes of the enigmatic declines in Mexico. We surveyed a total of 45 localities in 12 states across Mexico. We examined a total of 364 wild specimens representing 14 genera and 28 species by light microscopy to identify presence of *Bd* sporangia. We analyzed the positive samples from wild amphibians and an entire colony (90 specimens) of *Ambystoma mexicanum* by Real Time PCR. We found evidence of Bd infection in a total of 110 specimens, comprising 14 species from 12 localities. Eighty-five percent of the *A. mexicanum* from the colony were infected with *Bd*. The two most highly infected individuals were *Ambystoma mexicanum* and *Pachymedusa dacnicolor*, purchased at a pet shop.

Detection of *Batrachochytrium dendrobatidis* in four endemic Central Texas amphibians

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During the last 20 years, significant declines in populations of amphibians have been observed worldwide. Although many factors such as habitat loss, environmental toxins, invasive species, and UV radiation have been implicated in declines, some of the blame for recent losses is given to the emerging infectious fungus *Batrachochytrium dendrobatidis* (Chytrid fungus). Consequences of the occurrence of this pathogen in North America remain unclear, although the pathogen has been detected in many U.S. states. However, its presence is not well documented in Texas, which provides habitat for several endemic and threatened amphibians. The aim of our study was to screen individuals of four threatened amphibians in Central Texas for Chytrid fungus. Using PCR-based analyses, Chytrid fungus was detected on endangered Jollyville (*Eurycea tonkawae*), Barton Spring (*Eurycea sosorum*), and San Marcos (*Eurycea nana*) salamanders and also on Houston toad (*Bufo houstonensis*). While all analyses of salamanders were based on recently obtained samples, Houston toad samples covered a time range from year 2000 to 2006 with only the most recent samples being positive for Chytrid fungus. Sequence analysis of PCR products confirmed the detection of Chytrid fungus and, thus, demonstrated the presence of this pathogen in populations of endangered species in Central Texas.

Emergency translocation as a response to the detection of *Bd* into a naive threatened population of *Leiopelma archeyi* in New Zealand

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Leiopelma archeyi is one of four native frog species present in New Zealand. Due to the risk of significant population decline, it has been classified as "nationally critical" which is the highest threat category in NZ. It is a terrestrial frog and is located in two populations on the North Island: Coromandel and Whareorino. Between 1994 and 2002, a major population crash occurred on the Coromandel peninsula hypothesized to be due to *Bd* which was discovered in a few dead frogs by histology in 2001 (Bell 2004) and confirmed by Taqman real-time PCR in 2006. The Whareorino population was estimated to be around 5000 to 20,000 frogs and considered the last stronghold for the species. Routine *Bd* swabbing from four mark-recapture monitoring grids in November 2005 – March 2006 revealed a positive prevalence of 5-6% in the two Northern grids by Taqman real-time PCR in triplicate. Forty dead frogs collected from the area between 2000 and 2006 tested negative for *Bd*. Therefore, the new presence of *Bd* in a naïve population was considered a major threat. Our emergency response was to establish a new wild population in the hopes of spreading the disease risk over multiple populations. One hundred frogs from both North and South Whareorino were collected and quarantined for 90 days. Eleven frogs tested positive for *Bd*, and two others were positive in one out of three replicates. Two died in quarantine, one being chytrid positive. Seventy were translocated to a new site thought to be chytrid free (no frogs or running water present) although we were not able to prove this due to current constraints of testing for the presence of *Bd* in the environment. Sixteen frogs were added to a captive population, and some are now breeding. Twelve chytrid positive frogs went to the University of Otago and cleared the infection naturally.

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Evaluation of distribution limits of chytrid fungus in the Rocky Mountains and potential for transport by waterfowl

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Hopes for managing the effects of the chytrid fungus (*Batrachochytrium dendrobatidis* [*Bd*]) on amphibian populations, including repatriation of endangered species, depend on the absence of *Bd* from appropriate amphibian habitat and effectiveness of methods to prevent the spread of *Bd* into those habitats. However, recent surveys have found *Bd* already widely distributed among amphibian populations and habitats in western North America. If *Bd* can survive in habitats without amphibians and if it can be transported among wetlands by nonamphibian mechanisms, such as on the feet of waterfowl, then the prospects for locating *Bd*-free habitats and maintaining that status become very uncertain. In 2007, using recently developed methods for detecting presence of *Bd* in water, we sampled 28 water bodies in Colorado and Montana that are either above the known elevation limits for amphibians or are in areas recently deglaciated and not yet colonized by amphibians. We also collected standard *Bd* swabs from feet of ducks captured for avian influenza testing at urban ponds and a National Wildlife Refuge in Montana. We will present preliminary results and discuss the implications for the occurrence and spread of *Bd* in the Rocky Mountains.

The plight of native ranid frogs on the Coronado National Forest

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The Coronado National Forest of southeastern Arizona and extreme southwestern New Mexico is composed of numerous small mountain ranges that are separated by valleys. The Coronado National Forest manages these disjunct mountain ranges as 12 Ecosystem Management Areas (EMAs). All of the EMAs have been inhabited (at least historically) by one or more species of native ranid frogs. The area has the highest diversity of ranid frogs in western North America, including *Rana blairi*, *R. chiricahuensis*, *R. subaquavocalis*, *R. tarahumarae*, and *R. yavapaiensis*. All of these species have been in decline to various degrees. Lowland populations of all species are either gone or in jeopardy due to the effects of urbanization and agriculture. *Rana tarahumarae* was extirpated from the Coronado National Forest (and Arizona and the USA) in 1983, but efforts are underway to re-establish populations. The southern populations of *R. chiricahuensis* have their USA epicenter on and adjacent to the Coronado National Forest. This species was once widespread across most of the forest but is now down to a handful of localities. In recent years, it may have been extirpated from the Chiricahua Mountains (including the type locality) and Galiuro Mountains. *Rana subaquavocalis* is endemic to the Huachuca Mountains and was nearly extirpated but is making a slow comeback as a result of reintroductions and habitat improvements. These dramatic declines have been attributed to predation by nonnative species, chytridiomycosis, loss of habitat, and drought. Conservation programs are proceeding to stabilize and improve the status of these imperiled frogs.

The ubiquity of the chytrid fungus and the futility of fighting it: lessons for the prevention of future wildlife panzootics

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The human-mediated transport of amphibians is the most plausible driver for the intercontinental spread of chytridiomycosis. *Batrachochytrium dendrobatidis* occurs on six continents and infects over 260 amphibian species. It is widespread throughout Australia, Europe, and the Americas. *Batrachochytrium dendrobatidis* was detected across the altitudinal gradient in all five studies that have examined its altitudinal distribution. The apparent ubiquity of *B. dendrobatidis* suggests that, barring drastic and immediate amphibian trade restrictions, it is likely to invade any currently unoccupied areas of its bioclimatic envelope. It is not feasible to eradicate the fungus from infected sites and it is unlikely that suitable methods will be developed prior to the fungus' initial epidemic wave wreaking havoc on any remaining naïve and susceptible amphibian populations. The management of *B. dendrobatidis* in infected amphibian populations is thus futile, the only management of those populations being indirect, that is, mitigating all other threats to the affected populations. Protecting remaining uninfected but susceptible amphibian populations from the chytrid fungus will require drastic and immediate restrictions on both the domestic and global trade and transport of amphibians, on a scale unlike any that have been implemented in the past. That one of the most serious threats to biodiversity cannot be managed at affected sites should serve as a valuable lesson for the prevention of future panzootics. The continued intercontinental trade and transport of animals will inevitably lead to numerous extinctions caused by the introduction of infectious diseases. However, appropriate biosecurity measures can reduce the likelihood of these events.

A survey of *Bd* presence from randomly selected wetlands in the Winnebago River watershed, Iowa, USA

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Chytridiomycosis is not specifically linked with any amphibian population declines in the Midwest; however, the effects of this pathogen on amphibian populations in other parts of the world make it a potential threat that should not be ignored. To date, there are no published accounts of field surveys for *Bd* in the Midwest. A better understanding of the distribution of the disease can lead to more focused monitoring and study of *Bd* disease ecology and population impact assessment. From April to July of 2006, I collected swabs from northern leopard frog (*Rana pipiens*) tadpoles, metamorphs, and adults at randomly selected prairie pothole wetlands in north-central Iowa. I focused sampling on tadpoles and attempted to sample 60 tadpoles per wetland in order to detect at least one affected animal, with 95% confidence, given the assumption that *Bd* prevalence in the population was 5% (DiGiacomo and Koepsell 1986). I collected 755 swabs from 22 wetlands, sampled from 720 tadpoles, 25 metamorphs, and 10 adults. I used a quantitative real-time Taqman PCR assay and detected *Bd* on five swabs, all taken from adults. There were no detections of *Bd* on swab samples taken from tadpoles and metamorphs. Although less than 1% of all swabs were positive for *Bd*, the pathogen was detected at 23% of all wetlands sampled. I collected 200 additional swabs from larvae and metamorphs in 2007, which are awaiting analysis. The results of this survey will add to the growing body of knowledge about the geographic distribution and prevalence of *Bd*.

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Environmental substrates as a source of chytridiomycosis infection

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In the past few decades, a large proportion of amphibians have suffered decreases in population size with many facing possible extinction. Research has shown that these declines may be a result of chytridiomycosis, an infectious disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis*. Previous studies have found conflicting results as to the potential of the fungus to persist on environmental substrates; however, this topic remains a relatively unexplored area in the study of chytridiomycosis. The focus of this study was to test for the presence of *B. dendrobatidis* on environmental substrates during a chytridiomycosis epidemic to determine whether they could serve as potential sources of infection. The research was conducted at two sites in Panama and Real-Time PCR was to detect presence of *B. dendrobatidis* on environmental swabs. The percentage of positive environmental samples was then compared to the percentage of *Atelopus* skin swab samples that tested positive in the respective areas. Several streamside substrates tested positive for the fungus indicating that contaminated substrates may be an important mode of transmission for this disease. This finding provides new insight into the role that the environment plays in the spread of chytridiomycosis.

Experimental assay for the environmental transfer of amphibian chytridiomycosis in captively propagated alligator snapping turtles

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Chytridiomycosis is a highly infectious amphibian disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). The National Fish Hatchery system raises many aquatic species and frequently releases animals into wild waters as part of recovery and restoration efforts. *Bd* was recently found on a federal facility, causing concern that stocking practices may facilitate the movement of *Bd* to novel habitats. Tishomingo National Fish Hatchery is a warm water facility currently rearing alligator snapping turtles (*Macrochelys temminckii*) and has not been surveyed for *Bd*. As a first step, we chose to ensure that no passive transfer of *Bd* is occurring with the release of captively reared alligator snapping turtles. For this study, we used diagnostic quantitative polymerase chain reaction to determine the infection status of resident broodstock and production populations of alligator snapping turtles from Tishomingo National Fish Hatchery. The analysis of foot swabs from 100 alligator snapping turtles indicated no *Bd* pathogen was present, which suggests that alligator snapping turtles are not passively transferring *Bd* to novel waters. This study provides some assurance that in the alligator snapping turtle program at Tishomingo National Fish Hatchery, *Bd* is not hitchhiking into naive habitat with released turtles. We recommend the hatchery program continue to monitor animals for the presence of *Bd*, as well as testing amphibians in the vicinity of the station to determine the level of ambient infection. The U.S. Fish and Wildlife Service in Region 2 has taken the first steps to preemptively prepare for the potential impact of *Bd* on hatchery operations.

Chytrid fungus in Jollyville Plateau salamander populations in Texas

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The chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), appears to be a recently emerged worldwide pathogen that affects mainly adult frogs and toads. The chytrid fungus infects keratinized skin, which is found over the entire body of adult frogs and toads, and is believed to interfere with respiration and hydration. City of Austin biologists have documented *Bd* in the Jollyville Plateau salamander, *Eurycea tonkawae* (JPS) across its range in an area northwest of Austin, Texas. JPS is a neotenic plethodontid salamander found in springs and caves in the northern segment of the Edwards Aquifer. In contrast to frogs and toads, keratinized skin in aquatic salamanders is believed to be limited to the toes and does not appear to cause any noticeable health effects. In December 2005 and May/June 2006, City of Austin biologists submitted small amounts of toe tissue from 39 wild-caught JPS to Pisces Molecular (Boulder, Colorado) for chytrid PCR analysis. All samples, which were collected from 16 different sites ranging from urban to "pristine," tested strongly positive for *Bd*. Although sampling of anurans within the JPS's range has been very limited, tadpoles are noticeably absent from some of the urban sites, and one of two Rio Grande leopard frogs (*Rana berlandieri*) has tested positive for *Bd*. This appears to be the first documentation of *Bd* in Texas.

California Center for Amphibian Disease Control—a website resource

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Amphibian declines have been an area of intense research since the early 1990s; however, disease was never seriously considered a factor in the declines. That changed in 1998 when *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of amphibian chytridiomycosis, leaped onto center stage. Since then numerous amphibian declines and extinctions worldwide have been linked to the pathogen. We believe that effective management for this and other amphibian diseases will require a publicly accessible database system to track the taxonomic and geographic distributions of pathogens and to provide up-to-date information on sampling, collecting, and testing for pathogens and decontamination procedures. California Center for Amphibian Disease Control was designed in an attempt to provide state-of-the-art information on *Bd*, particularly in California amphibians, for professionals venturing into the field and management agencies.

Amphibian chytridiomycosis in Oregon spotted frogs at Conboy Lake National Wildlife Refuge

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The Oregon spotted frog (*Rana pretiosa*) is a candidate species for listing under the U.S. Endangered Species Act and is considered imperiled across its geographic range. The largest extant population of

Oregon spotted frog occurs at Conboy Lake NWR (Klickitat County, Washington State), where the species co-occurs with introduced American bullfrogs (*R. catesbeiana*). Conboy Lake has been surveyed on a continuing basis since 1997. Egg mass counts revealed a breeding population of *R. pretiosa* which increased from 2001 to 2005 but declined thereafter; a marked drop (i.e., 67%) in egg mass numbers occurred between 2006 and 2007. In 2006, we detected *Batrachochytrium dendrobatidis* (*Bd*) in *R. pretiosa* at Conboy Lake. Subsequent collecting in 2007 revealed that 100% of dead *R. catesbeiana* were also heavily infected with *Bd*. We here discuss the potential implications of these findings.

Treatment of chytrid infection in *Typhlonectes* spp. using elevated water temperatures

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A group of *Typhlonectes* spp. was brought into quarantine at the Bronx Zoo's Wildlife Health Center after being confiscated by the U.S. Fish and Wildlife Service at a local international airport. Animals were set up in aquaria and water temperature was maintained at 21 – 24.5°C with a pH of 5.8 – 6.5. Routine quarantine procedures were initiated consisting of foot baths, isolation from other amphibians, and handling/servicing the animals while wearing disposable gloves. All individuals were swabbed with dry, sterile swabs approximately 30 times on the ventral aspect of their bodies. Swabs were allowed to air-dry and then were placed into sterile dry vials for transport. PCR to detect Batrachochytrium dendrobatidis was performed by Dr. Alan Pessier at San Diego's Center for Research on Endangered Species. Thirteen of 24 animals tested positive for the fungus. Treatment, consisting of elevating to and then holding water temperatures at 32.2°C for 72 hours, was performed. At the end of 72 hours, animals were moved to clean enclosures and water temperatures were returned to normal. Repeat testings, 1 to 3 weeks after treatment, were negative for the fungus.

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Patterns of chytridiomycosis infection in Panamanian golden frog populations (*Atelopus varius* and *A. zeteki*)

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The Neotropical frog genus *Atelopus* is among the most imperiled of all amphibian lineages with 62 of 77 species classified as extinct or critically endangered. In Panama, the two sister taxa collectively called Panamanian golden frogs, *Atelopus varius* and *A. zeteki*, are in critical danger of extinction, likely due in large part to an epidemic wave of chytridiomycosis infection. While *A. varius* was historically found throughout much of montane Costa Rica and western Panama, it has disappeared from most of its former range. *Atelopus zeteki* has also recently disappeared from much of its range, which included the area in and around an extinct volcanic crater at El Valle de Anton, Panama. Knowing how population-level differences in behavior and natural history translate into variation in chytridiomycosis infection represents an important step toward understanding the relationship between this disease and patterns of declines and extinctions in *Atelopus* and other Neotropical amphibians. To test for correlations between patterns of *Batrachochytrium dendrobatidis* infection and variation in golden frog habitat use, behavior, and demography, we used mark-recapture studies and Real-Time PCR assays of two populations. The level of detail afforded by mark-recapture allowed us to test previously proposed hypotheses about the thermoregulation behavior and body condition of infected frogs, the spatio-temporal pattern of infection during an epidemic, and the relationship between susceptibility habitat use. We hope this information will not only aid understanding the observed pattern of *Atelopus* declines and extinctions but also help predict and perhaps prevent future extinctions through informed conservation management.

Climatic and spatial predictors of *Bd*-related amphibian extinctions in Central and South America

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In Central and South America, chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*), is the putative cause of 70 out of 113 extinctions in the toad genus *Atelopus*. Recent evidence suggests that these extinctions were associated with global climate change; however, alternative hypotheses have not been carefully tested. We evaluated the strength of the following four nonexclusive hypotheses for *Atelopus* extinctions: (1) The Epidemic-spread hypothesis, in which extinctions follow *Bd* introductions from nearby outbreaks, and the (2) Chytrid-thermal-optimum, (3) Mean-climate, and (4) Climate-variability hypotheses, in which changes in cloud cover, mean climate, and temporal variability in temperature predict extinctions, respectively. Our analyses revealed that the rate of *Atelopus* extinctions increased in the 1980s and decreased in the 1990s, consistent with epidemic dynamics following one or more recent introductions. However, we found no evidence that the distance between species ranges was predictive of the timing of *Atelopus* extinctions. We found no support for the Chytrid-thermal-optimum hypothesis because cloud cover was not predictive of extinctions. While some mean climate variables were predictive of *Atelopus* extinctions, none were as predictive as monthly variability in temperature. Monthly variability in temperature was greater in warmer years, and long-term increases in temperature variability were greatest during cooler months and at mid-elevations. These results are consistent with patterns of *Bd*-related declines, which are known to occur during warm years, during cooler months, and at mid elevations. These findings suggest that elevated climatic variability associated with climate change might be an important factor in *Bd*-related amphibian declines.

Banking amphibian genetic resources for research and recovery

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Bioresource banking supports in situ and ex situ conservation efforts by facilitating research programs, through curation of samples for ongoing and future investigations and for potential assisted reproduction efforts. The Zoological Society of San Diego's collections of viable, frozen, early passage

diploid cell cultures (known as the Frozen Zoo®) currently contains frozen cell cultures from more than 8,300 individual vertebrates comprising greater than 600 species. Only two of these are amphibians, and to our knowledge, no systematic effort is underway to preserve important bioresource materials for characterizing amphibian populations, identifying factors associated with susceptibility and resistance to chytrid infection, small population management applications, and other studies crucial to efforts to achieve long-term sustainability of endangered amphibian populations. Small population management efforts for endangered taxa routinely generate questions amenable to analysis with genetic studies, including phylogenetic systematics, population genetics, patterns of migration and dispersal, kinship and parentage confirmation. Furthermore, studies of host-parasite interactions typically involve utilization of well-defined in vitro systems for elucidating mechanisms of pathogenesis and therapeutic intervention. The establishment of a network for collecting specimens suitable for establishment of cell cultures and making resources, including derivatives such as DNA and cellular RNAs, available to the larger research community, is a crucial emerging need in support of long-term conservation efforts. The Amphibian Ark is supporting this conservation-focused resource by helping with the development and distribution of protocols for collection and storage of samples from the field.

High prevalence of *Batrachochytrium dendrobatidis* in wild populations of lowland leopard frogs *Rana yavapaiensis* in Arizona

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Batrachochytrium dendrobatidis (*Bd*) is a fungus that can potentially lead to chytridiomycosis, an amphibian disease implicated in die-offs and population declines in many regions of the world. Winter field surveys in the last decade have documented die-offs in populations of the lowland leopard frog *Rana yavapaiensis* with chytridiomycosis. To test whether the fungus persists in host populations between episodes of observed host mortality, we quantified field-based *Bd* infection rates during nonwinter months. We used PCR to sample for the presence of *Bd* in live individuals from nine seemingly healthy populations of the lowland leopard frog as well as four of the American bullfrog *R. catesbeiana* (a putative vector for *Bd*) from Arizona. We found *Bd* in 10 of 13 sampled populations. The overall prevalence of *Bd* was 43% in lowland leopard frogs and 18% in American bullfrogs. Our results suggest that *Bd* is widespread in Arizona during nonwinter months, and may become virulent only in winter in conjunction with other co-factors, or is now benign in these species. The absence of *Bd* from two populations associated with thermal springs (water >30°C), despite its presence in nearby ambient waters, suggests that these microhabitats represent refugia from *Bd* and chytridiomycosis. If other species of anurans are found to be tolerant of warm water temperatures, wildlife managers may consider exploring the use of artificially warmed waters as a refuge from *Bd* for critical populations.

Presence of *Batrachochytrium dendrobatidis* and amphibian ranaviruses in U.S. markets

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The international trade in live amphibians has been implicated in the global spread of *Batrachochytrium dendrobatidis* and ranaviral disease. The American bullfrog (*Rana catesbeiana*) is an efficient carrier of these pathogens and is globally traded as a live commodity and sold in U.S. markets. In this study, we obtained importation data for all live amphibians and parts thereof into three U.S. ports of entry (Los Angeles, San Francisco, and New York) to identify overall trends. The importation of live amphibians into these ports was calculated in the millions per year. Additionally, samples were collected from market frogs in each of these cities to determine disease prevalence. PCR analyses of samples yielded positive infections of both *B. dendrobatidis* and ranaviral disease. This study definitively identifies the presence of two pathogenic amphibian diseases in live market frogs and suggests that the amphibian trade may contribute to introductions of these pathogens into new regions. This data has been influential in advising the OIE Amphibian Working Group, a committee currently recommending that B. dendrobatidis and ranaviruses be listed as notifiable diseases.

Monitoring Georgia's amphibian populations for the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*): a comprehensive survey of five physiographic regions

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With one-third of all amphibian species threatened, the global decline of amphibians is of great concern to conservation biologists (Stuart et al. 2004; Mendelson et al. 2006). Although a large proportion of declines are contributed to anthropogenic factors, including habitat destruction and fragmentation, pollution, and over collection, "mysterious" declines are also occurring in protected and pristine sites around the world. These declines have been linked to the presence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al., 1998; Longcore et al. 1999). Despite amphibian populations being monitored for the occurrence of *Bd* in the neotropics, a center of anuran biodiversity, little has been done in the southeastern United States, a biodiversity hotspot for caudates. A comprehensive survey and sampling of amphibian populations for *Bd* was conducted throughout Georgia's five physiographic regions. Ten of 12 families of native Georgia amphibians (83%) were sampled for *Bd*, including 18 of 22 genera (82%) and 47 of 86 species (55%). Of the 460 samples taken, 10 were *Bd*-positive. Eight of these positive results were taken from six *Rana* spp. and two *Desmognathus conanti* from the same site in northwest Georgia. This is the first documentation of the presence of *Bd* from a *Desmognathus* species. The remaining samples were taken from two *Rana sphenocephala* from a site in the Coastal Plain. All of the *Bd*-positive individuals appeared healthy and did not exhibit physical symptoms of chytridiomycosis such as reddening of ventrum, sloughing skin, lethargy, loss of righting reflex and hemorrhaging (Daszak et al. 1999).

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Experimental infection of North American plethodontid salamanders with the fungus *Batrachochytrium dendrobatidis*

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Outbreaks of the amphibian fungal disease chytridiomycosis are causing sudden, localized extirpations of large proportions of the amphibian fauna in Central America and other regions. Population-level responses to *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of this disease, vary from severe declines and even extinctions in some species to apparently no effect in other species that may act as reservoir hosts. Recent surveys suggest this pathogen is widespread in the southeastern United States, but the implications for amphibian populations are unknown. From a conservation standpoint, it is critical to determine how much of a threat this pathogen may pose to the many endemic species of plethodontid salamanders in the Southern Appalachians. Ultimately, we need to understand which species are susceptible to infection and under what environmental conditions disease outbreaks might occur. As a first step to addressing these questions, we conducted a laboratory infection study of the susceptibility to *Bd* infection of two species of North American plethodontids, one highly aquatic (*Desmognathus monticola*) and the other terrestrial (*Plethodon metcalfi*). We predicted that at least *P. metcalfi* would become infected through contact with high concentrations of zoospores in water, because other researchers have infected the congeneric *P. cinereus* in the lab. Mortality of *P. metcalfi* due to *Bd* was 41.7% and was higher at 8°C than at 16°C. The mean time-to-death due to *Bd* was 36.0 days. *Bd* did not cause any mortality in *D. monticola*. None of the *P. metcalfi* in the control treatment died and only two control *D. monticola* died.

Batrachochytrium dendrobatidis infection of the Red List species _Alytes muletensis_ in Mallorca; routes of introduction and establishment of an itraconazole-based protocol for treatment

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In the early 2005, a specimen of the CITES Red List (IUCN VU D2) Mallorcan midwife toad, *Alytes muletensis* was found dead and quantitative PCR demonstrated infection by *Batrachochytrium dendrobatidis.* Here we present conclusive evidence for the presence of *B. dendrobatidis* in Mallorca. From the 17 sites analyzed, five were found to sustain *B. dendrobatidis* infection. In three of these sites, prevalence reached 100% and parasite load was extremely high. Potthoff-Whittinghil's test for overdispersion identified positive sites as distinct infection clusters associated with significantly increased risk of *B. dendrobatidis* infection. Demonstration of infection within Zoo populations that were used for re-introduction programs, and low genetic diversity of the pathogen within the infected sites, suggest that captive breeding programs may have introduced *B. dendrobatidis* into the infected natural populations.

In an effort to mitigate potential disease outbreaks, we have developed an effective method of clearing infection in infected *Alytes* larvae based on the antifungal itraconazole. An optimised protocol has shown that treatment with 0.5 mg/litre itraconazole applied in a 200 ml bath daily for 7 days results in complete clearance of infection, as demonstrated by qPCR. While further tests need to be undertaken to ensure that animals suffer no long-term side effects, antifungal treatment may be useful in clearing natural populations of *B. dendrobatidis*.

Mortality from chytrid fungus in California slender salamander, _Batrachoseps attenuatus_ (Plethodontidae)

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Batrachochytrium dendrobatidis (*Bd*), the causative agent of the fungal infection, chytridiomycosis, has been linked to amphibian declines across the globe. The pathogen has been primarily found and studied in anurans; however, there is increasing evidence for its presence in caudates. In 2006, *Bd* was found on the Plethodontid salamander, *Batrachoseps attenuatus*, in Berkeley, California. To assess the effects of naturally acquired *Bd* infection in *B. attenuatus*, a total of 27 infected (n = 18) and uninfected (n = 9) salamanders were collected and observed. During the period of study, zoospore levels for each individual were determined on a weekly basis using quantitative PCR. In infected salamanders, zoospore quantities increased following initial detection. Infected salamanders showed 100% mortality with death observed in 18 out of 18 infected as compared to 1 out of 9 uninfected salamanders.

SPONSOR ANNOUNCEMENTS

COLUMBUS ZOO AND AQUARIUM ANNOUNCES 2008 GRANT PROGRAM

For almost 20 years the Columbus Zoo and Aquarium has been providing modest grants for projects in central Ohio and throughout the world. In 2007 almost $700,000 will be given to other NGO's, graduate students, established field researchers, indigenous communities and artisan cooperatives working to benefit people and natural resources. The 2008 grant program will continue to support a wide variety of initiatives. However, research/conservation projects working with amphibians in peril will be of special interest in the coming year.

For more information about the Columbus Zoo and Aquarium Grant program visit www.columbuszoo.org. For grant applications contact Rebecca Rose at rebecca.rose@columbuszoo.org.

PIJAC ANNOUNCES NEW EDUCATION/OUTREACH CAMPAIGN

At this conference, The Pet Industry Joint Advisory Council (PIJAC) is launching an education/outreach campaign on Bd targeted at amphibian breeders, distributors, retailers, and hobbyists. The campaign slogan, "Keep Your 'Phibs *Bd* Free! Practice Safe Husbandry," and associated logo will be coupled with information on best management practices for amphibian quarantine, disinfection, and treatment. PIJAC welcomes campaign partners.

To learn more, participate in Working Group 2 on Day 3 or contact Jamie K. Reaser at pijacscience@nelsoncable.com.