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Promoting Conservation, Research and
Education for the World's Amphibians

SPECIAL EDITION

Scaling up the Global
Response to Emerging
Amphibian Diseases

Scientists Turn to Genetics
to Solve Chytrid's Puzzle

Trouble in the Aquatic
World: How Wildlife
Professionals are Battling
Amphibian Declines

Recent Publications

And Much More!

FrogLog

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Editorial

Dear FrogLoggers,

The challenges we face as a global community in the wake of emerging amphibian diseases may seem daunting and insurmountable at times. This is especially true in light of the threat that the recently discovered chytrid *Batrachochytrium salamandrivorans* poses to salamanders on both sides of the Atlantic Ocean. Sadly diseases like this are a reality of our time and the only way we will win the war against disease-driven declines and extinctions is through broad, collaborative and innovative approaches. We must pool our resources, expertise and our passions.

Researchers and conservationists are working around the clock pioneering cutting edge tactics to prevent the mass die-offs that we have witnessed before. This edition of *FrogLog* will put you right on the front lines in the fight against these deadly pathogens. Go behind the scenes, meet the people and learn more about the groundbreaking research and applications that may one day stop these diseases in their tracks. Below is a summary of some of the issues and questions you can read about in this edition:

- A new study discovered that frogs in captivity retain much of their pathogen-fighting microbial communities. Does this mean that one day the Panamanian natural golden frog could be returned to the wild? Researchers are investigating anti-*Bd* probiotics as a way to make this happen.
- How are wildlife professionals battling amphibian declines and what lies ahead through research, monitoring and on-the-ground management?
- How are scientists using genetics to solve chytrid puzzles?
- How do habitat destruction and chytrid add up to one thing: extinction?
- How can citizen scientists contribute to disease monitoring?
- What are the options for mitigating disease in places like Madagascar?

Through these pages you will quickly realize that there is unfortunately no such thing as a “one-size-fits-all” solution to this challenge. And that’s what makes this seem like an uphill battle. But it’s not a battle that each of us has to face on our own.

As we move into 2015, the Alliance has invested a total of \$427,211.28, awarded 22 grants (several of which you will read about in the following pages), supported 18 priority species and protected 5,860 hectares of important amphibian habitat worldwide. We’re making real progress and the momentum is continuing to build. All of this has only been made possible thanks to the dedicated commitment of Alliance Partners and individuals such as yourself working towards a shared goal. The Alliance is continuing to coordinate funding for disease monitoring and support the development of disease mitigation tools. There’s a lot more to do and we call on conservation-minded individuals like you to help in this fight. If your organization wants to be a part of this global movement please get in touch.

In the face of pathogens like *Bd*, Dr. Matt Becker makes a fantastic point in an article starting on page 31. “The more people we have working on a solution, the better. Maybe the solution doesn’t end up being probiotics. I really don’t care what the solution is, as long as we can stop the spread of *Bd*...” The Alliance supports Dr. Becker’s viewpoint as we support multiple strategies and combinations of strategies.

There’s a role each of us can play in saving these amphibians. Tell your friends. Make a [donation](#). Become a [partner](#). Sign up for our [mailing list](#). Follow us on [Facebook](#). Join us [Twitter](#). If we can all come together and find common ground then we will make real progress on the many threats amphibians face. It won’t be easy and it’s a long tough road ahead but as I always say, there is great strength in numbers. We can—and we will—win.

Candace M. Hansen-Hendrikkx

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AmphibiaWeb and the Amphibian Survival Alliance Partner to Collate and Share the World's Amphibian Data

By James Lewis & Michelle Koo

AmphibiaWeb, the web based platform that provides information on amphibian declines, natural history, conservation and taxonomy entered into a new partnership with the ASA at the end of 2014 to help fill the gaps in the coordinated sharing of amphibian related data.

AmphibiaWeb has been an active member of the Alliance since the beginning of 2014 but with an increasing need for a coordinated data resource it was natural for AmphibiaWeb to take on a more formal role for the Alliance.

As many of our readers know, the Alliance obtains many of its priority actions from the IUCN SSC Amphibian Specialist Group. One of the identified actions was to “create and promote the use of data sharing tools.” To address this action the Alliance has been working with a number of partners to determine what resources were currently available, as well as what options may exist for creating such tools.

A recognized need is a central authority on amphibian science. AmphibiaWeb's roots in the science community and in biodiversity informatics are important in this regard. Specifically, AmphibiaWeb tracks new species publications and localities, provides monthly amphibian declines literature, provides access to mapped species observations and range maps, as well as connects with amphibian experts around the world to provide vetted information on taxonomy, disease and natural history. With about 20,000 queries per day and scores of scientists and herpetology students contributing photos, information and accounts each year, AmphibiaWeb has a growing and solid community of international users who are demonstrating their concern for the conservation of these unique animals.

“By partnering with AmphibiaWeb to create the Alliance's ‘Science Zone’ we are helping to build the resources of this important website but also helping to provide the science community with a one stop shop for all things related to amphibian data,” said Don Church, executive director of the Alliance.

Most importantly, the new partnership of AmphibiaWeb and ASA will lead to new collaborative projects. One of the first projects will be an interactive and disease-monitoring portal. Working with groups like *Bd* Maps, *Bsal* Maps, Ranavirus Maps and a number of labs and organizations, the Alliance and AmphibiaWeb will collaborate to develop a tool that can link databases and provide a range of tools for both sharing and analyzing disease-related data.

AmphibiaWeb's professional steering committee has long envisioned creating such a portal to facilitate data exchange and visualization of disease outbreaks, especially important with newly emerging and quickly moving occurrences of chytrid fungus. “As we learn more about new strains of fungal disease and their disease dynamics,” David Wake, Director of AmphibiaWeb, states, “it is clear that monitoring with the most up-to-date data is key to making sound policy decisions to save amphibian species.”

The long-term goal of the Science Zone is the creation of a tool that will be as useful for amphibian researchers as it is for policy makers and educators alike, a truly collaborative platform that will be utilized by a range of stakeholders.

These types of projects are essential for the progress of amphibian conservation efforts around the world and the Alliance is eager to hear from groups that want to become involved in this project.



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SCIENCE ZONE
of the
asa
amphibian survival alliance



Photo: Robin Moore.

Carlos Vasquez Almazan Wins the 2014 Sabin Award for Amphibian Conservation

By Robin Moore

The Amphibian Survival Alliance is thrilled to announce Carlos Vasquez Almazan from Guatemala as the winner of the 2014 Sabin Award for Amphibian Conservation. Carlos is a rapidly emerging leader in the conservation of Central America's amphibians, effectively bridging the gap between science, conservation action and community engagement to identify and protect some of the most critical and imperiled habitats.

Carlos was instrumental in one of the landmark success stories of recent years: the creation of the [Sierra Caral Amphibian Reserve](#). The reserve, which protects the single most biodiverse forest remnant in Caribbean Guatemala and numerous amphibians found nowhere else, was recently expanded by the Guatemala National Congress to encompass 47,000 acres of lush forest.

Carlos continues to build on the success of the Sierra Caral by working with local and international partners towards the [protection of the rich forests of the Cuchumatanes mountains](#) of northwest Guatemala, home to several endemic and threatened salamanders and frogs.

What got you interested in amphibians?

When I was a child I used to go to my grandmother's house in the south coast of Guatemala. On nights when it rained I found there were many frogs and toads and I used to get wet walking and catching amphibians. My grandmother would always get an-

gry with me. I think my interest with these creatures, that sang so loudly on rainy nights in the tropical forest, were born out of these trips to my grandmother's.

What are your earliest memories of interacting with amphibians?

I remember one time when I was around 7 or 8, my father bought a piece of land in the city and started some construction. The workers found a white, soft and fat amphibian in the wet soil. It looked like a frog from the genus *Rhinophrynus* but white. I was so excited about this. I held it in my hand and wanted to keep it, but my moth-



Bradytriton silus. Photo: Robin Moore.

er wouldn't allow me. I am sure that the species I held, which was in the center of Guatemala City, was likely a new species.

When did you learn that amphibians were in trouble and how did this make you feel?

I started working at the Museum of Natural History and went to university to study biology around the same time. I started to work first with snakes, but I read more about and was conscious of the disappearance of forests and the threats to amphibian survival and I wanted to do something to preserve these amazing animals that made me so happy as a child.

Can you tell us a little about your current work and focus?

I work with a collection of amphibians and reptiles at the Museum of Natural History in the National University of Guatemala. I began working with colleagues from California and Mexico almost nine years ago, and we were all concerned about the importance of protecting habitats for the future. I worked also with a Guatemalan NGO called FUNDAECO working to protect some of the most fragile and important relicts of habitat and ecosystems throughout the country. I tried to focus all the knowledge and experience from my science background into actions to promote direct conservation actions. I really want children to have the opportunity to discover and experience the wonder of these amazing animals.



Photo: Robin Moore.

I am also working on a Ph.D. project focusing on a genus of endemic frogs from the genus *Plectrohyla*, that is very threatened by habitat loss, climate change and the amphibian chytrid fungus.

Do you have any funny stories from the field?

The first time when I camp in the field was in Laguna Lachua in Alta Verapaz Guatemala, I heard from the people that in the national park there are Jaguars. In the night we started to heard a loud noise like a big animal howling. I was so scared because I was thinking about the Jaguars and how big they are because the big noise. We were very vulnerable in tents in the field. After a sleepless night somebody told me that it was howler monkeys in the jungle.

The first time that I was in the lowlands in Peten Guatemala, one night I heard strange sounds in the leaves, close to where we would walk searching for animals. I pointed my light in the direction of the noise, but couldn't see anything. Again the sound started up again, really loud, and so I decided to walk to the source to see what was making the noise. There was nothing there. Later, the noise started again, as I was standing next to the spot and I discovered the source of the noise. It was crap from howler monkeys falling from higher branches in the trees. I almost took a shower in crap in the middle of the night.

What keeps you optimistic and positive?

Good memories from the past in Guatemala, and the memories of the forest from when I was a child. I want to give to my son, daughter and future children the opportunity to experience the beauty of the Guatemalan countryside. The smell of wet moss in the mountains, to see the mist in the Puerta del Cielo in the Cuchumatanes and to hear the cool streams running down from the mountains and swim in clean rivers and lakes. All the smells that I remember from nature make me happy and make my soul free and alive! Through my work I also meet many people from all over the world that have the same dream as me, and this is supportive and makes me believe in a better future for nature.



Scaling up the Global Response to Emerging Amphibian Diseases Through the Alliance

By James P. Lewis, Reid Harris, Candace M. Hansen-Hendrikx & Peter Jenkins

By now most of you will have read the highly anticipated paper¹ by Dr. An Martel from Ghent University in Belgium and her colleagues that reports on the new species of chytrid fungus, *Batrachochytrium salamandrivorans* (*Bsal*) which is now threatening salamander species around the world.

This recently discovered chytrid is lethal to salamanders such as Fire salamanders in Europe. Originating in Asia where it co-exists with local salamander species, this fungus was probably accidentally introduced by humans into Europe.

North America has the most biodiverse community of salamanders in the world and is home to many of the world's salamanders, including the diverse group of lungless salamanders that are abundant in the Appalachian Mountains, where they evolved and diversified. The biomass of terrestrial salamander in North American forests is huge and can equal or exceed the biomass of all the small mammal species. Thus, salamanders play a very important role in the ecosystem. Martel *et al.* report that the two tested members of this family in North America were not susceptible to *Bsal*, but they predict that species in this large family will vary in their susceptibility and therefore some plethodontids in North America are likely to be susceptible. The fungus was lethal to both eastern and western North American newts.

Dr. Reid Harris, Director of International Disease Mitigation with the ASA states, "*Batrachochytrium salamandrivorans* is indeed concerning. We have seen significant global declines and even the extinction of some amphibian species due to *Batrachochytrium dendrobatidis* (*Bd*), a similar species of fungus. Without action, the same fate awaits many species of salamanders in Europe and in the Americas due to *Bsal*. The difference with *Bsal* is that we are ahead of the curve at this point, and if we act quickly and decisively, investing in understanding which mitigation strategies are effective, monitoring and preventing movement of the pathogen around the world, we may be able to save many species from extinction. Some immediate actions to mitigate the effects of *Bsal* include testing all amphibians entering countries such as the United States for pathogens, including *Bd* and *Bsal*. Currently there is no mandated testing of amphibians entering the United States in the pet trade or via other pathways. In addition, antifungal skin probiotics are a promising preventative and treatment strategy and have been successful in some laboratory and field trials of frogs and salamanders with *Bd*."

Shortly after the publication of the paper, the Alliance and IUCN SSC Amphibian Specialist Group released a statement that in part highlighted the fact that, "Unregulated and unmonitored global amphibian trade is considered a major mechanism for dispersal of invasive species, including non-native emerging infectious diseases (EID). There are currently no global safeguard standards to ensure that amphibians in the international trade are monitored and tested for amphibian diseases. This means that amphibian populations in unaffected areas are at a very high risk of being impacted by EIDs that may be transported by amphibian hosts in the pet trade." Working with our Partners around the world we are exploring ways in which we can proactively address these issues.

Within the U.S. the Alliance submitted a letter on behalf of eigh-

teen U.S.-based Alliance Partners to the U.S. Fish and Wildlife Service (USFWS) requesting that the agency take emergency action to prevent the spread of *Bsal* into the U.S. We also organized a January 14 "*Bsal* Summit" with the USFWS leadership in which we presented advice from the top amphibian experts, as well as strong favorable input from conservation NGOs. We urged an emergency response and are hopeful the USFWS will act soon, but its next step remains unclear.

Working with a range of stakeholders, including members of the conservation and science communities and the pet industry, we are helping to provide recommendations to the USFWS as to reasonable measures that could be implemented to reduce the risk of *Bsal* entering the US. There are a number of different strategies on the table at this point but in essence we are looking for an option that would allow for a clean trade in salamanders. This is likely to include a mechanism to test imported salamanders for *Bsal*. How and where this happens is still to be worked out however the majority of those involved, including the Alliance, are eager to see the implementation of a system that will allow trade to continue but in a way that will prevent the spread of *Bsal*.

We already have a very sensitive test for *Bsal* which is a major step towards being able to effectively deal with this problem. The diagnostic test protocol is to swab individual salamanders and then use TaqMan qPCR with primers designed to detect *Bsal*. This method is published and uses the same qPCR technique used to detect *Bd*, except with *Bsal* primers instead of *Bd* primers. The technique is very sensitive for *Bd* and *Bsal* detection (down to 0.1 zoospore genome equivalents). TaqMan qPCR is the most sensitive method to detect *Bd* (as opposed to histology and immunology methods) (Hyatt *et al.* 2007)².

Effective treatments are still being developed and tested however a recent paper by Blooi *et al.*³ demonstrated that heat treatment might be an option for some species.

The question has arisen: would clean trade measures need to apply "order-wide" or only to some high-risk imported species? The



Eastern newt (*Notophthalmus viridescens*). Photo: Twan Leenders.

answer is complicated by the limited testing of salamander susceptibility to *Bsal* that has occurred to date.

Imagine this scenario: ten airliners arrive in the U.S. on the same day from the same country that is experiencing an outbreak of a lethal and highly contagious disease such as Ebola. Minimal testing is conducted of passengers on five of the planes and Ebola is found on four of those five planes. Would a Federal health official be comfortable allowing all the passengers on all ten planes to enter without further measures to ensure they did not have Ebola, even from the untested planes?

Of course the answer would be no, therefore we felt the situation should be no different for salamanders. Working with scientists, we identified two key reasons why we felt order-wide emergency regulations are necessary:

- **Evolutionary reasons:** A member of the family Sirenidae, Siren, and a member of the family Hynobiidae, Salamandrealla, were found to be a carrier of *Bsal*.¹ These are ancient families strongly suggesting that the ability to carry *Bsal* is an early-evolved, primitive trait, and therefore that it will occur in all species in all salamander families unless it is shown not to.
- **Statistical reasons:** There are ten families of salamanders of which five have been minimally tested for ability to be infected. Four of the five families had members that can be infected (carriers).¹

Importantly, a positive result with minimal sampling does prove members of the family can be infected, while a negative result with minimal sampling does not provide strong evidence that the family cannot be infected. In laboratory testing, only one family (the Ambystomatidae) out of five was found to be “not infectible”. However, it is important to note that this result is based on minimal sampling: ten individuals of two species in this family. With such a small sample size it is impossible to make a definitive conclusion about whether members of this family can carry infection, and it would be inaccurate to generalize this to all species in this family. Therefore, the best evidence is that species in four of four families can be infected, and we are not sure about the other six families because testing has not been completed or has not begun.

The statistical argument for order-level regulation is that we are sure that species in four tested families can be infected and act as carriers, including a species in an ancient lineage (Sirenidae). Thus, it is highly probably that the other six families for which testing is not complete will include species that can be infected and act as carriers.

At this point, we want to stress that all evidence currently available suggests that *Bsal* has not yet arrived in the U.S. and so our primary focus is to push forward policy actions and work with importers to keep it out. However, we just don’t know how effective existing voluntary measures are in keeping diseases from invading wild populations when amphibians are imported.

At the end of January we sent out free *Bsal* testing kits to owners of salamanders within the U.S. with the help of Alliance Partners. These kits will help provide us with a better understanding of whether *Bsal* is found on pet salamanders already in the U.S. In addition to this, we are also looking to work with the zoo community to undertake similar tests and there are a number of research groups now testing wild salamanders around North America.

Also by working with groups like USARK and companies such as Josh’s Frogs we are exploring ways to connect researchers with large amphibian importers so that we can get a better sense of the current effectiveness of disease mitigation measures.

“The Pet Industry must be proactive about emerging diseases that afflict the animals we love. There is no need for the pet trade to have a negative long term effect on wild populations,” said Zach Brinks from Josh’s Frogs.

As we work with our Partners to address the threats to wild populations we also want to help keep pet salamanders as safe as possible from this threat. Therefore we have suggested that all people with pet salamanders and newts:

- Bleach their wastewater. Before you dispose of any wastewater that might have come in contact with your salamanders be sure you add a little bleach before you dispose of it. Although the science is still out on the concentrations needed, the best evidence points towards a ratio of about one part bleach to ten parts water. Using this ratio should ensure any of the deadly *Bsal* fungus is killed before leaving your home.
- Don’t release captive salamanders into the wild. More important than ever, please don’t release your pets into the wild. If a captive salamander has *Bsal* and ends up in the wild it could be devastating for wild populations. If you have a salamander that you are no longer able to look after try contacting your local herpetological society, humane society or zoo.

We are also asking you to share this information with friends, online through social media and favorite forums, local herpetological societies and pet stores.

With the highest salamander biodiversity in the world, the U.S. has been our priority focal region. However we have also been working with Partners in Europe and Canada to explore options in these regions as well.

Although we are spending significant time trying to keep *Bsal* out of North America we are also very much looking to what needs to happen if it arrives. This preparation process will not only help us prepare for *Bsal* but also for any other amphibian disease that might make its way into novel communities, either in the U.S. or elsewhere around the world.

Monitoring global amphibian diseases will become increasingly important and for that reason the Alliance is working on the development on an international disease-monitoring portal in cooperation with Alliance Partner AmphibiaWeb. Working with groups like *Bd* maps, *Bsal* Maps, Ranavirus Maps and a number of labs and organizations we will be developing a tool that can link databases and provide a range of tools for both sharing and analyzing disease related data. We will keep everyone updated as the project moves forward and we would like to hear from other groups that are interested in becoming involved with this project.

Emerging wildlife diseases are a reality of our time. The only way we will effectively combat them is through a broad collaborative approach. If we can find common ground then real progress on *Bsal* and other amphibian issues will be made for the benefit of all of us and most importantly for the amphibians. To find out more about our work to save salamanders around the world and how you can get involved visit www.amphibians.org.

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ASA and BHS Partnering to Fund the Future of Amphibian Conservation, Research and Education Through Seed Grants

By James P. Lewis & Candace M. Hansen-Hendriks

Have you ever considered supporting a Seed Grant to support amphibian conservation efforts? The British Herpetological Society (BHS) has. To help global amphibian conservation efforts around the world they have teamed up with the ASA to leverage funds and support an increasing number of projects around the world.

We all know that funding for amphibian research is difficult to obtain, especially in developing countries and small territories. Seed Grants are an invaluable approach to supporting conservation efforts. Although the grants are often relatively small they can be vital for getting projects up and running and allowing researchers and conservationists to try new and innovative approaches.



Since its creation in 1947, the BHS has supported the conservation of endangered reptiles and amphibians. Through its publications—*The Herpetological Journal* and the *Herpetological Bulletin*—it strives to disseminate research and husbandry knowledge and its executive committees and grant

programs support conservation initiatives directly. While most BHS support has traditionally been directed to projects in Britain, some of its activities—notably the Captive Breeding Committee, Research Committee and Student Grant Scheme—have supported important research and conservation across the globe. The Society recently decided to increase its level of proactive support for crucial research and conservation of globally threatened herpetofauna and made an initial donation of USD \$1500 towards the ASA's Seed Grant program.

The BHS Council voted on a shortlist of applications supplied by ASA and chose two clear favorites. One is an amphibian survey and capacity-building projects in the British Virgin Islands, a UK Overseas Territory; the other will survey salamanders in remote parts of Algeria. The Council felt that an Overseas Territory project was a natural choice for the BHS, but also that the Algerian project was very worthy of assistance.

"We are very pleased to support the ASA's Seed Grant work and excited to be partnered with two excellent projects in the British Virgin Islands and Algeria. We look forward to championing these projects and working with them to achieve their goals of increasing knowledge on some of the world's most threatened amphibians," said Dr. Chris Gleed-Owen, BHS chair.

It is through collaboration like this that makes amphibian conservation possible. "As you can imagine the Alliance receives significantly more Seed Grant applications than we are able to fund on our own. Developing the partnership with the BHS was an important step forward to begin funding even more innovative projects that will help make a positive difference for amphibians on a truly global scale," said James Lewis, ASA Director of Operations.

But there's still so much more that needs to be and this is where we can all work together.

The Alliance would like to partner with more organizations as we did with BHS to combine resources in order to make more of these types of projects a reality and to create further collaborative Seed Grants.

If your organization believes that Seed Grants are critical to the future of amphibian conservation, research and education and have +\$500 to support a Seed Grant or help establish a fund for future applications, please contact us.

And with that, we are pleased to announce the winners of the second round of ASA Seed Grants:

- Of dogs and frogs: Sniffing out one of South Africa's most threatened amphibians;
- Ecology and populations status of two Endangered species of Urodela in Edough Peninsula (Annaba, Algeria); and
- British Version Islands amphibian conservation scoping project

Learn more about these exciting projects over the next few pages of this edition of *FrogLog*.

We also want to ensure that all applicants to our Seed Grants program are provided with an opportunity to fully realize their project's potential to positively impact the amphibians we're all working so hard to protect. To help with this we have developed an [online map](#) that highlights a growing number of projects around the world that are currently seeking funding. We encourage you to learn more about these projects and help by supporting them financially or sharing them with others who would be interested in championing these projects.

While we do accept and review Seed Grants throughout the year, grants are only announced four times per year in *FrogLog*. With the previous round over, we are now pleased to kick off another call for Seed Grant applications. Here are the deadlines for 2015 applications:

- March 1st for Seed Grants announced in the Spring
- June 1st for Seed Grants announced in the Summer
- September 1st for Seed Grants announced in the Fall
- December 1st for Seed Grants announced in the Winter

Our Seed Grants typically range between USD \$500–\$1,000 and are designed to help kick start projects or allow teams to try new approaches to address conservation, research and education challenges.

Visit the [Seed Grants](#) page on our website to learn more about this initiative and to apply for funding.



Jessie, the project dog, with her trainer Este Matthews searching for Giant bullfrogs. Photo: Jeanne Tarrant

Of Dogs and Frogs: Sniffing out one of South Africa’s Most Threatened Amphibians

By ^{1,2}Jeanne Tarrant, ²Ché Weldon, ²Este Matthews & ¹Christine Coppinger

The Critically Endangered Amathole toad, *Vandijkophrynus amatolicus*, is one of South Africa’s rarest frogs. Having disappeared for 13 years between 1998 and 2011, the species made it onto the IUCN SSC Amphibian Specialist’s Group Lost Frog search campaign list in 2010. Despite a concerted search effort in August of that year (and in years prior), the toad remained AWOL and rumors of its extinction emerged. Then, in 2011 Jeanne Tarrant and Michael Cunningham turned up a single female as well as a few egg-strings and tadpoles in a near-by road puddle, sparking new hope for the existence of the species.

In 2012 a single male was located at a new location, and in 2013 a total of three Amathole toads were found during the breeding season. So we know it’s still out there, but compared to historical reports of hundreds of individuals per sighting, the Amathole toad remains elusive. We’ve since developed an ecological niche model based on known records and ecological requirements and we are using this to guide ongoing surveys.

Independently, the North-West University’s African Amphibian Conservation Research Group (Potchefstroom, South Africa) has

started investigating the use of sniffer dogs to search for fossorial frog species. They have successfully trialed this novel approach on the Giant bullfrog, *Pyxicephalus adspersus*, a species locally threatened in Gauteng Province. The aim of this project is to make use of this “frog-sniffing” method to help in the search for the Amathole toad. This will be the first project of its kind in South Africa focused on a Threatened frog species.



Amathole toad (*Vandijkophrynus amatolicus*). Photo: Jeanne Tarrant.

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Filming the Amathole toad. Photo: Jeanne Tarrant.

The project will take place within the toad's very limited range, restricted to the Hogsback Region of the Eastern Cape in South Africa. The sniffer dog, a frisky border collie called Jessie, will be trained prior to the trip based on scent samples obtained from an Amathole toad (or the closely related Karoo toad). Using our predictive model as a guide, we will focus our searches within areas of probable toad habitat, during the breeding season for this species with an initial field trip planned for February/March 2015). This project will provide the necessary information to more fully understand the status of the Amathole toad and to help prioritize sites for long-term protection, management and monitoring strategies. Conservation plans are currently being developed and will be made available to the relevant land-owners on whose property the species is known to occur.



Amathole toad habitat. Photo: Jeanne Tarrant.



Jessie. Photo supplied by Este Matthew.



Jessie, Ché Weldon and Este Matthew. Photo supplied by Este Matthew.



Jessie and Este Matthew. Photo supplied by Este Matthew.

Ecology and Populations Status of two Endangered Species of Urodela in Edough Peninsula (Annaba, Algeria)

By Jihène Ben Hassine & Daniel Escoriza

Algeria is a key location as a biogeographical contact area between the Maghreb and Europe; however, data about the ecology of Algerian amphibians remain very scarce. In this country several genera of mesophilous amphibians show an endemic and restricted distribution, among them *Salamandra algira* and *Pleurodeles poireti*. *Salamandra algira* is generally considered to be a very rare species in Algeria by The IUCN and is listed as globally Vulnerable. According to The IUCN Red List of Threatened Species, *P. poireti* is listed as Endangered, given that its extent of occurrence of less than 5,000 km² and area of occupancy is estimated at less than 500 km², with all individuals clustered in fewer than five locations in Edough peninsula (Annaba, north-eastern Algeria). This newt is considered the most threatened amphibian in Algeria.

Despite the ecological significance and endemic status of *P. poireti*, there is no conservation plan for the species and no study of its ecology has been conducted. During our first field trip to the area of Edough peninsula, we were able to identify only 9 ponds where the species is still breeding, mainly in the lowlands, some close to urban areas. This newt is facing a continuous decline in the extent and quality of its habitat. Currently, this area is suffering from habitat disturbances linked to the expansion of urban structures. According to our preliminary observations, its breeding habitat may be also threatened by chemicals pollution and invasive species (*Gambusia holbrooki*). More field trips to the area are urgently needed in order to identify the presence of more suitable habitat for the species. Also, these surveys can provide more data about the ecology and the biology of this poorly known Endangered species. Even though the situation for *S. algira* could be less critical, as this species inhabits less impacted mountainous forests, the extant populations are facing deforestation throughout their limited distribution range.

The main goals of our project are (1) to collect occurrence data of both urodela species by means of systematic surveys of the wetlands in and around Mont Edough; (2) characterize species breeding site parameters (3) identify potential threats and causes of the potential decline of these species¹; and (4) to propose guidelines to stop this trend.

Given the threats that these species may be facing, the delimitation of the habitat preferences and the estimation of the population

sizes in Algeria could be very important for their future conservation. The information that we are going to gather will be extremely helpful to implementing adequate conservation strategies.

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Pleurodeles poireti. Photo: Jihène Ben Hassine.



Pleurodeles poireti breeding habitat in Algeria. Photo: Jihène Ben Hassine.



Pleurodeles poireti breeding habitat: temporary pond inside an urban area (Algeria). Photo: Jihène Ben Hassine.



Salamandra algira habitat in Algeria. Photo: Jihène Ben Hassine.

Boophis elenae. Photo: Franco Andreone.



Frogs of Madagascar: A Second ACSAM Workshop to Prevent a Crisis

By Franco Andreone, John E. Cadle, Angelica Crottini, Jeff Dawson, Eileen Larney, Falitiana C. E. Rabemananjara, Sahondra Rabesihanaka, Nirhy Rabibisoa, Eric Rabsomanitrdrasana, Tsanta F. Rakotonanahary & Gonçalo M. Rosa

In November 2014 a critically important workshop to develop the next actions for the conservation of Madagascar's unique amphibians took place in Centre ValBio, Ranomafana, Madagascar (1).

Madagascar is home to a notably high number of unique species including the brightly coloured Mantellas (*Mantella* spp.) and the iconic Tomato frogs (*Dyscophus antongili* and *D. guineti*), which are integral parts of Madagascar's biodiversity and a draw for tourists and visitors from around the world.

Madagascar's 300 described species plus many more yet to be named are facing a myriad of growing threats to their survival including emerging disease, invasive species, deforestation and habitat alteration.

Between the 18th and 22nd November, 2014, a meeting called "A Conservation Strategy for the Amphibians of Madagascar 2" (ACSAM2), saw the mobilization of more than 70 local and international conservationists and experts for the second time in eight years (the first one was in 2006 in Antananarivo) to discuss and develop new strategies to tackle these diverse threats and prevent the extinction of these amphibians.

Understanding the wealth of Madagascar's native amphibian fauna is critical to their future conservation. By identifying key research areas, conservationists will be able to take action for poorly

known species and undertake long-term monitoring at key sites to understand how emerging diseases or climate change may impact the countries amphibians.

Talks and discussions led to a number of key outputs addressing global infectious disease threats such as that caused by the chytrid fungus which has caused mass extinctions of amphibians in other



Franco Andreone at ACSAM2. Photo: Jeff Dawson.



Mantella baroni. Photo: Angelica Crotini.



Spinomantis sp. aff. fimbriatus. Photo: Franco Andreone.

countries. These were enhanced by the presentations and input of researchers who have had direct experience of working in these countries. A commitment to maintain the national monitoring program (2) and the creation of a rapid response strategy will enable Madagascar to effectively detect and respond promptly to chytrid should it emerge there.

Another important point of discussion was the approach to captive breeding. Participants agreed to a number of actions to strengthen the captive breeding capacity within Madagascar. It was also recognized that it is crucial to develop the local capacity in Madagascar to implement these actions, from field research to captive breeding to coordination, which was a theme that ran through all discussions.

The key output from ACSAM2 will be the release of the updated National Action Plan for Madagascar's amphibians (Sahonagasy Vaovào) which will incorporate all the actions identified during the meeting. This will form the framework for amphibian conservation efforts in Madagascar over the next five years.

Many organizations and individuals supported ACSAM2 both financially and academically, from natural history museums to zoological parks, universities, governmental institutions and individual people who are concerned about the future of amphibians worldwide. They will all play a crucial role in helping ensure the follow through of actions developed during this meeting and help to make this global effort to support the amphibians of Madagascar a model for future conservation efforts.

The meeting itself received widespread press coverage within Madagascar, in the national press, on national radio and television. The national TV news piece can be seen here: <https://www.youtube.com/watch?v=KCiKKS-dBh0> (starting approximately 5:00 minutes in).

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Coffee break at ACSAM2. Photo: Lance Woolaver.



Mdm Sahondra Rabesihanaka from the Ministry of Environment at ACSAM2. Photo: Tsanta Rakotonanahary.



Ranomafana National Park, Madagascar. Photo: Jeff Dawson.

Taxonomic Changes: An Attempt to Overcome the Linnean Shortfalls for Brazilian Amphibians

By ^{1,2}Felipe Siqueira Campos & ³Mirco Solé

Ten years after the first official Brazilian list of amphibian species was published, the Brazilian Society of Herpetology has acknowledged a 36 percent increase in listed amphibian species in the country. The updated numbers reflect a progressive increase in the number of researchers active in Brazilian herpetology but it's going to take continued commitment and additional resources to gain a full understanding of the amphibian diversity in this largely unexplored region.

The first official Brazilian list of amphibian species was published during the First Brazilian Congress of Herpetology, in July 2004 (1). In this meeting, a group of herpetologists announced 751 species of amphibians for the country.

After some updates with descriptions of new species, new genetic allocations and other taxonomic changes, the Brazilian Society of Herpetology announced the occurrence of 1,026 amphibian species with confirmed records for the country (1). Studies conducted on Brazilian amphibians between July 2004 and July 2014 added 275 new species, including 265 anurans, six caecilians and four salamanders.

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This high number of new amphibian species described from Brazil has been influenced by the growth of local investment in research infrastructure in recent years. One measure of a nation's knowledge base is its output of Ph.D. students, which is directly associated with the growth of infrastructure investment. In this example, the science budget allocated in Brazil rose from US\$575 million in 2002 to more than US\$3.3 billion in 2010 (2). The constant increase in the number of researchers holding a Ph.D. in Brazil is still not enough to solve the demand for amphibian diversity studies needed in the country. Brazil is one of the largest ecosystems in the world and many more species undoubtedly remain unknown to science. It is incumbent on policymakers, conservationists and scientists to continue to direct resources toward the research of amphibian diversity in Brazil, ensuring the protection of that biodiversity before it is lost.

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Salamanders: World Icons of Aquatic Biodiversity in Forests

By Deanna H. Olson

Salamanders are well-known denizens of northern hemisphere forests, with bi-phasic life histories bridging aquatic and terrestrial habitats. Today, we are still discovering the extent of forest salamander biodiversity, and charting their losses simultaneously. With close ties to forest, water, and climate, salamanders are emerging as contemporary symbols of world forestry sustainability and its complexity over the next millennia as we strive for wise stewardship inclusive of both water and land resources. Salamanders-in-forests was a session theme at the 24th IUFRO World Congress in October 2014 (IUFRO = International Union of Forest Research Organizations) and the theme of the November 2014 issue of the [Salamander News](#), both as part of the “Year of the Salamander” activities sponsored by Partners in Amphibian and Reptile Conservation (PARC). To further commemorate the year and forest-dwelling salamanders in particular, a special issue of the journal *Forests* showcases forest salamander research. Articles span management implications of forestry practices for salamanders, riparian buffer efficacy, forest salamander habitat associations and population ecology and climate niche models and climate refugia of conservation priority species. The following publications are currently available [online](#):

- Clipp H. L., Anderson J. T. 2014. Environmental and Anthropogenic Factors Influencing Salamanders in Riparian Forests: A Review. *Forests* 5(11):2679-2702.

- Homyack J. A., Kroll A.J. 2014. Slow Lives in the Fast Landscape: Conservation and Management of Plethodontid Salamanders in Production Forests of the United States. *Forests* 5(11):2750-2772.
- Olson D. H., Burton J. I. 2014. Near-Term Effects of Repeated-Thinning with Riparian Buffers on Headwater Stream Vertebrates and Habitats in Oregon, USA. *Forests* 5(11): 2703-2729
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Surveillance Methods for Amphibian Ranaviruses—Call for Participation

By Yvonne Black

Ranaviruses pose a significant threat to the global diversity of amphibians, reptiles and fish having been implicated as a contributing factor in the global decline of amphibian populations and resulting in moderate fish loss or mass mortality events.

Two surveys have been set up to collect data on the global surveillance of amphibian ranaviruses, a project being undertaken as part of the MVetSci in Conservation Medicine at University of Edinburgh. The projects are supervised by Dr. Stephen Price (UCL) and Dr. Anna Meredith (University of Edinburgh). Dr. Price is the lead author of a recent paper that indicates that two viruses of the genus Ranavirus have had dramatic effects on whole communities of amphibians in Northern Spain (1). If we are to limit amphibian declines, it is important to understand the origins and movements of this group of viruses in the context of its effects on animal populations. Currently, a Global Ranavirus Reporting System (GRRS) is being established by the Global Ranavirus Consortium (co-ordinated by Dr. Amanda Duffus; <http://fwf.ag.utk.edu/mgray/ranavirus/Ranavirus.htm>), US Forest Service and the EcoHealth Alliance.

This reporting system is intended to serve as an information portal for scientists and natural resource managers. The purpose of this project is to review the current methods of sampling and testing for amphibian ranaviruses. One survey aims to collect information about laboratory screening methods. The other seeks to define the contribution made by citizen science projects in the area.

Responses to the surveys will help to clarify how much testing is being carried out and its methodology. By participating in the survey, scientists will hopefully get the chance to share their views on appropriate sampling and testing techniques with colleagues and optimize surveillance effort. If you are involved in diagnostic testing of amphibian ranaviruses and/or an amphibian citizen science project and you haven't already filled in a questionnaire, please contact Yvonne Black at: s1267294@sms.ed.ac.uk for further details and a link to the short online survey(s).

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A Selection of Smithsonian Herpetological Information Services Publications on Amphibians

By George R. Zug

The Division of Amphibians and Reptiles of the National Museum of Natural History has produced the Smithsonian Herpetological Information Services (SHIS) series since 1965. This series distributes translations, bibliographies, indices and similar items judged useful to individuals interested in the biology of amphibians and reptiles and unlikely to be published in the normal technical journals. The series continues under that tradition.

Because I suspect that many readers of *FrogLog* are unfamiliar with this resource of herpetological information, I have listed a few of the more recent SHIS issues that focus on amphibians. All SHIS numbers are distributed as PDF documents and are available from two Smithsonian outlets:

Biodiversity Heritage Library [www.biodiversitylibrary.org/bibliography/15728]. All numbers from 1 to 131 [1968–2001] available in BHL.

DIVISION'S WEBSITE

vertebrates.si.edu/herps/herps_NMNH_herppubs/herps_herps.html

Numbers 84 to 145 available as pdfs in the herpetological publications section of the website.

SHIS

- 109 Amphibians of Northwest Africa. Alfredo Salvador. 1996
- 111 Bibliography and indices to the herpetological papers published in freshwater biology and limnology journals 1950–1995. Dreux J. Watermolen. 1996.

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SPECIAL PUBLICATIONS - SHIS

- Bibliography of the frogs of the *Leptodactylus* clade – *Adenomera*, *Hydrolaetare*, *Leptodactylus*, *Lithodytes* (Amphibia, Anura, Leptodactylidae). Volume 1. References. Miriam H. Heyer, W. Ronald Heyer & Rafael O. de Sa. 2009.
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Photo: Ben Taylor.

Getting Physical for Amphibians

By Rob Gandola

Northwest of Madagascar, the villages of Mariarano, Tanandava and Antafiameva are surrounded by remnant patches of western dry forest, with adjacent wetlands that are fed by the Mariarano and Antsena Rivers. Some of the lower-lying areas close to these rivers form the only sources of water during the long dry season, as even the Mariarano River runs dry in some years. It is at these water sources, mainly comprised of rice paddies and irrigation canals with a number of small freshwater ponds and lakes, where the nine currently known species of amphibians congregate. And it is here where we go “frogging” as part of the Monitoring Mariarano’s Amphibians (MMA) program.

THE METHODOLOGY—AND THE FUN

MMA has been part of the herpetological monitoring methodology at our field site in the Commune of Mariarano, Mahamavo, since 2012. We employ time-defined surveys to pit students (both secondary and university level) against staff and local guides to catch as many amphibians as they can. The team with the highest tally is declared the winner. In the event of a tie, the team with the higher diversity of species wins. During the friendly competition, we record detailed morphological data and health assessments for each individual frog caught. This, in addition to the raw species



Heterixalus tricolor. Photo: Rob Gandola.



Heterixalus tricolor. Photo: Rob Gandola.

counts, allows us to monitor amphibian community composition change over time, as well as to give an indication of the overall health of the amphibian community. So, not only is this good fun, it is a great way for the students to learn about the local amphibian species and get hands-on experience.

The concentration of water and therefore frogs, in places that are relatively easy for human surveyors to access, means that other fauna can access them too. Because of this, MMA also observes the natural behavior of frog predators. For example, we regularly see the Malagasy cat-eyed snake (*Madagascarophis colubrinus*) preying on a number of species.



Photo: Ben Taylor.

THE RESULTS ARE (NEARLY) IN

To date, more than 300 secondary- and university-level students have assisted with the collection of data from almost 2,000 frog captures, comprising nine species (distributed across seven genera). The majority were the widespread, non-endemic Mascarene grass frog (*Ptychadena mascareniensis*). Preliminary analysis suggests a dynamic system with patterns of change in amphibian community diversity that may be driven by climatic events and alterations to irrigation regimes. “Frogging” will continue to be an important part of our methodology into the future and hopefully we will be able to encourage a number of students to begin looking at the community ecology of the amphibians in these dynamic water bodies.

Acknowledgements

The Mahamavo Herp team would like to thank all the schools, volunteers, students, and staff of Operation Wallacea, Development Biodiversity and Conservation Action for Madagascar (DBCAM); Prof. Felix Rakotondraparany (DBA, University of Antananarivo); GIZ Mahajanga (Oely and Eric) and DREF Boeny. We would particularly like to thank the members of VOI Tanteraka; VOI Tanandava; our guides and the people of Mariarano, Tanadava and Antafiameva for welcoming and assisting us with this project every year.

WWF Education for Nature Program Supports Conservationists Worldwide

By Alex Batka



Ngo Van Tri conducting research in the field in Vietnam. Photo: Ngo Van Tri.

Ngo Van Tri is known as the Gecko King in Vietnam. He earned the title by discovering more than 10 different species of gecko and countless other types of reptiles while working in some of the most remote forests in Vietnam.

His fascination with geckos began many years ago while he was working at a non-governmental organization. One day Tri was conducting research in the field and a gecko ran across his path. Intrigued by the creature, he left his job and dedicated his life to researching and documenting geckos in his native country. With no external financial support, Tri used his own funds to conduct his work for several years. He exemplifies the hard work and perseverance of scientists conducting important research on biodiversity despite having very limited external resources to do so.

In 2004, Tri received funding from the WWF Russell E. Train Education for Nature Program (EFN) to attend the 20th Annual Meeting of the Society of Conservation Biology. Soon after, EFN awarded him a second grant to support his research and provide funding to help publish work on his most recent discoveries. Since then, Tri has discovered more new species and his research has appeared in numerous scientific journals.

Finding sufficient funds for research and education is a constant struggle for conservationists like Tri. In 1994, WWF established EFN to address the problem of lack of funding. The program was named

for WWF's founder and chairman emeritus, Russell E. Train, who early on recognized the enormous need for conservation capacity on a global scale. Train felt that without education and training, the full potential of promising conservationists would not be achieved, and the planet would suffer as a result.

For the past 20 years, EFN has worked tirelessly to achieve Train's vision of building local conservation capacity in Asia, Africa and Latin America. To date, the program has provided funding for over 2,000 conservationists to receive crucial training and to obtain advanced degrees in conservation-related fields. After completing their degrees or trainings, a full 99 percent continue to work in conservation and more than 90 percent of grantees are working to advance conservation initiatives in their home country. This year, EFN has a number of funding opportunities available for conservationists around the globe.

FELLOWSHIP OVERVIEW

Today's conservation challenges are more complex than ever before and require advanced skills and knowledge. Many committed conservationists cannot reach their full potential due to limited opportunities for higher education in their home countries. To reduce these barriers, EFN provides fellowships to rising leaders to pursue graduate degree (Master's and Ph.D.) studies in conservation-related fields.

Applicants can apply to attend any university around the world and then must return to their home countries to work in conservation for at least two years after completing their degree. New fellowship topics are selected each year depending on WWF priorities. The EFN 2015 fellowship opportunities are listed below and detailed eligibility requirements can be found at: www.worldwildlife.org/efn. The deadline to apply for all fellowships is February 28, 2015.

OCEAN MANAGEMENT FELLOWSHIP

WWF's mission in the Pacific and in the Indian Oceans is to ensure strong stakeholder participation to conserve rich oceans and coastal diversity while also addressing threats from climate change, overfishing and unsustainable marine resource management. To build local capacity in the marine community, EFN is offering this fellowship to empower individuals to advance marine conservation in their home countries.

VALUING STANDING FORESTS FELLOWSHIP

The full value of forests is not currently recognized by global or local economies, nor is it effectively safeguarded in public policies and governance systems. The result is the loss of high-value forests replaced by other land-uses that generate higher short-term financial returns often with disregard for the long-term, high-cost impacts. This year, EFN is offering a fellowship opportunity for research that enhances the understanding of the true value of forests and investigates mechanisms and strategies to communicate, facilitate and implement standing forest conservation.

CURRENT & ASPIRING FACULTY FELLOWSHIP

Strong university faculty is fundamental for effective higher



The Lipstick gecko (*Dixonius aaronbaueri*) is one of the 145 new species featured in the WWF report *New Blood: Greater Mekong new species discoveries 2009*. Photo: Ngo Van Tri/WWF-Greater Mekong.

education. Faculty members inspire students, design curricula, promote learning, lead research and publish scientific results. EFN is proud to offer this opportunity to support current and aspiring university faculty that are affiliated with or currently employed at African, Asian and Latin American universities. After completing a degree, the individual must enhance, expand or establish a graduate-level conservation program at a university in their home country. By doing so, WWF aims to improve the capacity of these local and regional universities and encourage future conservationists to study in their home countries.

ADDITIONAL FUNDING OPPORTUNITIES

In addition to fellowships, EFN offers Professional Development Grants (PDGs) and Conservation Workshop Grants. The PDG program provides support for mid-career conservationists to pursue short-term, non-degree training to upgrade their knowledge and skills through short courses, workshops, symposiums, conferences (if presenting) and professional exchanges. A limited number of PDGs are awarded throughout the year on a rolling basis.

Conservation Workshop Grants support non-governmental organizations, community groups, government agencies and educational institutions. These grants support training courses and workshops in WWF priority ecoregions on topics of importance for local and regional conservation efforts. Applications are accepted on a rolling basis and reviewed four times per year.

To apply for any of the opportunities listed here or to learn more about EFN and other funding opportunities, please visit: www.worldwildlife.org/efn. For questions, please email: efn@wwf.us.org.



A new gecko species discovered by Ngo Van Tri in Dong Nai Province in Southeastern Vietnam. Photo: Ngo Van Tri.



Russell E. Train
Education for
Nature Program



Boophis luteus handimal, created by Guido Daniele and Ginevra Daniele. Hand models: Giulia Fossati and Serena Crystal Andreone. Photos: Michael James Daniele and Franco Andreone.

Non-Traditional Ways of Raising Awareness for Frog Conservation: Guido Daniele’s Handimal of *Boophis luteus* From Madagascar

By Franco Andreone

Considerable momentum behind addressing the conservation status of the nearly 500 Malagasy frog species, of which only 300 have been described so far, has come out of the ACSAM2 workshop held in Ranomafana’s Centre ValBio, Madagascar back in November, 2015. So far approximately 300 species have been described and there are at least another 200 to be assessed as possible candidate species.

Making an emotional connection with common people and stakeholders often creates a much stronger awareness of the conservation needs of amphibians than pure science, lectures and meetings in both Madagascar and elsewhere. As such, one of the most creative outcomes from the Ranomafana meeting has been a recent collaborative project with one of the most renowned body painters in the world.

Guido Daniele is well known for the creation of his “handimals,” which are hands painted in a way that mimic animals, many of which are threatened. Together with the assistance of Guido’s sons Ginevra and Michael James, a rich and populated park of animals is born out of his delicate brushes. This process is typically carried out in collaboration with conservationists and zoologists.

Guido’s most recent exhibit, “Handimals” at the New York Rockefeller Center from 15–25 January, was dedicated to the great prima-

tologist and conservationist Jane Goodall.

Now, through ACSAM Guido has created a handimal that is dedicated to one of the most outstanding frogs of Madagascar—*Boophis luteus*. While this species is far from being classified as under threat on The IUCN Red List of Threatened Species, it is a true symbol of Madagascar’s frog wildlife. It is a typical “skeleton” frog, with a delicate body profile and arboreal habitat and is closely associated with the fragile and vanishing rainforests of Madagascar’s eastern belt. Even more wonderful is the fact that its three major color shades, green of the body and red-white of the protruding eyes, are also the colors of Madagascar’s national flag. This species was also the logo of both editions of ACSAM (in 2006 in Antananarivo and in 2014 in Ranomafana). These same colors have a magical effect on the hands of the two models who gave life to this new handimal.

Guido has created this artwork for free, which he often does when there are clear conservation benefits and also because of his passion for Madagascar, a country he has visited several times.



Photo: Franco Andreone.



Trouble in the Aquatic World: How Wildlife Professionals are Battling Amphibian Declines

By ¹Deanna H. Olson & ²Tara Chestnut

A parasitic fungus, similar to the one that caused the extinction of numerous tropical frog and toad species, is killing salamanders in Europe. Scientists first identified the fungus, *Batrachochytrium salamandrivorans*, in 2013 as the culprit behind the death of Fire salamanders (*Salamandra salamandra*) in the Netherlands (11) and are now exploring its potential impact to other species. Although the fungus, which kills the amphibians by infecting their skin, has not yet spread to the United States, researchers believe it's only a matter of time before it does and, when that happens, the impact on salamander populations could be devastating (12).

Reports of worldwide declines of amphibians began a quarter of a century ago (2). Globally, some amphibian population declines occurred in the late 1950s and early 1960s, and declining trends continued in North America (8). In the earlier years, population declines



Fig. 1: Author Tara Chestnut samples wetlands for the amphibian chytrid fungus in Klamath Basin National Wildlife Refuge, Oregon. Although the fungus has been around for decades, researchers are investigating how factors such as land use change, climate change and pollutants could be making frogs and salamanders more vulnerable to the disease. Photo: Charlie Crisafulli.

were attributed primarily to overharvest due to unregulated supply of species such as the Northern leopard frog (*Lithobates pipiens*) for educational use (4). In later years, however, causes of declines were less evident. In 1989, herpetologists at the First World Congress of Herpetology traded alarming stories of losses across continents and in seemingly protected landscapes, making it clear that amphibian population declines were a “global phenomenon.” In response to these reports, in 1991,

the International Union for Conservation of Nature (IUCN) established the Declining Amphibian Populations Task Force to better understand the scale and scope of global amphibian declines. Unfortunately, the absence of long-term monitoring data and targeted studies made it difficult for the task force to compile information. Today, according to AmphibiaWeb.org, there are 7,342 amphibian species in the world—double the number since the first alerts of declines—making the situation appear deceptively less dire. In fact, our understanding of genetic diversity significantly raises the stakes, and we are at risk of losing far more species than we believed only a few years ago. According to the IUCN, amphibians now lead the list of vertebrate taxa affected by the larger “biodiversity crisis” and sixth major mass extinction event on Earth (9; 15).

DECLINE AND MITIGATION

Across the world, numerous factors are responsible for the ongoing decline in amphibian populations such as habitat loss, invasive

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species, chemical contaminants, diseases, climate change and synergisms among several of these factors.

The Oregon spotted frog (*Rana pretiosa*)—recently listed as threatened under the Endangered Species Act (Federal Register 2014)—is an example of a species facing combined threats. Once common in large, relatively warm wetlands with permanent water across the Pacific Northwest, Oregon spotted frog populations are believed lost from at least 78 percent of their former range. Factors—several of which are driven by human-caused changes to the landscape—such as loss of wetlands, hydrological changes, disease and depredation by non-native predators, including introduced trout and bullfrogs, have contributed to declining populations.

Yet, for each of these threat factors alone or in concert, science, management, and the public are playing key roles in the form of research, management and monitoring. The following is a sampling of ongoing efforts to address and mitigate threats facing amphibians in the United States.

RESEARCH

One stressor that has been gaining attention—with significant research contributions over recent years—is the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis. This pathogen, described 15 years ago (10), can be lethal under some circumstances, which has been the case for the federally Endangered Mountain yellow-legged frog (*Rana muscosa*). The species appears to be more susceptible to chytridiomycosis than other frogs in the region (California), and exposure to pesticides may weaken its immune mechanism.

In response, researchers are working to understand geographic and biologic occurrence of the fungus along with pathogenicity patterns (Fig. 1). The U.S. Forest Service—with the help of world scientists, professional ecologists, resource managers and volunteer citizen scientists—has developed occurrence maps reflecting the 1,377 species that have been inventoried for the fungus (Fig. 2). It's widespread—found about half the time overall—yet at most sites with the fungus, amphibians do not show symptoms of chytridiomycosis. Now, emerging science suggests that some strains of the fungus may be located exclusively in North America (13). Consequently, it's likely that some North American amphibians co-evolved with some of these strains, which would explain why we see amphibian populations that test positive for chytrid infection, but without disease-related die-offs. Given the ubiquity and antiquity of the global pandemic lineages of the amphibian chytrid, the question of whether some subtle environmental or strain change has occurred to trigger symptoms becomes extremely relevant.

New research aimed at studying the ecology of the fungus has also helped us to understand patterns of occurrence. This aquatic fungus appears to be sensitive to temperature conditions, and is not as prevalent in areas that get extremely hot and cold. In at least one strain of the fungus, researchers found that differences in frogs' generation time and fecundity were observed in response to different thermal regimes (14). We pursued the fungus at high latitudes to see if its range might be limited there due to extreme cold temperatures. In Alaska, we found the fungus throughout the range of the Wood frog (*Lithobates sylvaticus*), the only amphibian species

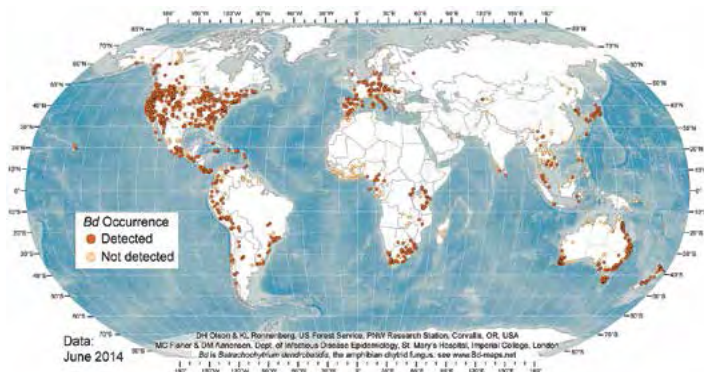


Fig. 2: This map shows the global distribution of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. The fungus has been detected in 71 of 105 sampled countries. [reprinted from Olson and Ronnenberg, *FrogLog* 22(3), Number 111 [July 2014]: 17–20. Available for downloading at: <http://www.fs.fed.us/pnw/lwm/aem/people/olson.html>].

that inhabits the Alaska interior and a unique frog species in that they “freeze” in winter. Our research showed that frogs can largely clear the pathogen during this process, although upon warming, lingering fungal zoospores appeared to be able to quickly re-establish infection (Fig. 3).

In another recent advance in research, the occurrence of the amphibian chytrid fungus can now be detected as a free-living form by filtering water, in addition to swabbing animals to detect it on their skin. As part of our research, we described water sampling across the United States where we reported this fungus persists year round, with variable densities in the environment (3; Fig. 4). Water is moved between watersheds for a number of management and conservation needs such as fire-fighting, fish hatchery production and reintroduction programs. Well-meaning nature enthusiasts and teachers have relocated animals and released pets and classroom animals that may be infected with or carry the amphibian chytrid fungus. In addition, amphibians are part of an enormous world trade for food and pets, with hundreds of millions of animals, and the water they live in, moving across borders every year. Hence, infected waters may be a concern for amphibian health as well as infected individual amphibians. In fact, the stakes for salamander conservation have been raised this year, given the deadly consequences of the newly described chytrid fungus, *B. salamandrorans*, for many of the world’s salamanders. Researchers note that movement of animals or the water in which they’re kept could prove lethal to native U.S. species such as forest-dwelling newts. As a result, they are exploring the possibility of modifying existing methods used to sample wetlands for amphibian chytrid fungus in an effort to provide early detection for this and other aquatic diseases.

MONITORING AND MODELING TOOLS

Using occupancy modeling—statistical methods that account for imperfect detection—in 2013, researchers with the U.S. Geological Survey (USGS) Amphibian Research and Monitoring Initiative provided the first estimate of the rate of amphibian declines in the United States (1). They studied amphibian occupancy of sites on federal lands and reported that those populations from across the nation were declining at a rate of 3.7 percent per year, noting that salamanders were declining at a faster rate than frogs. Further, their research showed that amphibian occupancy of sites declined in all parts of the United States, with the south experiencing greatest declines.

Today, researchers can better quantify species decline metrics with monitoring conducted by a diversity of professional ecologists, citizen scientists and land managers. Standardized survey protocols have been established based on habitats and life history attributes of species in various regions and compiled into a manual developed by Partners in Amphibian and Reptile Conservation (6). In 2000, Congress established the USGS Amphibian Research and Monitoring Initiative to investigate the status and trends of amphibians, identify causes of amphibian declines and provide critical information to natural resource managers to support effective management actions that address declines. However, with our current knowledge of a variety of threat factors and their potential interactions, wildlife professionals might consider increasing the scope and scale of routine amphibian monitoring.

ON-THE-GROUND MANAGEMENT

With ongoing management measures, wildlife professionals are already seeing signs of success. For example, research shows that habitat restoration can result in increases in Spotted frog populations, and scientists noted that between 1991 and 2011, Columbia Spotted frog populations (*Rana luteiventris*) in the northwestern United States grew rapidly in response to wetland restoration in areas with historical population declines (7). Still, long-term benefits of management efforts for many species are currently unknown; however, experts agree that no single action is enough to recover most species. Further, some threats are challenging to control such as the effects of non-native fish and bullfrogs. For example, the Oregon spotted frog requires permanent year-round water and, as a result, management actions such as altering hydrologic regimes that would reduce or eradicate harmful predators could also hurt the frogs and other aquatic organisms.

In terms of threats from disease, federal and state wildlife agencies, in collaboration with the Woodland Park Zoo in Washington State, have implemented reintroduction programs that screen Oregon spotted frogs for the amphibian chytrid fungus before release to new locations. In the wild, Oregon spotted frogs have tested positive for several diseases of concern including the amphibian chytrid fungus, the fungus-like pathogen *Saprolegnia* and trematode parasite *Ribeiroia ondatrae*, which causes limb malformations. However, it isn’t clear if these diseases are a threat to populations because the strains that occur throughout the range along with the effect of co-infections are not known. Strain differences warrant identification and study for differential pathogenicity. Screening animals



Fig. 3: A wood frog sits frozen in a test tube as part of a study on the effect of cold temperatures on the amphibian chytrid fungus. Wood frogs are found at higher latitudes and have a unique ability to freeze in winter months—a characteristic that offers some protection from the fungus. However, warming temperatures caused by climate change could make the species more vulnerable to disease. Photos: Tara Chestnut.

prior to reintroduction is a sound precaution that will prevent the introduction of virulent strains that may be present in the area where animals were collected but may be novel to areas where they are released.

COLLABORATIVE EFFORTS

Ultimately, partnerships and joint efforts are critical in managing amphibian populations. For a host of considerations such as ethical, aesthetic, biomedical, ecological or “One-Health”—a worldwide strategy for expanding interdisciplinary collaborations and communications for the health of humans and the environment—we are no longer free to consider species as we once have. Our role has shifted from exploiting species for various uses toward becoming their stewards. The bridging of science, management and the public to address amphibian declines is creating a new platform for conservation biology, where partnerships and open communication pathways expedite the pace of science and its application to field settings. It’s a bottom-up approach where local human communities are making great strides to affect their local wildlife communities, and a top-down approach where programs that span regions and continents can have strong ripple effects. This is especially evident in the United States, where a variety of state, federal, tribal and private lands are being managed for ecosystem services



Fig. 4: A researcher swabs a Rough-skinned newt for traces of the chytrid fungus *Batrachochytrium dendrobatidis*. Authors Olson and Chestnut recently completed a study in the Willamette Valley, Oregon, investigating the relationship between *B. dendrobatidis* in the environment and the occurrence of disease in amphibian populations. Photo by Tara Chestnut.

inclusive of amphibian diversity. In some cases, these cooperative efforts and conservation strategies have made formal protections such as listing under the Endangered Species Act (ESA) less necessary.

A prime example of this process has been the development of multi-agency conservation strategies among federal agencies that have helped preclude formal decisions to list species under the ESA. The tri-agency conservation strategy for the forest-dependent Siskiyou Mountains salamander (*Plethodon stormi*) is one example of this approach. In fact, targeted surveys for animals and habitats resulted in discovery of a new species—the Scott Bar salamander (*Plethodon asupak*)—and the designation of high-priority sites for long-term management of the Siskiyou Mountains salamander

across a swath of Forest Service and Bureau of Land Management Lands, in cooperation with the U.S. Fish and Wildlife Service.

WHAT LIES AHEAD

We are entering a new age of information transfer about wildlife threats and population status, which enables an improved response of both research and management to a variety of stressors. E-communications and real-time web portals for information are being developed for a variety of purposes, and this will change how we aggregate and assess data, conduct risk assessments and respond to critical issues. The fate of amphibians and other imperiled species is not random. There is a human link to most known amphibian threats and, as a result, we have a role in both their imperilment and stewardship. We expect to continue to test new tools for amphibian conservation that may have application for broader wildlife consideration. Amphibians also serve as ideal tools to teach future generations how wildlife are integrated with their environment and how our actions affect their futures and ours. In this way, amphibians are helping to bind science, management and the public into a new alliance for conservation.

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The Disappearing Frogs Project: Call for Artists

By Terry Thirion



Some 300 species of frogs may become extinct within our lifetime if we do not change our behaviors. The chemicals we use daily and seasonally in our gardens and lawns dramatically affect the way frogs are able to reproduce. Changes in their habitats are also drastically affecting their numbers.

The mission of The Disappearing Frogs Project is to create an interactive art installation that brings awareness to the decline/disappearance for frogs and other amphibians and inspire people to take personal action to protect our environment.

The first multimedia frog project was assembled by artist Terry Thirion of Charlotte, North Carolina in February 2014 at the Charlotte Art League.

In partnership with Thirion, the Arts Council of York County extends an Artists Call to Action. The project will be on exhibit at the Community Performance Center during Rock Hill's Come-See-Me Festival, April 16-25, 2015. Curated by Terry Thirion, this exhibition will include frog images submitted by artists and students, video and digital presentations on the plight of amphibians. Patrons will have a chance to create their own frog art, attend several lectures on frogs for both children and families, a frog puppet show and a public reception. Specific information can be found at: YorkCountyArts.org

All proceeds from this exhibition will benefit the Amphibian Survival Alliance.

GET INVOLVED

- Create your own interpretation of a frog on one or more surfaces of your choice (canvas, fabric, wood, paper) in one of the following sizes: 4" x 4" | 4" x 8" | 8" x 8"

- Sign the artwork and write your name, address, telephone number, and email address on the back.
- Download and print the Artist Entry Form found [here](#). Include completed Artist Entry Form with your Disappearing Frogs Project artwork. (PDF Format)

KEY DATES

- Artwork Submission Period February 9 – March 30, 2015. Mail or deliver in person. Deliveries made in person are by appointment only. Contact [Erica Welzenbach](#) to schedule a drop off time.
- The Disappearing Frogs Exhibition: April 16–25, 2015.
- Green Heaven Royal Spa & Resort Puppet Show Saturday, April 18, 2015
- Artist Reception Saturday, April 18, 2015.
- Lecture With [Robin Moore](#) Saturday, April 18, 2015.
- Green Heaven Royal Spa & Resort Puppet Show Wednesday, April 22, 2015.
- A Perilous Time For Frogs In The Piedmont, Lecture By Dr. Bill Hilton, Jr. Thursday, April 23, 2015.
- Artwork Pickup Friday, May 1, 2015



arts
council
of york county



Call for Applications: 2015 Conservation Team Awards

Deadline: 23rd February 2015

Applications for the 2015 Conservation Leadership Programme (CLP) Conservation Team Awards are now open! CLP aims to advance biodiversity conservation globally by building the leadership capabilities of early-career conservation professionals working in high-priority places with limited capacity to address conservation issues. This partnership initiative, including BirdLife International, Fauna & Flora International and the Wildlife Conservation Society, has been helping young conservationists across the world to achieve their goals for 30 years. The programme works toward its aims by offering awards, training and mentoring support. CLP offers three levels of Conservation Awards:

- **Future Conservationist Awards:** Approximately 18 awards of up to \$12,500 each
- **Conservation Follow-up Awards:** Approximately 2 awards of up to \$20,000 each (available only to previous CLP Future Conservationist Award winners)
- **Conservation Leadership Awards:** 1 award of \$40,000 (available only to previous CLP Follow-up Award winners)

In 2015 CLP will only be accepting proposals for projects to be implemented in the following 22 countries: **Algeria, Angola, Azerbaijan, Brazil, China, Egypt, Georgia, India, Indonesia, Iraq, Kuwait, Libya, Malaysia, Mexico, Mozambique, Oman, South Africa, Thailand, Trinidad and Tobago, Turkey, UAE and Vietnam.**

The application deadline for full proposals is **23rd February 2015 for ALL applications.** Those applying for **Conservation Follow-up** and **Conservation Leadership Awards** must submit a Logical Framework and the Final Report of their previous CLP project as part of their application materials. Awards will be announced in April 2015. Please visit the CLP website (www.conservationleadershipprogramme.org) for detailed eligibility criteria, guidelines and application form.

Successful applicants will: 1.) Develop the knowledge, skills and abilities of team members; 2.) Implement a focused, high-priority conservation project combining research and action; and 3.) Contribute to the long-term success of local conservation efforts. Applicants can request feedback on their proposals from CLP Alumni Ambassadors in their region prior to submission. More information on Alumni Ambassadors can be found on the CLP website. Applicants may also contact a member of the CLP team well before and up to two weeks before the application deadline for advice on project eligibility, methods and project activities. CLP may be able to put teams in touch with local partner offices or other experts who can provide additional advice.

A representative from each award-winning team will be invited to attend an international training event in June/July 2015 organised by CLP to share ideas and develop skills, knowledge and contacts. Additionally, winning teams are able to network with experts from within each of the partner organisations and past winners. CLP management team members are available to advise during project implementation, including guidance required during the planning and team training stages.

Have additional questions or seeking advice? Email clp@birdlife.org for more information. Please forward this announcement to other potentially interested individuals, organisations or academic institutions.





Panamanian golden frog in a probiotic bath. Photo: Brian Gratwicke, Smithsonian Conservation Biology Institute.

New Study Finds That Frogs in Captivity Retain Much of Their Pathogen-Fighting Microbial Community

By Lindsay Renick Mayer

More than a decade after the Maryland Zoo in Baltimore was approved to establish a captive population of adult Panamanian golden frogs (*Atelopus zeteki*) to get them out of the path of the deadly fungal disease *Batrachochytrium dendrobatidis* (*Bd*), only ghosts of the black-spotted yellow beauties now haunt the riverbanks of western Panama. But while captivity has been the species' only defense from extinction, it may also be fundamentally altering the animals in ways scientists are striving to understand.

Dr. Matt Becker, a postdoctoral researcher at the Smithsonian Conservation Biology Institute who has focused his research on the use of probiotics to help frogs fight off *Bd*, is among those ranks. In 2011, he set out to determine whether eight years in captivity could change the diverse bacterial community found on the skin of amphibians that act as a first line of defense against pathogens.

What he found surprised him.

"After eight years in captivity and even after one generation, 70 percent of the bacteria on their skin was the same as it was in the wild," Becker says. "It was more than I expected. There were some obvious differences, but if they're maintaining that much of their microbial community, that's a good sign."

Becker published his findings recently in *Biological Conservation*, adding to a growing body of knowledge related to the microbiomes of animals, including humans. Researchers are interested in learn-

ing more about how probiotics—or beneficial bacteria—confer health benefits to the hosts on which they live. Probiotics are used in agriculture and aquaculture and even in human medicine.

For Becker, probiotics may also be the key to someday putting the frog species most susceptible to *Bd*—like the Golden frog—back into their native habitat. In one study, Becker and colleagues successfully transferred probiotics from the Four-toed salamander (*Hemidactylium scutatum*), which can effectively fight off *Bd*, to the Mountain yellow-legged frog (*Rana muscosa*), giving the frog the anti-fungal properties necessary to survive the disease.

While treating every frog in the wild with probiotics may be unrealistic, Becker says probiotics could be the key to returning captive populations to the wild. The probiotics method could also help prevent epidemics before they spread in places like Madagascar, where chytrid was recently detected.

"There are a number of different potentially effective disease mitigation strategies that could work together with a probiotic biotherapy," says Reid Harris, the Amphibian Survival Alliance's director of international disease mitigation and one of the first scientists to look at probiotics as a potential defense for amphibians against chytrid. "But for now the probiotic biotherapy is the only field-tested method that has worked to mitigate the disease threats."

In recent probiotic trials with the Panamanian golden frog, the



Matt Becker prepares a probiotic bath for Panamanian golden frogs. Photo: Brian Gratwicke, Smithsonian Conservation Biology Institute.

results haven't been as encouraging, however. Becker has tried five different probiotics that don't seem to colonize the skin of the frogs, either because the frogs themselves or the bacteria on their skin are inhibiting the bacteria from colonizing. Becker's ongoing studies at the Smithsonian Conservation Biology Institute aim to identify why some individuals are able to survive the pathogen—whether it has to do with probiotics, with genetics, or with a combination of the two—and why others succumb to the disease.

Becker's finding that Panamanian golden frogs retain 70 percent of their microbial community in captivity will help him find the right kind of bacteria to transfer to the golden frog—one that will persist both in captivity and in the wild. And ultimately it serves as a reminder to animal keepers and collection managers that they should do whatever they can to protect the frogs' microbial community. This could include using the same soil and other elements from the animals' natural environments. Recent studies have shown that 16 to 90 percent of bacteria are shared with the amphibian's surrounding environment.

"I don't think many people think about what's going on with an animal's microbiome when we bring them into captivity," Becker says. "But we have to think not just about preserving the genetic diversity of these captive populations, but about their microbial communities, too. If we're affecting the microbial communities dramatically, this may have consequences down the road."

Becker says when he started researching probiotics with Reid Harris in 2006, very little was known about the microbiome of amphibians. Now he hears about new people working on probiotics every month, he says. This, combined with research into potential vaccines and genetic resistance—gives Becker hope that his favor-

ite species—the Panamanian golden frog—may someday return to its riverbanks.

"The more people we have working on a solution, the better," Becker says. "Maybe the solution doesn't end up being probiotics. I really don't care what the solution is, as long as we can stop the spread of *Bd* and get these guys back in the wild. It breaks my heart that they're not out there anymore."



Anti-fungal bacteria that are applied to frogs to see if they can "stick." Photo: Brian Gratwicke, Smithsonian Conservation Biology Institute.



Mantella cowani. Photo: Molly Bletz.

What are our Options? An Overview of Disease Mitigation Strategies for Madagascar

By Reid N. Harris

Chytridiomycosis is a devastating disease of amphibians that can result in widespread die-offs and extinctions, such as those seen in the mountains of Panama (3). A recent paper found *Batrachochytrium dendrobatidis* (*Bd*) on some frogs exported from Madagascar in the pet trade, suggesting that *Bd* is in-country. This is of great concern since Madagascar is home to 500 endemic species of frogs (4) and many occur in upland habitats where *Bd* is expected to thrive.

As an emergency response to disease and other recent threats, the Amphibian Survival Alliance (ASA) helped organize and fund the ACSAM2 meeting (A Conservation Strategy for the Amphibians of Madagascar), which was held recently at Centre ValBio, Ranomafana, Madagascar. This meeting brought together experts in Malagasy frogs and in conservation biology. As ASA's Director of International Disease Mitigation, I presented an overview of disease mitigation strategies that can be considered now that *Bd* has been found in-country, including probiotic bioaugmentation, vaccination, heat therapy and biocontrol using aquatic invertebrates.

Probiotic bioaugmentation is the only disease mitigation for *Bd* that has worked in a field trial. This method increases the protective locally occurring bacteria that naturally occur on the skins of amphibians. These protective bacteria secrete antifungal compounds that kill *Bd* and that deter zoospores from colonizing. Importantly, a field experiment involving bioaugmentation of an anti-*Bd* species,

Janthinobacterium lividum, on *Rana muscosa* in the Sierra Nevada of California showed that frogs treated with probiotic baths had lower peak infection loads than untreated controls (8). One year after treatment, untreated controls were not recovered whereas 39% of probiotic-treated individuals were recovered (Vredenburg, pers. comm.), suggesting that probiotic treatment allowed individuals to persist by preventing *Bd* from reaching a lethal threshold. This is the only field trial of which I am aware, but a number of laboratory trials have shown mixed results. To more consistently find effective probiotics, my colleagues and I devised a probiotic selection protocol that aims to identify the most effective probiotics (1). With support from the ASA, The Mohamed bin Zayed Species Conservation Fund, Chester Zoo and other agencies, Molly Bletz at Technische Universität Braunschweig is currently leading an effort in collaboration with the Cellule d' Urgence Chytride en Madagascar, to find effective probiotics for the frogs of Madagascar.

Other strategies show some promising results in laboratory experiments or field surveys. Vaccination is a method that should work since amphibians have a complex mammal-like adaptive immune system. Early attempts at vaccination were not effective, perhaps because we now know that *Bd* secretes a compound that kills the lymphocytes of the immune system, which are an important part of the amphibian response to pathogens. A recent study applied live *Bd* to the skins of the Cuban tree frog and then cleared the

infection with heat therapy. After applying and clearing *Bd* three times, a 30% improvement in survival was achieved (5). Similar results were reported using dead zoospores. Once optimized, vaccination can play a role in repatriating amphibians back into nature from survival assurance colonies.

Some individuals within susceptible species are resistant, which can be due to individuals having certain Major Histocompatibility Complex (MHC) alleles, an effective combination of antimicrobial peptides (AMPs) that they can secrete onto their skins, or an effective community of defensive bacteria (6). Resistant individuals can be bred, with the expectation that their offspring will be resistant also. Considerably more research is needed in this area before we understand the genetic basis of resistance. However, this strategy can also play a role in getting amphibians from assurance colonies back into nature.

Zoospores can re-infect the same host individual on which they developed, but they can also swim away from their host to find another one. A recent laboratory study has found that aquatic micro-invertebrates, such as rotifers and ciliates, consume *Bd* zoospores and that water bodies with high population densities of these invertebrates had lower zoospore populations, which led to a lower chance of infection of amphibians (7). This leads to the possibility of augmenting predatory invertebrates in water bodies to reduce the probability of infection by *Bd*. Field experimentation is needed to assess its efficacy and to optimize protocols for such a strategy. This method would probably work best in still water bodies, and therefore could be effective for pond dwelling and breeding am-

phibians.

Bd grows best at cooler temperatures and dies when temperatures exceed 30 °C. Many species of amphibians can tolerate high temperatures, and heat treatment has been used to clear individuals of infection in the laboratory. There is some evidence from field studies that warmer areas within the habitat range of a susceptible species offers a refuge from *Bd* (11). Some have suggested creating warm spots by tree trimming and cutting. Additional mitigation strategies are outlined in Woodhams *et al.* (10).

With so many species in Madagascar, it is necessary to determine which species and locations are most at risk from *Bd*. A recently published method allows species to be ranked by susceptibility to *Bd* by doing a simple lab test (10). Molly Bletz has begun these studies in Madagascar, and pending funding there are plans to greatly expand these surveys with additional capacity provided by an international team of researchers in collaboration with the Cellule d'Urgence Chytride en Madagascar. With these data, a Landscape of Risk can be mapped and mitigation efforts can be focused in areas and species predicted to be of highest risk.

In Madagascar, the optimal disease control strategy may rely on using multiple methods to effectively conserve its hyper-diverse and endemic amphibian fauna.

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Field sampling in Ankaratra, Madagascar. Photo: Molly Bletz.



Platypelis grandis. Photo: Molly Bletz.



Rana temporaria mating mass, Glendalough, Wicklow Mountains National Park, Ireland.
Photo: Rob Gandola.

The Contribution of Citizen Scientists to Amphibian Disease Monitoring in Ireland

By Robert Gandola, Catriona Hendry, Collie Ennis & J.P. Dunbar

One of the benefits for a given species of occurring on the island of Ireland—and any other island—is that it is usually more difficult for pathogens to colonize, particularly those that are not wind-borne. That said, there is always the potential for diseases to be transported by migrating avifauna or via introductions of non-native animals (for examples see 4, 6). The open-trade agreement within the European Union means that the number of tourists and haulage moving back and forth between Ireland and the rest of Europe has greatly increased in past decades, thereby also increasing the chances of accidental introductions of non-native animals or diseases. The recent economic downturn in Europe is having an unintentional effect on disease monitoring, in that monies that may have been available to be more proactive in screening for disease has now dried up. Even so, it is crucial to the health of the Irish native wildlife that monitoring continues, and so it has become a priority for volunteer organizations such as the Herpetological Society of Ireland (H.S.I) to encourage members of the public to become involved in safeguarding amphibian popula-

tions. We ask that people report anything that they think is unusual in relation to amphibians, either in their garden ponds, local parks, public walking routes or National Parks.

SURVEYING FOR DISEASE

In 2012 we undertook the Irish Amphibian Chytrid Survey (IACS), the first major disease-screening survey of native amphibian populations on the island. The aim of this project was to generate a baseline status for the chytrid fungus (*Batrachochytrium dendrobatidis*) against which future investigations could be compared (for more details and results see 3). This initiative relied heavily on volunteers to survey and collect the necessary samples.

INVESTIGATING REPORTS OF DEAD AMPHIBIANS

In the last two years the H.S.I has investigated a total of eight reports of dead amphibians, with seven in 2014. Most cases were easy to rule out as a result of natural causes. However two major die-offs involving the European common frog (*Rana temporaria*) (one in Co. Waterford in 2013 and the other in Co. Kildare in 2014), were serious and required more detailed investigation, including carcass collection, water analysis and PCR-based disease screening. This suggests that the public will take the time to report incidents once they know there is a dedicated outlet for their information or at least somewhere they can get advice.

Robert Gandola is the Senior Scientist for the Herpetological Society of Ireland and a Ph.D student at the University of Southampton, Catriona Hendry is a Science Officer for the Herpetological Society of Ireland and a Ph.D student at the George Washington University, Collie Ennis a Science Officer for the Herpetological Society of Ireland, and JP Dunbar is a Science Officer for the Herpetological Society of Ireland (Corresponding Author Email: rgandola@thehsi.org)



Rana temporaria, Glendalough, Wicklow Mountains National Park, Ireland. Photo: Rob Gandola.

NON-NATIVE SPECIES

Thankfully Ireland does not have any major issues with non-native herpetofauna, but there are a couple of cases in which non-native populations have come to our attention and again it was vigilance by members of the public that alerted us to the situation. As a result we were able to identify isolated cases of non-native Alpine newts (*Ichthyosaura alpestris*) and Common toads (*Bufo bufo*) in Irish waterways, situations that we will continue to monitor in future seasons. Both of these cases were assessed for disease risk, and once again are in part the result of the generosity of ZSL/IoZ in accommodating and facilitating the screening of samples particularly under the recent Garden Wildlife Health initiative (<http://www.gardenwildlifehealth.org/>).

There is always a low risk, but a threat nonetheless, of emergent amphibian diseases arriving via the pet trade (1, 2), as has been the suggested route for the newly discovered *Batrachochytrium salamandrivorans* (5). At this time there are no known cases of other amphibian species from the pet trade, or otherwise, having been released here in Ireland, but again it is likely that members of the public will be the first to notice (and hopefully report!) such an event.

USEFUL TECHNOLOGY

The widespread use of smart phones with built-in cameras has made it easy to rapidly deal with any reports of dead or dying

animals. Current smart phones have relatively high-resolution imaging, which, in addition to the GPS capabilities of most models, means that members of the public can provide verifiable images at the time of discovery of a die-off event. This is crucial for assessing what has actually happened at a site. With this in mind, and the spring spawning season approaching, we continue to appeal to members of the public to submit any sightings of dead or dying amphibians to the H.S.I, with images and GPS location whenever possible. This is particularly important when significant numbers of animals are involved.

To report any dead or dying amphibians please contact science@thehsi.org

For more information on the H.S.I visit www.thehsi.org or <https://www.facebook.com/hsi.fan>

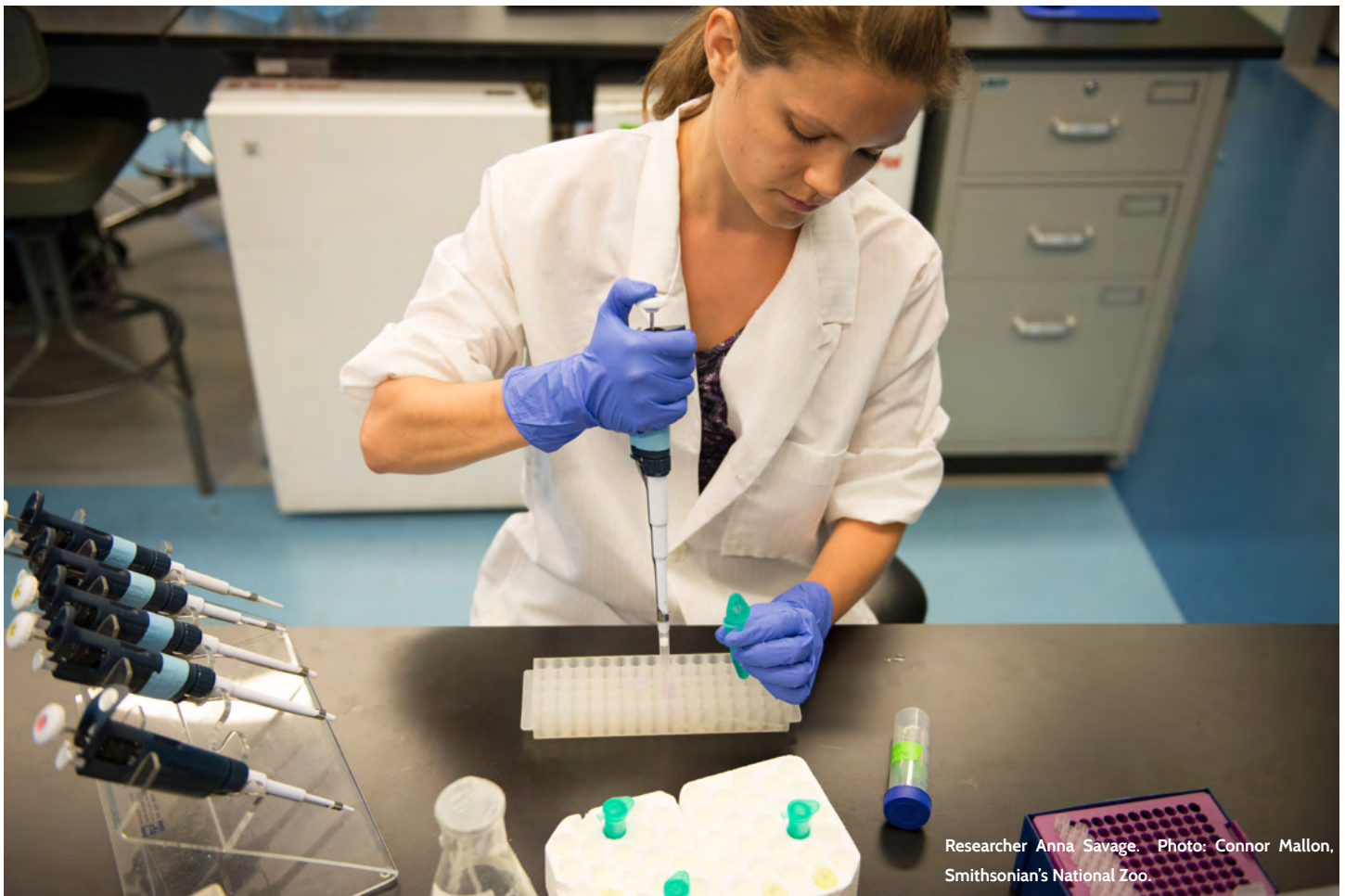
Acknowledgements

We offer our utmost thanks to our volunteers and members of the public who collect and submit sightings data to the H.S.I. We also thank the National Parks and Wildlife Service of Ireland (particularly David McNamara and Ferdia Marnell for their continued support), the Northern Ireland Environment Agency, ZSL/IoZ, Felicity Wynne, Stephen Price, Trent Garner, Becki Lawson and Prof. Andrew Cunningham.

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Researcher Anna Savage. Photo: Connor Mallon, Smithsonian's National Zoo.

Scientists Turn to Genetics to Solve Chytrid's Puzzle

By Lindsay Renick Mayer

Imagine trying to solve a jigsaw puzzle with 6,500 pieces, all of which are dramatically different, scattered throughout the world on every continent and apt to change shape in response to a varying environment. That kind of complexity is at the root of what scientists face as they piece together a comprehensive picture of the fungal disease *Batrachochytrium dendrobatidis* (*Bd*, or chytrid), even as it continues to decimate populations of amphibians around the globe.

"Part of why the amphibian disease crisis is such a crisis is because we have thousands of amphibian species all over the world with very different life histories living in different environments," says Dr. Anna Savage, molecular evolution postdoctoral fellow with the Smithsonian Conservation Biology Institute and assistant professor at the University of Central Florida. "There are just so many unique combinations of conditions leading to different disease outcomes in a thousand different scenarios."

A number of researchers, including Savage, are turning to a field that has not traditionally focused on amphibians—that of genetics—to start to solve the puzzle of chytrid: how it has evolved, how it spreads, how (and why) its strains vary, how it affects each frog species and individual differently, where it came from and, most importantly, how to stop its deadly global rampage.

THE EMERGING DISEASE TRIFECTA

When Savage set out to work on her dissertation, she focused on looking at the differences in the immune system genes between

Lowland leopard frogs (*Rana yavapaiensis*) that effectively fight off chytrid and those that succumb to it. Conservationists and population managers could then translocate individuals with the identified "good genes" or even breed for those genes in captive populations. This idea, however, gets complicated quickly, Savage says.

"Although we have actually found some genetic variants that we know in the lab are associated with very high survival rates against *Bd* infections, we don't necessarily want to just breed that genotype into every population," she says. "That could erase other local adaptations that help fight off other pathogens. We don't have enough information to say that would be the right thing to do."

So now Savage has changed her focus, expanding her scope beyond DNA variance. Today her research looks at gene expression—the combination of genes in an individual frog that gets turned on or off—while the frog mounts an immune response to fight off chytrid. Specifically, Savage and colleagues are looking at the intersection of three factors that can manipulate the on and off switch: the genetic diversity of the individual frog, the environment in which that frog lives and the genes of the pathogen infecting the frog.

The collection of the results from these kinds of studies could be tremendous. Conservationists could turn to a database or catalog that outlines the genetic and environmental factors that may make a specific population of frogs more or less susceptible to chytrid. It could also help conservationists at the start of an endemic, such as the recent discovery of chytrid in Madagascar, determine where to focus their efforts.



The Lowland leopard frog (*Rana yavapaiensis*).
Photo: Brian Gratwicke, Smithsonian's National Zoo.

“Eventually we’ll be able to say the specific things that you should do as a population manager or conservationist to promote the right environmental conditions to help your specific populations fight off disease,” Savage says. Until that time, the most important thing conservationists can do for amphibians is protect their habitat, she adds.

“The more protected habitat a population has, the more breeding success it will have,” Savage says. “The bigger your effective population size, the more genetic diversity you have in a population. And the more genetic diversity you have, the better you are at fighting off pathogens.”

LOOKING BACK AND LOOKING BEYOND

Like Savage, Dr. Erica Bree Rosenblum, assistant professor at the University of California at Berkeley, is interested not only in the host’s genes, but the genetic make-up of chytrid itself. Her pathogen work revolves not only around identifying the particular genes chytrid needs to successfully attack its host, but also on answering basic questions about the fungal disease: where did it come from, where geographically has it had a longer history, where has it spread more quickly, what pathways has it travelled and what about its genome allows it to evolve so rapidly.

“What we call *Bd* has a lot of diversity inside of it,” Rosenblum says. “Recognizing that complexity is critically important so we do not assume there will be a one-size-fits-all conservation strategy. For example, our conservation strategies are going to be different in places where *Bd* has had a longer history than in places where it

is a new arrival.”

This type of research is about more than benefiting amphibians, Rosenblum says, especially as emerging infectious diseases are on the rise globally. Researchers studying white-nose syndrome in bats, for example, have been able to mobilize quicker, in part, because of the lessons the amphibian community has learned about fungal disease.

“What we learn about a particular emerging infectious disease becomes part of the collective database to inform how we will respond to wildlife disease threats in general over the next century,” says Rosenblum.

Are researchers optimistic that their efforts today will help solve the puzzle of these emerging infectious diseases in time to stop some species from going extinct? Yes and no, Rosenblum says.

“I am troubled by what is likely to happen to amphibian biodiversity over the next 50–100 years,” she says. “I think many people who work in the field are concerned about the state of biodiversity on the planet and humans’ role in that capacity. But, even so, I would say there are a few rays of hope.”

Is Habitat Destruction More Dangerous for *Atelopus* Than Chytrid?

By Rebecca D. Tarvin, Diego Acosta & Santiago R. Ron

Population declines have strongly affected a group of frogs known as Jambatos, or Harlequin frogs, of the genus *Atelopus* (3). Many of these declines have been purportedly caused by chytridiomycosis (3). However, our recent publication in *Journal of Herpetology*, “Changes in population size and survival in *Atelopus spumarius* (Anura: Bufonidae) are not correlated with chytrid prevalence,” presents a case where habitat destruction rather than chytrid was responsible for population decline (6).

Our 22-month mark-recapture study followed the population size and survival of *A. spumarius* at a locality in Amazonian Ecuador. Six months into the study, the opening of a road allowed timber extraction that resulted in trees being logged along our study site. Thereafter we witnessed a slow decline in estimated survival rates and population size. Three years later in September of 2014, a group of students from the Zoology Museum at Pontificia Universidad Católica del Ecuador returned to the site. Since the conclusion of our study in 2010, the road had advanced closer to the *Atelopus* population and houses and crops had overtaken what was previously prime *Atelopus* habitat; the students did not find any remaining individuals.

We found that early habitat degradation resulted in a significant decline in male body condition. Additionally, following habitat degradation, rates of emigration decreased, suggesting that the population was not moving out of the area despite deteriorating conditions. This was likely compounded by the high site fidelity we observed in both males and females.

Bd is present at our study site, but the population of *A. spumarius* appeared to be unaffected, as we found no evidence of chytrid-infected individuals despite genetic screening of all individuals found. This was contrary to our expectations because *Atelopus* has been considered one of the groups most severely affected by chytrid (3). There are two potential explanations for this apparent lack of effect: (1) a long history of coevolution between *A. spumarius* and an endemic *Bd* leading to stable coexistence, or, (2) the population declined after the arrival of a novel *Bd* but later recovered by evolving resistance. In addition to our data, growing support for an endemic *Bd* that has long been in South America (5) lead us to suggest that the first hypothesis is more likely than the second. Moreover, recent population studies with other species of *Atelopus* show a similar pattern: stable populations in the presence of *Bd* (1,2,4).

It is now apparent that at least some species of *Atelopus* can survive with ambient levels of *Bd*. Regardless of which hypothesis explains *Atelopus* resistance to *Bd*, we highlight that at present, habitat destruction may be the most significant threat for the survival of these unique and Critically Endangered anurans.



Photo: Rebecca Tarvin.



An *Atelopus spumarius* population near Puyo, Ecuador, existed in sympatry with *Bd* since at least 2008, but logging in 2011 resulted in their disappearance. Photo: Santiago Ron.

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Fig. 1: A pond home to American toads and Northern leopard frogs in southwest Ohio. Photo: Samantha Rumschlag.

When Infection is Common but Declines are not Apparent: The Influence of *Batrachochytrium dendrobatidis* Exposure on Midwestern Amphibians

By 'Samantha L. Rumschlag & 'Michelle D. Boone

Pathogens have the potential to shape populations and communities; through effects on host growth, survival, and reproduction, pathogens can influence community interactions such as competition, facilitation, predation and invasion. Although we understand the potential for the effects of disease, we lack the ability to predict the impact of specific pathogens on host populations and communities.

Although much attention has been given to the disease ecology of chytridiomycosis in regions in which mortality events have been sudden and widespread like Central and South America and Australia, relatively little focus has been given to the role of *Batrachochytrium dendrobatidis* (*Bd*) infection in populations in which mortality events are not apparent, which undermines our ability to understand the range of host-pathogen interactions that could help reduce outbreaks in disease-prone areas. For instance, in the Midwestern United States, surveys for *Bd* in amphibian populations suggest that *Bd* exposure and infection are common. Furthermore,

laboratory exposures of these amphibian species to *Bd* indicate that *Bd* could have impacts that affect amphibian populations in the wild, which supports the need to investigate the effect of *Bd* on these populations.

Our research shows that *Bd* can cause infection and impact host growth and survival of species not generally thought at risk to *Bd*-related declines. For example, *Bd* exposures of Northern leopard frog juveniles (*Lithobates pipiens*) can reduce growth over a 42-day period by about 30 percent with no effects on survival (Fig 2., [1]). Reductions in growth have been shown to have population-level impacts by reducing fitness through increased time to first reproduction and decreased fecundity. For American toads (*Anaxyrus americanus*), *Bd* can cause infection and drastically reduce survival by about 75 percent (Fig. 3, [2]). These huge reductions in survival, if sustained over multiple generations, could have serious consequences for population persistence if they occur in the field. But to date, there are no records of American toad and Northern leopard frog population declines caused by *Bd*. The lack of these records does not suggest that *Bd* does not cause population-level impacts.

¹Department of Biology, Miami University, Oxford, Ohio.

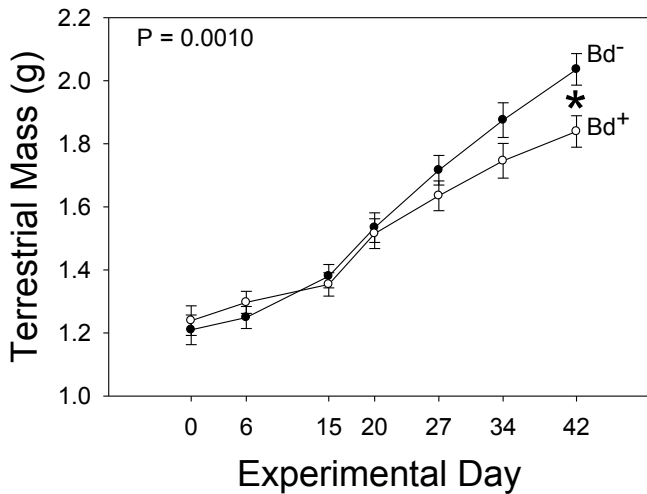


Fig. 2: Terrestrial growth of juvenile Northern leopard frogs over time significantly changed between *Bd* absent (*Bd*-) and present (*Bd*+) treatments. Northern leopard frogs were exposed to *Bd* just after metamorphosis and were reared for 42 days in the laboratory. Error bars represent + 1 standard error. *Indicates significant treatment difference at a given time point. (Caseltine *et al.* in review).

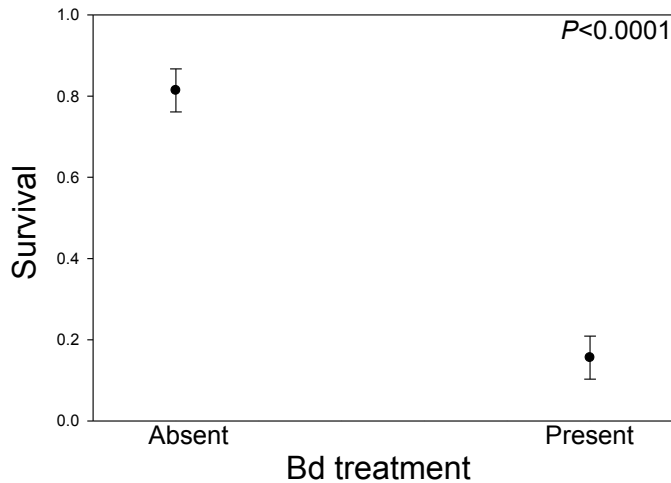


Fig. 3: American toad terrestrial survival in the presence or absence of *Batrachochytrium dendrobatidis* (*Bd*). American toads were exposed to *Bd* just after metamorphosis and reared for 51 days in the laboratory. Error bars represent ± 1 standard error. (Wise *et al.* 2014)



Fig. 4: American toad (*Anaxyrus americanus*). Photo: Melissa Youngquist.

Rather, the absence of *Bd*-related declines could be explained by lack of survey efforts that has led to the effects of *Bd* going unreported. A need exists for comprehensive surveys and long-term monitoring of populations to determine the effects of *Bd* on population persistence of species that appear sensitive to *Bd* exposures.

Amphibian populations in temperate regions such as the Midwest may be faced with additional factors that populations in tropical regions do not encounter and that could compound the effects of *Bd* infection and exposure. The overwintering phase of the amphibian life cycle is associated with a natural reduction in the immune system, which could cause increased susceptibility to *Bd*. If *Bd* infection causes decreased overwintering survival, this effect would be difficult to observe in the field and then attribute to *Bd* since many species overwinter underground.



Fig. 5: Northern leopard frog (*Lithobates pipiens*). Photo: Melissa Youngquist.

American toad and Northern leopard frog populations may be affected by *Bd*, but we are currently unaware of the effects of *Bd* on these populations in the wild. There is a need to survey and monitor species like these for long-term effects of *Bd* on population growth and persistence and to evaluate factors that may influence outbreaks. Understanding the distribution of *Bd* within the range of these species and the influence on growth and survival of hosts in the field will lead to a better understanding of the role of host-pathogen interactions in populations where *Bd* persists. This may also offer insights into how populations that recover from *Bd*-related die-offs may persist with this pathogen.

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Frog Body Temperature Influences How the Chytrid Pathogen Grows and Reproduces

By Lisa A. Stevenson, Elizabeth A. Roznik, Ross A. Alford & David A. Pike

Understanding the impacts of the chytrid fungus (*Batrachochytrium dendrobatidis*) on amphibians is inherently difficult because the life history of the pathogen depends on temperature (1). Subtle changes in body temperature that amphibian hosts experience in nature (e.g., over hourly, daily or seasonal timescales) likely influence how the pathogen grows and reproduces (2). Unfortunately, we know relatively little about the thermal biology of amphibians, limiting our understanding of how temperature influences disease susceptibility. Recent advances in miniaturized tracking devices are now allowing us to better quantify the temperatures experienced by free-living amphibians in nature (Figure 1; reviewed by 3). We have used this technology to record, in detail, the temperatures experienced by three sympatric species of rainforest frogs at both low and high elevations (Fig. 1; 2). We then—for the first time—incubated the chytrid fungus in the laboratory under the mean temperatures experienced by each of these three frog species in the wild (2). Our aim was to test whether patterns of decline in nature were related to the relative temperatures experienced by each species, and whether exposure to these temperatures enhances rates of chytrid growth in culture.

We re-created conditions emulating the mean thermal regimes of three rainforest frog species (*Litoria nannotis*, *L. rheocola* and *L. serrata*) from high- and low-elevation populations (2). These species are stream-associated rainforest specialists with broadly overlapping distributions in the Wet Tropics region of northern Queensland, Australia (4) and differ substantially in their ecology, behavior and microhabitat preferences (Fig. 2). The Waterfall frog *L. nannotis* is stream dwelling; both males and females spend much of the day sheltering behind waterfalls or wedged between rocks in the stream, and do not often venture far from streams (Fig. 2; 5-7). The Green-eyed treefrog *L. serrata* reproduces in streams, but can often be found in or on surrounding vegetation (Fig. 2; 6, 8). The Common mistfrog *L. rheocola* is intermediate in habitat use between *L. nannotis* and *L. serrata*, remaining near the stream and using both rocks and vegetation (Fig. 2; 9, 10).

The rainforest frogs *Litoria nannotis*, *L. rheocola* and *L. serrata* maintained thermal regimes within the optimal range for chytrid growth (15–25 °C) at both low- and high-elevation rainforest sites. The species that has recovered from *Bd*-related declines, *L. serrata*, maintained the warmest and most variable body temperatures

that resulted in the slowest pathogen growth. The other two species, which have experienced more long-lasting declines, maintained cooler and less variable thermal regimes, resulting in faster pathogen growth. We also found that the chytrid fungus has a faster life cycle when maintained in the thermal regimes corresponding to high elevations than in those corresponding to low elevations, where frogs maintained warmer body temperatures. Many of these findings match ecological patterns documented in nature (6-9), which suggests that interspecific differences in the microhabitat preference of sympatric frogs can influence disease susceptibility (2).

The majority of what is known about the life history of the chytrid fungus, and most strategies to predict and mitigate its impacts, are based on the results of constant-temperature experiments (2). In our latest research, all of the thermal regimes we tested—based on real frog body temperatures in the wild—resulted in pathogen growth rates that were equivalent to, or faster than, rates expected from constant-temperature experiments (2). This demonstrates that the mean thermal regimes that frogs maintain, and the associated variation around the mean, can have

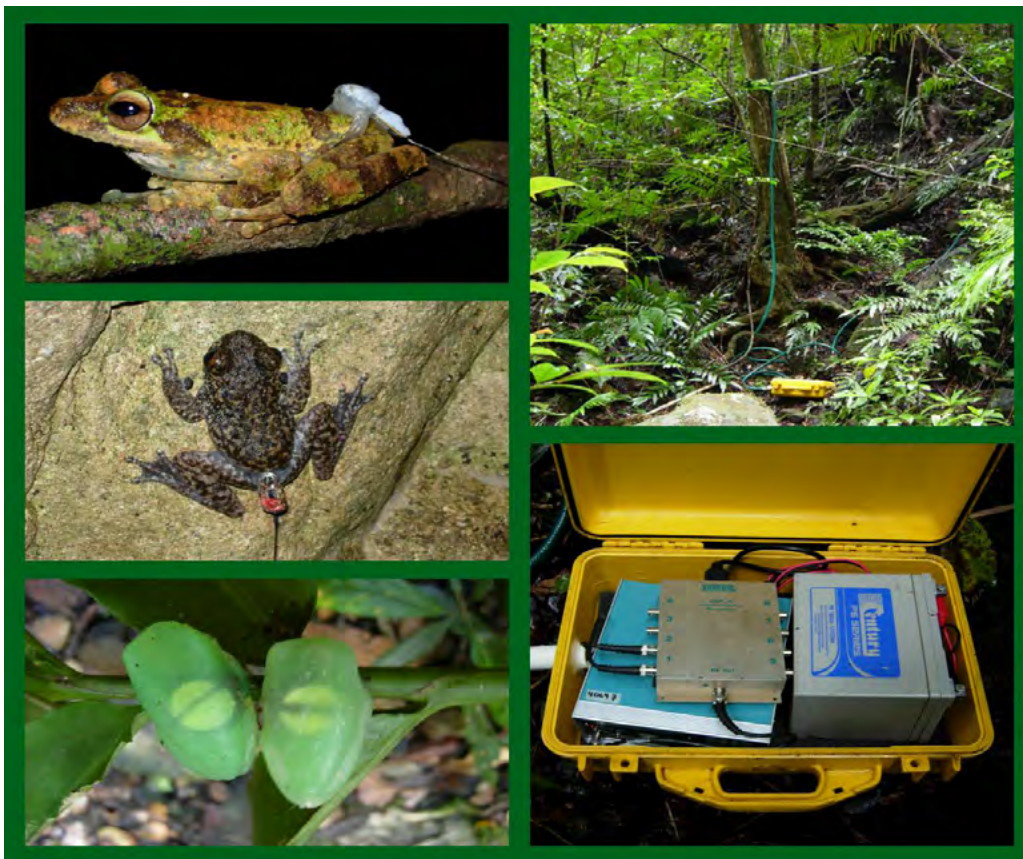


Fig. 1: We used miniaturized tracking devices to follow individual rainforest frogs to better quantify their microhabitat use, including patterns of daily body temperature fluctuations at both low and high elevation sites. We captured frogs, fitted them with miniature radiotransmitters (left, top is a male *Litoria serrata* and left, middle is a male *L. nannotis*, both with radiotransmitters affixed around their waists). An automated receiver antenna (right, top) connected to a datalogger (right, bottom) recorded external body temperatures of frogs at 15-minute intervals during the day and night. Frog body temperatures collected using automated radiotelemetry are tightly correlated to the data obtained by placing pairs of physical models in locations used by frogs (left, bottom; fully described and explained in 3). Photographs: Elizabeth Roznik.

important effects on the life cycle of the chytrid fungus. Fluctuating temperatures cause the pathogen to grow faster than at constant temperatures because of the asymmetrical nature of how *Bd* responds to temperature. When fluctuations in frog body temperature exceed the overall mean value for a given species, chytrid growth accelerates more rapidly than when temperatures drop below the mean by the same amount. This biological phenomenon results in growth rates in a fluctuating thermal environment that can be higher than expected based on the mean temperature of that environment (2, 11).

The effects of host body temperature on the life history of the chytrid fungus can explain many of the broad ecological patterns of population declines in our focal species, via direct effects on pathogen fitness (2). Our study highlights an urgent need to study the microhabitat use and thermal biology of amphibians to better understand how these attributes influence disease susceptibility. Experiments testing the susceptibility of live amphibians to this pathogen will be more realistic when they can incorporate species-specific thermal regimes. This will improve our ability to predict not only the spatial and temporal spread of disease, but spatial and temporal susceptibility of hosts. Understanding the functional responses of the chytrid fungus to the conditions that are experienced by amphibian hosts is crucial for determining the ecological drivers of disease outbreaks and mitigating the impacts of disease.

Acknowledgements

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Figure 2: We studied three species of rainforest frogs in the Wet Tropics of northeastern Australia: (left, top) *Litoria serrata*, (middle) *L. rheocola* and (bottom) *L. nannotis*. These species span a gradient of habitat use ranging from relatively warm and dry (*Litoria serrata*) to cool and wet cool and wet (*L. nannotis*). The Green-eyed treefrog (*L. serrata*) is often found in or on vegetation surrounding the stream, and thus maintains relatively warm body temperatures. The Common mistfrog (*L. rheocola*) remains near the stream and uses both rocks and vegetation, which results in intermediate body temperatures. The waterfall frog (*L. nannotis*) spends much of the day sheltering behind waterfalls or wedged between rocks in the stream, and do not often venture far from streams at night, resulting in cool body conditions. Typical rainforest stream habitat is shown at right. Photographs: Elizabeth Roznik.

Bd in Nicaragua: Confirmation of the Inevitable

By ¹Roberto García-Roa, ²Javier Sunyer, ³Andrés Fernández-Loras & ⁵Jaime Bosch

Nicaragua is the largest and middlemost country in Central America, and the Nicaraguan Depression constitutes the transitional area between Nuclear and Lower Central America, which has acted as a bottleneck in the dispersal routes of mainland American species (1). However, the herpetological biodiversity of Nicaragua compared to its neighboring countries (Honduras in the north and Costa Rica in the south) is considerably low (2). During the last 15 years there has been a substantial increase in herpetological research in Nicaragua, which has resulted in the description of several endemic species adding to the total of Nicaragua's herpetofauna species. Nevertheless, Nicaragua still has a relatively unknown and poorly understood herpetological species composition. To date, 74 amphibian species (9.5% of which are endemic) corresponding to three orders, 13 families and 35 genera have been recorded in the country, some of which have some degree of endangerment or vulnerability (3). Several anuran species are expected to be found in the country as research continues developing. For example, the frog genera *Duellmanohyla* and *Isthmohyla* as well as the species *Anotheca spinosa* are known to occur in countries both to the north and south of Nicaragua, but their presence have not yet been confirmed in the country and therefore constitute a gap in their respective distributions (4). In addition, far too little attention has been paid to amphibian diseases in Nicaragua. Chytridiomycosis is an infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and known to be the most serious disease affecting amphibian species worldwide. Its ability to decrease and cause populations to disappear precipitating extinction in amphibian species has been confirmed (5). This introduced pathogen has been recorded in almost all Central America apart from Belize and Nicaragua. Therefore, during 2011 we initiated some fieldwork in three different Nicaraguan localities with the aim to make a preliminary assessment about the presence of the lethal fungus in the country, and to identify which species are affected by this pathogen.

The surveys were carried out in three localities of Nicaragua along a north to south transect with an altitudinal gradient between 810–1470 m asl: 1) Cerro Jesús, Dept. Nueva Segovia; 2) Reserva Natural Datanlí-El Diablo, Dept. Jinotega; and 3) Reserva Natural Volcán Maderas, Reserva de la Biosfera Isla de Ometepe, Dept. Rivas. We analyzed samples from 18 amphibian species corresponding to nine families and three orders. Our results confirmed for the first time the presence of *Bd* in Nicaraguan amphibians, filling in a gap in the distribution of this lethal fungus in Central America (6). We confirmed the presence of *Bd* in four amphibian species for the first time: *Dendropsophus microcephalus*, *Craugastor lauraster*, *Smilisca baudinii* and *Lithobates brownorum*. All three localities were *Bd*-positive, although the percentage of infected amphibians varied greatly, with Volcán Maderas the highest with a 72% infection prevalence. This locality is greatly visited by tourists and is the only locality in Nicaragua where numerous anuran deaths have been recorded (7), in addition to the presence of ranavirus in 75% of the anuran

tadpoles in a remote crater lagoon near the volcano's summit (8). Further research in Nicaragua is urgently needed in order to understand the distribution, spread and potential impact of amphibian pathogens in the country before it is too late.

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Bolitoglossa insularis in Isla Ometepe (Nicaragua), the only locality known for the species. Photo: Roberto García-Roa.



The Leopard frog, *Lithobates brownorum*, one of the *Batrachochytrium dendrobatidis* positive species sampled in Cerro Jesús (Nicaragua). Photo: Roberto García-Roa.

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A Q&A with Amphibian Immunologist Dr. Louise Rollins-Smith

By Lindsay Renick Mayer

When Dr. Louise Rollins-Smith first heard about chytrid in 1998, it seemed to her like an intriguing scientific challenge. She had a master's degree and Ph.D. in zoology and had developed a keen interest in immunology, specifically that of amphibians. Since the discovery of the fungal disease, Rollins-Smith has published more than 50 papers related to the immunology of amphibians infected with chytrid, including most recently in *Science* and *Nature*. Her lab at Vanderbilt University continues to investigate some of the most pressing questions about chytrid, answering questions about the pathogen's defense mechanisms, amphibians' antimicrobial peptides and potential management tools.

"Louise has been a great collaborator and mentor to so many in our community," says colleague Brian Gratwicke, amphibian conservation biologist at the Smithsonian Conservation Biology Institute. "She has significantly helped us improve our understanding of this problem and potential solutions."

For this special issue of *FrogLog*, we sat down with Rollins-Smith to talk more about her career, and where her work is heading.

How did you develop an interest in amphibians specifically?

I've always enjoyed all things natural. I grew up on a farm in the southern part of Minnesota long ago and was always interested in biology. In graduate school I loved embryology and wanted to work more on developing systems and amphibians have such beautiful developing embryos. I was trying to go in that direction and I ended up in a frog development lab. I think that's where it all crystalized.



Photo: Courtesy of Louise Rollins-Smith.

At some point I was going to be a virologist or cancer biologist. My mom used to tell people that I was a cancer biologist long after I'd kind of given that up. I worked on that a little bit more as a postdoc, but then I left virology and turned toward straight immunology, understanding how the immune system develops in frogs and how it changes at metamorphosis.

What is the focus of the research that you're currently doing?

We're trying to understand how the chytrid fungus circumvents the immune system. We know the amphibian has a very complex and robust immune system, nearly as complex as yours and mine, and still in many species it doesn't seem to do a very good job of clearing a skin fungus. So we're trying to figure out how the fungus protects itself from the immune system.

When you started your work in this field, were there many others looking at amphibians?

There were not many amphibian immunology groups. When chytridiomycosis came into the picture, I came to know a lot of those herpetologists and disease biologists, ecologists, endocrinologists. Trying to understand the whole problem introduced me to a lot of different kinds of amphibian biologists that I hadn't known about.

How did you feel when you first learned about chytrid?

I was really intrigued with trying to get to the bottom of it. Was it a developmental problem? Was it disease? Before that there was evidence for toxic effects of agricultural chemicals—you had these poor little deformed frogs that we all worried about. That was another area I was interested in. I might have gone in that direction looking at some of the toxicology and how that led to deformed frogs, but the disease question was more attractive.

Since then, what has happened in the field? What kind of knowledge have we gained, and are we closer to being able to mitigate this?

Since about 1999 there have been annual meetings of a group of amphibian biologists that occur once a year in Arizona in November. Those of us who were there at the beginning recently remarked how far we've come in understanding the causes of amphibian declines and what we've learned about disease and what we now know about mitigation. I think we all feel like there's an exponential growth in information in really only the 15 or 16 years since we started working on this.

Through the course of your work, what has surprised you the most?

It has always been a long-standing puzzle why the immune system doesn't do a better job of clearing this fungus. And so getting at that was a real pleasure, to finally be able to demonstrate that the fungus is in fact fighting back. It's not just this little commensal organism in the skin; it has its own weapons to protect itself from destruction.

When you got into this field and got interested in chytrid specifically, were you in it for the love of solving this puzzle or helping save species or a combination of both?

I think it was a combination of both. We're scientists and so here's a challenge, this is an important scientific problem and we need to solve it to fix things. We hope we can fix things, but in order to fix things we have to understand them. So it's a combination of wanting to solve that puzzle and also a genuine interest in preserving biodiversity.

What's next in your work?

I hope we'll be able to identify this toxic factor that inhibits the immune system. Then maybe we can work with people who manipulate the fungus to find the pathway that makes the toxin and knock out those genes so we have a less potent organism. I think everybody is concerned at the moment about Madagascar because there are so many unique amphibians there. So we hope to work with people who are studying the bacteria there.

What do you love most about the work you do?

Interacting with lots of other interesting amphibian biologists around the world. Training students. Making these discoveries. You have bad days in the lab when the experiments aren't working or you get a paper rejected or your grant proposal doesn't get funded and those are the bad, discouraging days. But then the other days the students come in with a really nice experiment that shows you something new that you didn't know before that will advance our understanding of the whole problem. Those are the good days.

Are there any applications of your work for non-amphibian species?

We had one publication in the past that shows that some of these antimicrobial peptides are good inhibitors of the HIV virus, so they could potentially be used as microbicides. That's not an area I've had a lot of time to pursue, but I think they could be part of a cocktail of microbicides.

I think there are potential human medicines that could be developed. Even this fungus factor that we're working with, it not only inhibits amphibian lymphocytes, but it inhibits mouse and human lymphocytes, too. So if it turns out to be something unique, something unusual, it has the potential to have medical applications as well. It kills leukemia cells.

What advice would you give a student wanting to get into this field?

If they love amphibians and if they love solving puzzles, it's a good area to do research. There are still a lot of fundamental questions we have to answer to better understand how to protect amphibians broadly and how to understand their diseases.



The African clawed frog (*Xenopus laevis*) has traditionally been an immune system model. Photo: Brian Gratwicke, Smithsonian Conservation Biology Institute..

Ranavirus Outbreaks in Northwest Spain: Time to Act

By ¹Cesar Ayres, ²Jaime Bosch & ³Stephen J. Price



Fig. 1: *Triturus marmoratus* sharing a refuge with a dead *Lissotriton boscai*. Photo: C. Ayres.

Recent research has reported on the collapse of amphibian communities in northwest Spain due to *Ranavirus* (1). Two areas were studied, a national park in Asturias, and a small reservoir in Galicia. The results of the National Park monitoring show a dramatic decline in the populations of several amphibian species.

Here we will focus on the second area (Galicia). Although we do not have the ongoing host population counts for this region available for the national park, observations of disease and periodic carcass counts show that at least two newt species (*Triturus marmoratus* and *Lissotriton boscai* Fig. 1) have been seriously affected by ranavirus (Bosca's newt virus, BNV). Additionally, spillover into two species of water snake appears to have occurred as they scavenged on infected newts.

Currently, we have no data on the impact of BNV for other amphibian species inhabiting the reservoir and surrounding areas. If the Galician outbreak follows the same pattern as the national park, where all six common species of amphibian have been affected by mass-mortality events, then *Pelophylax perezi*, *Alytes obstetricans*, *Hyla molleri* as well as protected species, *Rana iberica* and *Chioglossa lusitanica*, could be decimated by ranavirus. Further research is needed to answer these questions.

However, urgent action is needed to mitigate this serious threat posed by the *Ranavirus*. At present there is neither an adequate monitoring program nor mitigation measures in place, in spite of the reservoir being used as an important resource for leisure activities and competitive sports events. Official accounts of moni-

toring at the reservoir for the period 2010–2013 do not match our own observations, which include counts of approximately 1,700 dead newts.

The approach to recreational use of the reservoir is also in need of review. To our knowledge, there is no cleaning and disinfection area in the reservoir. This is very important as the reservoir is used daily for canoeing. Regional and national canoeing championship events are held there several times per year. *Ranavirus* may persist and remain viable in water and sediment for days, weeks, or months (2) and sports equipment therefore presents a potential means for translocation of this pathogen. The scale of this risk is apparent when the number and origin of teams attending regional (36 teams, Fig.2) and national (74, Fig.3) championships during 2014 is considered. Equipment (canoes, kayaks, paddles, lifejackets) was transported as far as 1,000 km from the reservoir back to base. While much of this equipment is disinfected prior to arrival at the reservoir in order to avoid the dispersal of Zebra mussel (an invasive aquatic mollusk native to the Black Sea but now widespread in Europe and North America), no such measures are in place for the return journey.

BNV is a known pathogen of reptiles, *Natrix* sp., while related viruses are driving outbreaks of disease and mass-mortality in chelonians in North America. Native pond turtles are therefore also



Fig. 2: Dispersal of canoe teams from the reservoir after the regional championship.

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Adult Cascades frog (*Rana cascadae*) at Carter Meadow, Lassen National Forest, California. Photo: Bennett Hardy.

Itraconazole Treatment Reduces Chytrid and Increases Overwinter Survival in Wild Frogs

By ¹Bennett M. Hardy, ²Karen L. Pope, ³Jonah Piovia-Scott, ⁴Richard N. Brown & ⁴Janet E. Foley

The amphibian disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), has been implicated as a major factor for amphibian declines in pristine environments around the world (1–5). Recent efforts aimed at mitigating the impacts of *Bd* on amphibian populations have focused on lab trials of anti-fungal drugs and developing safe treatments for captive amphibians (6, 7). No studies have experimentally tested the use of anti-fungal drugs in wild frog populations and released them back into their *Bd* positive environment. Based on previous research, low doses of itraconazole (Sporanox, Janssen Pharmaceutica) appear to be a safe and effective anti-fungal treatment (8, 9). In this study we tested the efficacy of a low-dose Itraconazole treatment on wild-caught recently metamorphosed Cascades frogs (*Rana cascadae*) and monitored amphibian survival and *Bd* prevalence and intensity after release back into a natural,

Bd-positive environment.

The Cascades frog is endemic to montane environments of the Pacific Northwest of the United States and ranges from the Olympic Peninsula of Washington to the southern Cascade Mountains in northern California. Significant declines of Cascades frogs in the southern Cascades of California, primarily attributed to chytridiomycosis, has left only 11 populations remaining (10). First year *R. cascadae* have extremely low overwinter survival (Pope *et al.*, unpubl. data) and juveniles (including previous years' young) have the highest *Bd* prevalence and infection intensity of all life stages (11). Recently-metamorphosed frogs are more vulnerable to infection (12) and are likely to be heavily exposed to *Bd* in the cool fall period between metamorphosis and overwintering. Under this scenario, large numbers of first year frogs are predicted to acquire the disease prior to the following springs' snow melt. If true, treating metamorphs with an anti-fungal agent in the fall could potentially impact the population by increasing recruitment into more resilient life stages.

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METHODS

In September 2012 we collected 60 newly metamorphosed Cascades frogs from a *Bd*-positive population at Carter Meadow, Lassen National Forest, California. Frogs were collected by dip-net and were placed in individual Ziploc bags that were filled with a small amount of local water before being placed in portable coolers and kept cool with ice packs. After being immediately transported by car to Humboldt State University (HSU; ~5 hr drive) in Arcata, California, the frogs were individually placed in sterile plastic Tupperware housing in a temperature controlled room. The containers were filled with filtered tap water and placed at a slight angle to provide basking area both in and out of water. A small dish with a hole cut in the side was placed in the dry portion of the container to provide refuge.

On the first day of treatment, all frogs were randomly assigned to the itraconazole treatment group (n=30) or control group (n=30) and swabbed with a sterile swab along the following areas of the frog five times: the left and right inner thighs, the left and right webbing of the hind feet and the ventral surface of the abdomen. For 10 minutes a day over four days, frogs in the treatment group were treated with a 0.01% solution of itraconazole in reverse osmosis water. The animals in the experimental control group were given a sham treatment in reverse osmosis water only. We used the same 0.01% concentration of itraconazole as other studies have (13, 14), but reduced the number of treatment days from 11 to 4 and doubled soak time from 5 min to 10 min to minimize the length of time frogs were held in captivity. On the last day of treatment (Day 4) all frogs were swabbed for *Bd*, weighed and injected with a visual implant elastomer (VIE) dye that was color-coded by treatment group for recognition in the field. Frogs were then transported back to Carter Meadow and released. Two frogs in each group died during the treatment phase of the study resulting in the release of 28 animals in each group.

Quantitative PCR (qPCR) was used to identify presence and intensity of *Bd* infection. These qPCR reactions followed a slightly modified standard protocol (15). The DNA quantity found by qPCR was multiplied by 160 to account for dilutions that occurred during processing, producing an estimate of the number of *Bd* zoospore equivalents (ZE) in each sample.

Since experimental animals were not given individual marks (a precaution aimed at minimizing the effect of marking on animals used in the study) we were not able to use repeated-measures analyses. Instead, we conducted separate analyses for each post-treatment time point and used a sequential Bonferroni correction to evaluate statistical significance (for $\alpha=0.05$) while accounting for experiment-wise error rate.

CONCLUSION

This study found evidence for decreased prevalence of *Bd* in newly metamorphosed frogs treated with itraconazole and then released back into the wild. Both treated animals and experimental controls showed low prevalence of *Bd* on the last day of treatment, but after release, prevalence in the control group increased more rapidly than in the treatment group so that at five weeks post-release 67% of experimental controls tested positive compared to 13% of the treated frogs (Fig. 1a). The development of high prevalence in experimental control frogs is consistent with the hypothesis that newly-metamorphosed frogs rapidly develop infections in late summer and early fall and mirrors the prevalence of infection in non-experimental field controls (Table 1). We were not able to in-

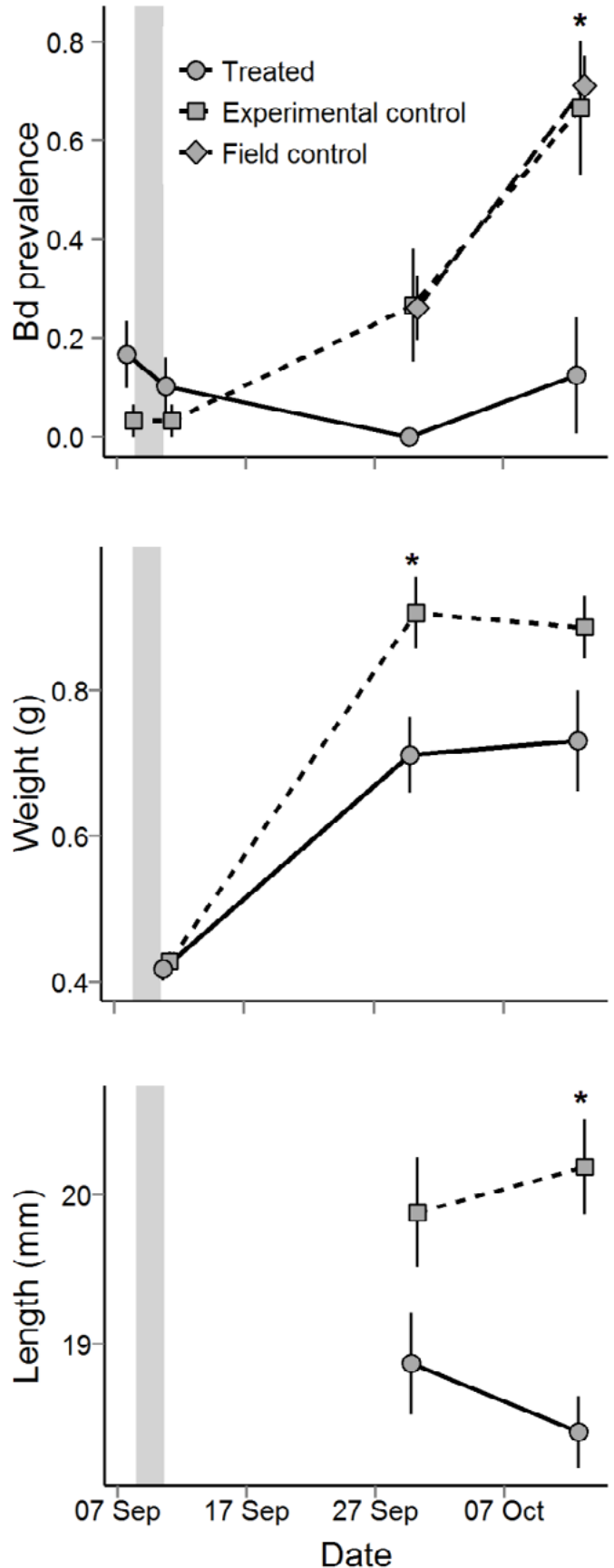


Fig 1. Effects of itraconazole treatment on Cascades frog metamorphs: (a) *Batrachochytrium dendrobatidis* (*Bd*) prevalence, (b) frog weight, and (c) length. Asterisks (*) denote significant differences between treated and experimental control frogs. Grey bars indicate the treatment period.



Cascades frog metamorphs being treated with itraconazole. Photo: Karen Pope.

clude treated animals in analyses of *Bd* load because there were not enough *Bd*-positive animals. Frogs that were treated with itraconazole weighed 22% less and were shorter in length than experimental controls three and five weeks after treatments were applied (Fig. 1b, 1c). This finding of reduced growth of itraconazole-treated frogs was also observed in Mountain yellow-legged frogs (*Rana muscosa*) (16). A significantly greater proportion of treated frogs (seven out of 28) than experimental controls (zero out of 28) were recaptured in seven surveys conducted during the spring and summer of 2013; only three field controls were found during these surveys. While itraconazole increased apparent winter survival in 2012–2013, reduced growth could limit any benefits arising from treatment in more severe winters when survival may be more tightly linked to

body size.

Treatment with itraconazole may have delayed *Bd* infection, preventing the pathogen from reaching detectable levels in most treated frogs during the 2012 sampling period. While this hypothesis seems likely, it is clear that further research is needed to determine the mechanisms responsible for the patterns observed in our study. While we did not completely clear infected individuals and observed negative effects on frog growth, it may be possible to achieve complete clearance of *Bd* infection and fewer negative effects for recently metamorphosed Cascades frogs using a lower concentration of itraconazole administered for a greater number of days (17).

Our study suggests that the positive effects of itraconazole treat-

Experimental stage	Date (2012)	Itraconazole treatment		Experimental control		Field control	
		Prev.	(ZE)	Prev.	(ZE)	Prev.	(ZE)
Capture	7 Sep	5/30	849 ± 505	1/30	1688	–	
Release	11 Sep	3/28	494 ± 389	1/28	419	–	
3 wk post-treatment	30 Sep	0/13	NA	4/15	92 ± 52	12/46	1305 ± 933
5 wk post-treatment	13 Oct	1/8	1952	8/12	4076 ± 1521	42/59	4959 ± 1264

Table 1. *Batrachochytrium dendrobatidis* (*Bd*) infection in three groups of Cascades frogs (*Rana cascadae*). Treated frogs were bathed in a solution containing the antifungal drug itraconazole between 7 and 11 September 2012, experimental controls were bathed in water, field controls were not subjected to any treatment. Data are prevalences (Prev. = frogs that tested positive for *Bd* / no. sampled) and number of zoospore equivalents (ZE) in positive samples (mean ± SE).



Researcher Karen Pope weighs a Cascades frog metamorph before swabbing for *Batrachochytrium dendrobatidis* (*Bd*) at Carter Meadow, Lassen National Forest, California. Photo: Sherilyn Munger.

ment outweigh the negative effects for wild juvenile frogs known to have a low chance of survival due to chytridiomycosis. A quarter of the treated frogs survived the winter while survival could not be confirmed for any of the untreated experimental control frogs. An itraconazole treatment could be used on juvenile frogs in critically at-risk populations to increase recruitment into more resilient older life stages and may stave off extirpation. While our study has shown that itraconazole treatment has a prolonged effect of *Bd* reduction when in a natural *Bd*-positive environment (at least five weeks), further work must be done to determine the mechanism for this effect and the length of treatment efficacy in additional species and environments.

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Conservation and Ecology

Activity patterns and fine-scale resource partitioning in the gregarious Kihansi spray toad *Nectophrynoides asperginis* in captivity

Alfan A. Rija, Ezekiel M. Goboro, Kuruthumu A. Mwamende, Abubakari Said, Edward M. Kohi & Shombe N. Hassan

We studied the behaviour of an extinct in the wild amphibian species *Nectophrynoides asperginis* from two captive centers to document activity patterns and to provide insights into the ongoing conservation program for this species. The behavior of the Kihansi spray toad was recorded by following up the individuals of different sex, age class and potentially different generations. Diurnal activity budgets and use of various habitat structures in glass terraria were recorded daily for two weeks each at the University of Dar es Salaam and Kihansi toad breeding arks where toads are bred to increase population for potential reintroduction in the Kihansi gorge. We also profiled laboratory weather parameters of temperature and relative humidity to understand their influence on the toad behavior and habitat use. Summarizing the key findings, our study shows similarity in the toad behaviors displayed by individuals of potentially different generations, possession of important survival strategies particularly the feeding, reproduction and ability to escape from potential dangers similar to their ancestors. Further, the use of habitats was different among toads of various age classes, sampling time and subpopulation. There was evidence of segregation in resource use among individuals across age classes consistent to their wild ancestors. These results suggest that the ongoing reintroduction may greatly benefit from the toad's retained behaviors. Our study recommends to the managers and conservation practitioners four key points; (i) increase monitoring of the reintroduced population and further research to understand how the toads adapt to the area and with a particular attention paid to potential predators that may have intruded the area following habitat deterioration, (ii) managers should maintain and improve existing habitats by encouraging structural diversity within the habitats both in captivity and the wild, (iii) given the high vulnerability of the toad and its habitat to damage due to elevated temperatures, it is crucially important for the managers to monitor and maintain favorable environmental conditions in captivity and the wild. This could be achieved through ensuring continued and adequate sprays to

the toad's core habitats, and, (iv) consider securing the area a full protection status so that the species and its habitats could gain appropriate mainstream attention for conservation.

A. A. Rija, E. M. Goboro, K. A. Mwamende, A. Said, E.M. Kohi, *et al.*, *Zoo Biology* 33(5): 411–418 (2014).



The Mountain chicken being represented in the 2012 carnival, Dominica. Photo: Marta Gaworek-Michalczenia.

An overview of current efforts to conserve the Critically Endangered Mountain chicken (*Leptodactylus fallax*) on Dominica

Benjamin Tapley, Luke Harding, Machel Sulton, Stephen Durand, Minchinton Burton, Jenny Spencer, Reginald Thomas, Trevorne Douglas, Jaqueline Andre, Randolph Winston, Meckleith George, Marta Gaworek-Michalczenia, Mike Hudson, Alex Blackman, James Dale & Andrew A. Cunningham

The Mountain chicken (*Leptodactylus fallax*) is restricted to Dominica and Montserrat, populations on both islands have undergone significant declines associated with the arrival of chytridiomycosis. Remaining wild populations are estimated to be no more than a few hundred individuals. *L. fallax* is of cultural importance to the people of Dominica, where the frog was the national dish. It is rare that an amphibian features so prominently in the culture and identity of a country and this, coupled with the incentive to protect it as an important food source, has been advantageous to the conservation of the species. The immediate future of the species is uncertain with the most realistic hope being through captive breeding and release. In the face of the chytrid epidemic, *L. fallax* from Montserrat were air-lifted to bio-secure facilities in European zoos, where they are now breeding. On Dominica, an in-country captive breeding program was established, over the coming years it is hoped that the frogs will reproduce and their progeny will eventually be released back into the wild.

Local capacity building is a vital component of the project. Dominican forestry staff were trained in amphibian monitoring, disease surveillance techniques and captive husbandry. A molecular laboratory was built established and a local molecular biologist was trained to analyse samples for the

presence or absence of *Batrachochytrium dendrobatidis*. Public engagement is a major component of the project in order to build and maintain a large constituency for *L. fallax* conservation. This is achieved with the involvement of local contributors. This helps to foster a sense of local pride and ownership for saving the species.

The project is guided by scientific research which have underpinned decisions on surveys and interventions required to ensure the long term survival of this species. There is ongoing research on the emergence, epidemiology and impact of chytridiomycosis in *L. fallax*. On Dominica, this work is assessing the current size and disease status of the remnant *L. fallax* population and sympatric amphibian species. As the crisis has deepened over the last few years for the *L. fallax* UK NGOs have worked together and, with the Dominican and Montserratian governments merged their different projects on Dominica and Montserrat into a collaborative effort to develop a more cohesive *L. fallax* conservation project and have developed a long-term action plan.

B. Tapley, *et al.*, *Herp. Bull.* 128 (2014).

Decline of the Cheat Mountain salamander over a 32-year period and the potential influence of competition from a sympatric species

Whitney A. Kroschel, William B. Sutton, Christopher J.W. McClure & Thomas K. Pauley

We evaluated trends in occupancy of the Cheat Mountain salamander (*Plethodon nettingi*) over a 32-yr period and examined the potential influence of competition by sympatric salamander species on these changes. We conducted surveys at 36 locations along four transects on an elevational gradient in the Appalachian Mountains geographic province of West Virginia, USA. We used occupancy modeling to examine patterns in species distributions for three focal species: *P. nettingi*, a federally threatened species, the Eastern red-backed salamander (*Plethodon cinereus*), and the Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*). The probability of occupancy for *P. nettingi* was considerably lower in 2011 compared to 1978–79 at medium and high elevations (1,169–1,378 m). Additionally, occupancy of *P. nettingi* was associated negatively with *P. cinereus* at the highest elevations. These data suggest that these *P. nettingi* populations have declined and *P. cinereus* have possibly expanded their vertical distribution. Thus, *P. cinereus* may be negatively affecting mid- and high-elevation populations of *P. nettingi*. Alternatively, environmental changes (*e.g.*,

habitat disturbance and altered weather patterns) may have contributed to declines of *P. nettingi* at mid- and high-elevation sites, facilitated by colonization by *P. cinereus*. Due to the endemic and federally threatened status of *P. nettingi*, conservation efforts to avoid fragmentation of *P. nettingi* habitat should be maintained and possibly enhanced, as ecological impacts of environmental changes can be exacerbated in high-elevation habitats.

W. A. Kroschel, W. B. Sutton, C. J. W. McClure, T. K. Pauley, *Journal of Herpetology* 48(3): 415–422 (2014).

Bioregional monitoring design and occupancy estimation for two Sierra Nevada amphibian taxa

Cathy Brown & Anthony R. Olsen

Land-management agencies need quantitative, statistically rigorous monitoring data, often at large spatial and temporal scales to support resource-management decisions. Monitoring designs typically must accommodate multiple ecological, logistical, political, and economic objectives and constraints.

We present a long-term bioregional monitoring program to assess the status and change in populations of the federally listed candidate frog species, Yosemite toad (*Anaxyrus* [*Bufo*] *canorus*) and Mountain yellow-legged frog (*Rana muscosa/sierrae* complex), on USDA Forest Service lands in the Sierra Nevada, California. The program takes advantage of advances in survey design and analysis to: 1) collect data at a metapopulation scale (i.e., small basins); 2) provide occupancy data on ≥ 2 species with overlapping ranges with the same field-monitoring protocol; 3) provide occupancy estimates applicable to the entire range of each species in the study region; 4) incorporate information from historical occupancy records; and 5) link the survey design to an existing survey design. We estimated occupancy assuming imperfect detection by extending existing procedures for maximum likelihood estimation to incorporate the unequal probability of selection used in the survey design. From 2002 to 2009, we estimate that the Yosemite toad used 0.25 ± 0.01 (SE), 0.86 ± 0.04 , and 0.86 ± 0.03 of basins over its range with historical presence, and with presence since 1990, respectively, and the Mountain yellow-legged frog used 0.04 ± 0.01 , 0.43 ± 0.04 , and 0.47 ± 0.04 of basins over its range, with historical presence, and with presence since 1990, respectively. Survey date and snow pack affected detection of the Yosemite toad but not of the mountain yellow-legged frog. Monitoring costs were reduced by using a complex survey design with panels that required generalizing existing methods

for estimating occupancy under imperfect detection.

C. Brown and A. R. Olsen, *Freshwater Science*, 32, 675 (2013).



Female of *Anotheca spinosa* taking care of her eggs in the protected area of La Pera in 2009. Photo: Leticia Ochoa-Ochoa.

Spatial and temporal variation in amphibian metacommunity structure in Chiapas, Mexico

Leticia M. Ochoa-Ochoa & Robert J. Whittaker

Amphibians are known to be sensitive to environmental changes however their responses to short-term environmental variability at the level of metacommunities are poorly understood. Amphibians may cope, adapt or move depending on the constraints factors that limit, and/or the facilitators, factors that assist, distribution. Our hypothesis is that if the conditions of the environment change we expect that metacommunity structures will change as well. Here we explored how meta-communities' structure varies when certain aspects of the environment change in two protected areas in south Mexico, Chiapas, La Pera and Nahá. We used field-data to test for variation in metacommunity properties for two consecutive years (2009 and 2010). We sampled amphibians, only at night during rainy seasons, and accompanying environmental aspects such as latitude, longitude, altitude, forest disturbance status, patch location in the landscape (matrix, edge or interior), canopy cover, litter depth, grass-height, presence of body waters, temperature and rain intensity for 30 and 31 patches, respectively. We employed coherence, turnover and boundary clumping metrics to analyze meta-community structure using reciprocal averaging (RA) ordination, with Spearman rank correlation used to examine relationships with environmental variables. The total number of individuals increased greatly but the most abundant species between years varied slightly, in both landscapes. The metacommunity structure varied differentially among the landscapes between years, being classed as quasi-Gleasonian in La Pera in both

years, but Clementsian for Nahá in 2009 and Gleasonian for Nahá in 2010. In further illustration of the variation between years, in 2009 the principal community gradient (RA axis 1) for La Pera was significantly positively correlated with altitude ($r = 0.36$), forest disturbance status ($r = 0.78$), mean canopy cover ($r = 0.79$) and mean litter depth ($r = 0.67$), while in 2010 it was correlated with latitude ($r = 0.38$), mean grass-layer height ($r = 0.38$), incidence of rainfall prior to sampling ($r = 0.35$) and presence of temporary ponds ($r = 0.45$). Our findings support the notion that amphibians respond to short-term environmental changes by individualistic movement within the landscape as well via population dynamic responses. Moreover, these responses vary greatly from landscape to landscape. We echo urgent need to develop a fuller understanding of temporal patterns of diversity. It will be the only way to be aware of under-laying processes that shape and maintain biodiversity.

L. M. Ochoa-Ochoa, R. J. Whittaker. *J. Trop. Ecol.* 30: 537–549 (2014).

Shifty salamanders: Transient trophic polymorphism and cannibalism within natural populations of larval ambystomatid salamanders

Dale M. Jefferson, Maud C.O. Ferrari, Alicia Mathis, Keith A. Hobson, Eric R. Britzke, Adam L. Crane, Andrew R. Blaustein & Douglas P. Chivers

Many species of ambystomatid salamanders utilize temporary wetlands as habitats for breeding and subsequent larval development. Such habitats are inherently variable and require larvae to exhibit plasticity in foraging, growth and development. In instances where larval densities are relatively high some species may rapidly adapt a distinct head morphology that could facilitate cannibalism. Relatively few studies have characterized structural cannibalism of larval salamanders within natural populations. In this study we examined two species of larval salamanders, Long-toed (*Ambystoma macrodactylum*) and Ringed salamanders (*A. annulatum*), collected from distinct populations where the cannibalistic morphology had previously been observed. Both species are explosive breeders and individuals produce large numbers of offspring in each breeding period. However, while Long-toed salamanders breed concurrently within wetlands, ringed salamanders stagger their breeding over a period of two months. This results in an inherent size hierarchy among larval ringed salamanders that does not exist among Long-toed salamander larvae. Head morphometrics and stable isotopic values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were used to identify the presence or absence of

structural cannibalism. Weather conditions for collection sites and years were also analyzed as a potential factor associated with the expression of cannibalistic morphology. Populations of salamander larvae did not consistently exhibit cannibalistic morphologies throughout collection periods. Trophic polymorphisms were observed among larval Long-toed salamanders during collection years when precipitation for the collection area was relatively low. Larval Ringed salamanders did not exhibit polymorphisms in this study and were cannibalistic. Our results suggest that structural cannibalism could be transient within populations for both species. This morphology appears necessary to facilitate cannibalistic behavior in Long-toed salamanders, whereas the size hierarchy within populations of larval Ringed salamanders facilitates cannibalism without morphological adaptations. However, the expression of the cannibalistic morphology in larval Ringed salamanders may prolong the viable time period in which individuals may consume conspecific prey. Additionally, expression of structural cannibalism may be related to weather conditions that result in increased population densities through reductions in wetland hydroperiod.

Jefferson *et al.*, *Front. Zool.* 11: 76 (2014).

Body size, nuptial pad size and hormone levels: Potential non-destructive biomarkers of reproductive health in wild toads (*Bufo bufo*)

Frances Orton, Alice Baynes, Frances Clare, Amanda L.J. Duffus, Severine Larroze, Martin Scholze & Trenton W.J. Garner

Amphibians are declining and fertility/fecundity are major drivers of population stability. The development of non-destructive methods to assess reproductive health are needed as destructive measures are fundamentally at odds with conservation goals for declining species. We investigated the utility of body size, nuptial pad size and forelimb width as non-destructive biomarkers of internal reproductive physiology, by analyzing correlations with commonly used destructive methods in adult male toads (*Bufo bufo*) from a low human impact (LI) and a high human impact (HI) site. Principal component analysis revealed that size was the most important variable for explaining inter-individual differences in other measured endpoints, both non-destructive and destructive, except for hormone levels and nuptial pad, which were independent of size. Toads from the LI and the HI site differed in almost all of the measured endpoints; this was largely driven by the significantly smaller size of toads from the HI site. Correlational analysis within sites revealed that size was correlated with several

reproductive endpoints in toads from the HI site but not the LI site, indicating a possible limiting effect of size on reproductive physiology. Intersex was observed in 33% of toads from the HI site and incidence was not related to any other measured endpoint. In conclusion, we provide evidence that size is associated with reproductive physiology and that nuptial pad/hormone levels have potential as additional markers due to their independence from size. We also show that human activities can have a negative effect on reproductive physiology of the common toad.

F. Orton *et al.*, *Ecotox.* 23: 1359 (2014).



Representative photographs of one *R. pretiosa* female detected along the Wood River in Klamath County, Oregon, USA. Photographs show identifying characteristics of the species, including venter coloration (upper left), upturned eyes (lower left and center) and extensive webbing between digits of rear foot (upper right). Photo: Luke Groff.

Using ecological niche models to direct rare amphibian surveys: A case study using the Oregon spotted frog (*Rana pretiosa*)

Luke A. Groff, Sharyn B. Marks & Marc P. Hayes

Rare amphibian species pose significant survey challenges because of their limited distributions and cryptic behavior. Using the Oregon spotted frog (*Rana pretiosa*) as a case study, we developed ecological niche models (ENMs) to guide surveys across the southern extent of the species' geographic distribution and assess the species' presence in California, where some regard it as extirpated. We used MAXENT to generate three ENM variants with 17 verified localities and a unique subset of environmental variables describing land cover, soil, topography, and climate. We applied jack-knife analyses to evaluate the estimates produced by each ENM. All ENMs predicted similar core areas of suitable habitat, but the spatial distribution of each suitability class differed among models. Land cover, particularly emergent herbaceous vegetation, and soils-derived variables most influenced all ENMs. Model evaluations produced moderately high prediction success rates (71%). We generated a consensus model using all ENM

variants to facilitate survey site selection, and investigated 44 sites predicted as moderately and highly suitable between 2 April and 17 August 2010. Based on initial on-site evaluations, we repeatedly surveyed 18 of these sites. We detected *R. pretiosa* at one previously unrecognized locality in Oregon. We show that ENMs generated from small datasets are useful for directing exploratory surveys for rare amphibians, and that variables derived to correspond with a species' ecology can contribute disproportionately to ENMs.

L. A. Groff, S. B. Marks, M. P. Hayes, *Herpetol Conserv Biol* 9: 354 (2014).

Comparing the status of two sympatric amphibians in the Sierra Nevada, California: insights on ecological risk and monitoring common species

Cathy Brown, Lucas R. Wilkinson, & Kathryn B. Kiehl

Basic information on species distribution and abundance is often lacking, even for common species. The historically abundant Mountain yellow-legged (*Rana* sp.) and Pacific treefrog (*Pseudacris* sp.) species complexes occur sympatrically and are exposed to similar threats. We evaluated the status of these species complexes on national forest lands in the Sierra Nevada, California as part of a long-term bioregional amphibian monitoring program. To determine occupancy and abundance indices, we surveyed all lentic and a sample of lotic habitats in 208 small watersheds (2–4 km²) selected using an unequal probability design where watersheds occupied previously had a higher likelihood of being selected. For *Rana* sp., results corroborated other assessments that the taxon has declined substantially. We found breeding activity in an estimated 0.04 (SE = 0.01) of watersheds rangewide, 0.47 (SE = 0.04) of watersheds with locality data from 1990–2001 and 0.02 (SE = 0.03) of watersheds with locality data prior to 1990. Less than 10% of the watersheds contained large numbers of frogs (>500 tadpoles or 100 adults + subadults). In contrast, treefrogs were still distributed widely, with breeding occurring in an estimated 0.25 (SE = 0.01) of watersheds rangewide and 0.95 (SE = 0.02) of watersheds where the taxon was assumed to have occurred historically. The status of *Rana* sp. is consistent with broad-scale predictions that highly aquatic, specialized anuran species have an increased extinction risk. The delayed recognition of the severity of *Rana* sp. declines underscores the value of an early warning monitoring system for all species including common ones.

C. Brown, L. R. Wilkinson, K. B. Kiehl, *J Herpetol*, 48: 74 (2014).

Do hormone-modulating chemicals impact on reproduction and development of wild amphibians?

Frances Orton & Charles R. Tyler

Globally, amphibians are undergoing a precipitous decline. At the last estimate in 2004, 32% of the approximately 6,000 species were threatened with extinction and 43% were experiencing significant declines. These declines have been linked with a wide range of environmental pressures from habitat loss to climate change, disease and pollution. This review evaluates the evidence that endocrine-disrupting contaminants (EDCs)—pollutants that affect hormone systems—are impacting on wild amphibians and contributing to population declines. The review is limited to anurans (frogs and toads) as data for effects of EDCs on wild urodeles (salamanders, newts) or caecilians (limbless amphibians) are extremely limited. Evidence from laboratory studies has shown that a wide range of chemicals have the ability to alter hormone systems and affect reproductive development and function in anurans, but for the most part only at concentrations exceeding those normally found in natural environments. Exceptions can be found for exposures to the herbicide atrazine and polychlorinated biphenyls in Leopard frogs (*Rana pipiens*) and perchlorate in African clawed frogs (*Xenopus laevis*). These contaminants induce feminising effects on the male gonads (including “intersex” – oocytes within testes) at concentrations measured in some aquatic environments. The most extensive data for effects of an EDC in wild amphibian populations are for feminising effects of atrazine on male gonad development in regions across the USA. Even where strong evidence has been provided for feminizing effects of EDCs, however, the possible impact of these effects on fertility and breeding outcome has not been established, making inference for effects on populations difficult. Laboratory studies have shown that various chemicals, including perchlorate, polychlorinated biphenyls and bromodiphenylethers, also act as endocrine disrupters through interfering with thyroid-dependent processes that are fundamental for amphibian metamorphosis. Perchlorate has also been shown to induce these effects in wild anuran populations from perchlorate-contaminated environments. Overall, the published data available suggest that some health effects observed in wild anuran populations, most notably intersex, likely have a chemical aetiology; however they derive only from very few anuran species and for a few pesticides at field sites in the USA. To understand better the impacts of EDCs on wild anuran populations, as well as other amphibian groups, assessment of fertility in exposed

animals are required. Development of non-destructive biomarkers that are indicative of specific EDC-effect mechanisms are also needed to allow the study of vulnerable populations. This will help to distinguish the effects of EDCs from other environmental and/or genetic influences on development and reproduction.

F. Orton & C. R. Tyler, *Biol. Rev.* Online: 21 Oct. 2014. DOI: 10.1111/brv.1214f

Jelly secretion by a foam-nesting tree frog *Chiromantis simus* (Anura: Rhacophoridae): An unreported behaviour

Ananda Banerjee

Chiromantis simus, a foam-nesting rhacophorid frog, previously considered extinct from India, was re-discovered in 1998. Surprisingly it is abundant at the village of Rajpur and its surroundings. This species is a true monsoon breeder and produces foam nests between June and October. Generally, during foam-nesting the female initially lays an egg mass without foam coating (i.e., “uncovered”). Later, she produces a foamy liquid and evenly covers the egg mass with it. I collected an uncovered egg mass before a female went to the water source below to absorb water. After returning, the female waited for 4 hours when she did not find the egg mass near the twig and then, by a process of continuous rubbing of her hind limbs, she secreted a thick jelly-like substance from the cloaca, instead of the foamy substance. Additional observations on the egg laying behavior showed that uncovered egg masses were always attacked by ants, while those egg masses covered by foam were never attacked. *Chiromantis simus* foam-nesting is mostly polyandrous but, when a female has to deal with too many males in amplexus, she leaves the egg mass without depositing an additional foam coating, which may be why some clutches can be found uncovered.

A. Banerjee, *Alytes* 31(3-4): 77-82 (2014).

Recent elevational range expansions in plethodontid salamanders (Amphibia: Plethodontidae) in the southern Appalachian Mountains

Matthew Moskwik

Approximately 32.5% of all amphibian species are globally threatened, and amphibian extinctions have been recorded worldwide. Individual studies rarely give full consideration to multiple potential drivers of observed biological change, instead tending to attribute changes to a single driver. Here, I tested for impacts of land use and climate change on range changes in a community of plethodontid

salamanders living from 518 to 2,036 m elevation in a global hotspot for amphibian diversity. I resurveyed 18 elevational transects in the southern Appalachian Mountains that had originally been surveyed by Nelson Hairston in 1940, 1947 and 1949. I recorded range changes for nine species that Hairston studied and determined the extent to which the recorded expansions or contractions could be attributed to changes in forest stand, climate and competition, singly or in combination. “Montane” species, which occur only on mountaintops, had expanded their lower elevational limits downwards. “Foothill” species, which occur at lower elevations than montane species, had expanded their upper elevational limits upwards. For montane species these downward expansions appear to be the result of two processes: local cooling of the climate and maturation of forests after the cessation of large-scale logging operations c. 80 years ago. In contrast, changes in upper elevational range limits of foothill species are probably the result of a suite of complex and interacting drivers, including, but not limited to, maturation of forests, changes in climate and interactions with other species.

M. Moskwik, *Journal of Biogeography* 41: 1957 (2014).



Growling grass frog *Litoria raniformis* from the northern outskirts of Melbourne, Victoria, Australia. Photo: Daniel Gilmore.

First documented use of underpass culverts by the Endangered Growling grass frog (*Litoria raniformis*) in Australia

Sally L. Koehler & Daniel C. Gilmore

We documented evidence of the use of culverts by the threatened Growling Grass Frog *Litoria raniformis*. Our study site was a wetland consisting of two ponds constructed on either side of a road connected by four box culverts. Movement through the culverts was established from mark-recapture data. Fifty-three individuals collectively undertook 63 movements through the culverts. We established that *L. raniformis* does use culverts of the design used at our site and the installation of these structures may assist with maintaining population connectivity and reduce road mortalities. While further studies are

required, we suspect that for highly aquatic species like *L. raniformis*, culverts may be most effective when they are permanently inundated (with air space) and are hydrologically linked to aquatic habitat on either side of a roadway.

S. Koehler, D. Gilmore, *Herpetol. Rev.* 45(3): 404–408 (2014).



A Western toad (*Anaxyrus boreas*) wearing a radio-transmitter. Photo: Constance Browne.

The influence of habitat composition, season and gender on habitat selection by Western toads (*Anaxyrus boreas*)

Constance L. Browne & Cynthia A. Paszkowski

The Western toad (*Anaxyrus boreas*) is a species of conservation concern in much of its range. We used radiotelemetry to track 116 adult toads in three study areas that were dominated by parkland, pasture, or boreal forest, respectively. We created resource selection function (RSF) models to evaluate the influence of habitat composition, season, and gender on habitat selection by toads. Overall, toads were found more often in open habitats (e.g., wet shrubland, crop/hay fields) than predicted by their availability. This pattern was most evident during the foraging season (July–August). Toads were more likely to be found close to breeding ponds in May–June and near hibernation sites in September–October. Female toads selected open habitat more than males, whereas males were more closely associated with water. Habitat in the northern part of the Western toad’s range is undergoing rapid change associated with resource development. Our study identifies key habitat features that should help managers protect this species in these landscapes.

C.L. Browne, C.A. Paszkowski. *Herp. Con. Bio.* 9(2): 417–427 (2014).

Riparian disturbance restricts in-stream movement of salamanders

Kristen K. Cecala, Winsor H. Lowe & John C. Maerz

Long-term population persistence or population rescue require dispersal from other source populations. Barriers to movement can effectively fragment and

isolate populations, reducing persistence and recolonisation. For stream organisms that depend heavily on movement within dendritic networks, research is needed to identify and estimate the effects of such barriers on connectivity.

We used capture-mark-recapture of displaced larval and adult salamanders to estimate return rates across gaps (length 13–85 m) in the riparian canopy and thus to assess the fragmentation of salamander populations within otherwise fully forested catchments.

Relative to salamanders in fully forested reaches, displaced salamanders were 86% less likely to return to their capture location when required to cross gaps in the canopy as short as 13 m, and the likelihood of return to their capture location declined with increasing gap length. The effects of gaps on return rates were consistent among life stages and for up- and downstream movement.

Our study suggests that riparian disturbance can reduce permeability to salamanders, even in the absence of additional land-use change. Because anthropogenic features, such as roads and power-lines, frequently cross small streams, the accumulation of apparently small land-cover changes has the potential to reduce continuous populations to small fragments with limited connectivity.

K. K. Cecala, W. H. Lowe, J. C. Maerz. *Fresh. Biol.* 59: 2354–2364 (2014).



Dorsal view of the holotype of *Rana neba* sp. nov. Photo: Masashi Ryuzaki.

A new brown frog of the genus *Rana* from Japan (Anura: Ranidae) revealed by cytological and bioacoustic studies

Masashi Ryuzaki, Yoshinori Hasegawa & Mitsuru Kuramoto

The Japanese brown frog from Nagano Prefecture, previously reported as *Rana tagoi* with $2n = 28$ chromosomes, is described as a new species. The new species differs only slightly in morphology from topotypic *R. tagoi tagoi*. It has a greater snout-nostril length, smaller fourth finger length, smaller fourth and fifth toe lengths (all relative to snout-vent length) and a narrower web, but is practically indistinguishable from a neighboring *R. tagoi tagoi* population. However, distinct acoustic differences in their advertisement calls clearly separate

the two taxa, and may serve as an effective pre-mating isolation mechanism. Karyotypic difference between the new species and *R. tagoi tagoi* results in nearly complete hybrid sterility. Because the new species is nested within a clade comprising neighboring *R. tagoi* populations, the new species must have originated rather recently by chromosome reconstruction, and subsequent acoustic divergence would have facilitated conspecific mating. This species is an example of an anuran sibling species that is unrelated to molecular phylogeny.

M. Ryuzaki, Y. Hasegawa, M. Kuramoto, *Alytes* 31(3–4): 49–58 (2014).



Natural markings in ventral view of *Melanophryniscus montevidensis*. Photo: E. Elgue.

Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae)

Ernesto Elgue, Gisela Pereira, Federico Achaval-Coppes & Raúl Maneyro

Individual identification is useful for answering a variety of biological questions about animal life histories. Most of the techniques used to mark amphibians are invasive and can cause negative effects, compromising individual survivorship and biasing studies. Photo-identification consists in the identification of specimens based on photographic records of unique color-design patterns. This technique has been used with success in several amphibian species. *Melanophryniscus montevidensis* is an endangered anuran species inhabiting the Uruguayan Atlantic coast. The general pattern of coloration is black with red and yellow blotches on the belly. In this study, we validated the technique of photo-identification assisted by software for individual recognition in *M. montevidensis* using natural markings. Field trips were performed over 16 months during which, the ventral color pattern of specimens was photographed. The photos were edited and analyzed with the Wild-ID 1.0 software for photographic reconnaissance. An efficiency of 100% was obtained in the visual recognition and 90% in the detection of recaptures using the software. The use of

photo-identification using natural marks is an effective technique in this species, because the color pattern of the belly was highly variable among individuals and remained unchanged in individuals over the 16 month period. In this evaluation the use of software for photo-identification was necessary for the treatment of large databases.

E. Elgue, G. Pereira, F. Achaval-Coppes, R. Maneyro, *Phyllomedusa* 13: 59–66 (2014).



A Red-bellied newt (*Taricha rivularis*) from Santa Clara County, California. Photo: Daniel Portik.

Discovery of a new, disjunct population of a narrowly distributed salamander (*Taricha rivularis*) in California presents conservation challenges

Sean B. Reilly, Daniel M. Portik, Michelle S. Koo & David B. Wake

The geographic distribution of amphibians in California is thought to be well resolved due to the large amount of attention biologists have focused on the region. However, there are still remote areas in California that are rarely accessed by biologists, and many amphibians are difficult to detect unless conditions are optimal. Recently a population of Red-bellied Newts (*Taricha rivularis*) was discovered in the Santa Cruz Mountains (Santa Clara Co., CA), representing a southerly range extension of approximately 130 km from the nearest records to the north. To investigate the origin of this population we sequenced two mitochondrial and one nuclear gene from the Santa Clara County population and from the main portion of the range including Sonoma, Mendocino and Humboldt Counties. We estimated phylogenetic relationships, historical demography and genetic diversity of *T. rivularis*, and these results were used to infer the origin of the newly discovered population and to elucidate the evolutionary history of the species. Surprisingly, this species exhibits the lowest genetic diversity of any salamander in coastal California, and contains a unique signature of population expansion not found in sympatrically occurring salamander species. The newly discovered population, characterized by a ubiquitous mtDNA haplotype found throughout the main range, is not genetically

divergent from the main portion of the range and could be a result of a recent human introduction or a relatively recent natural dispersal or vicariance event. Although it may be unconventional to protect a population that is possibly introduced, this newly discovered population could represent an assurance colony that could aid in the long-term persistence of this rapidly declining species. Given that *T. rivularis* lacks genetic variation, has a restricted range, and has experienced high levels of habitat disturbance, we recommend that it receive protection throughout its range.

S. B. Reilly, D. M. Portik, M. S. Koo, D. B. Wake, *Journal of Herpetology*, 48: 371–379 (2014).



Color variety of *Nyctimystes papua* from Ubai Gubi. Photo: J. Menzies.

Notes on tree frogs, *Nyctimystes* species (Anura: Hylidae) of New Guinea; the *Nyctimystes papua* species group

James I. Menzies

The definition of the *Nyctimystes papua* Species Group, as created by Zweifel (1983), is further elaborated and the differences between *N. papua* and *N. disruptus*, including morphology and geography, are discussed. The possibility that *Nyctimystes disruptus*, as currently recognized, includes more than one species, with either green or brown eyes, is also investigated. The diagnostics of *Nyctimystes oktediensis* are reviewed leading to the conclusion that it is not distinct from *N. disruptus*.

J. I. Menzies, *Alytes* 31(3–4): 59–76 (2014).

Mountaintop removal mining reduces stream salamander occupancy and richness in southeastern Kentucky (USA)

Brenee' L. Muncy, Steven J. Price, Simon J. Bonner & Chris D. Barton

Mountaintop removal mining with valley fills (MTR/VF) is a ubiquitous form of land conversion in central Appalachia, USA and threatens the integrity of stream ecosystems. We investigated the effects of MTR/VF on stream salamander occupancy and overall community composition in

southeastern Kentucky by conducting area constrained active searches for salamanders within first-order streams located in mature forest (i.e., control streams) and those impacted by MTR/VF. We found high mean species occupancy across 5 species at control streams, ranging from 0.73 (95% CI 0.41 to 0.96) to 0.90 (95% CI 0.77 to 0.98). Occupancy was lower at MTR/VF streams, with mean estimated occupancy probability ranging from 0.23 (95% CI 0.04 to 0.51) to 0.62 (95% CI 0.36 to 0.86). Additionally, the mean species richness for MTR/VF streams was 2.27 (± 1.27 SD) whereas richness was 4.67 (± 0.65 SD) for control streams. Numerous mechanisms may be responsible for decreased occupancy and species richness at MTR/VF streams, although water chemistry may be particularly important. Indeed, mean specific conductance was 30 times greater, sulfate (SO₄) levels were 70 times greater, and concentrations of dissolved ions (Ca, Mg, K, Na) were greater in MTR/VF streams than in control streams. Our results indicate that MTR/VF operations lead to significant decreases in salamander occupancy and species richness.

B. L. Muncy, S. J. Price, S. J. Bonner, C. D. Barton. 2014, *Biol. Con.* 180: 115–121 (2014).



Adult female of *Ichthyosaura alpestris* during terrestrial phase. Photo: L. Gvoždík.

Individual variation in amphibian metabolic rates during overwintering: Implications for a warming world

Peter Kristín & Lumír Gvoždík

Metabolic rates vary consistently among individuals within a population, providing raw material for natural selection. Although individual energy demands may play an increasingly important role for ectotherm survival under warmer and more variable winter conditions, whether individual variation in metabolic rates persists during overwintering is virtually unknown. Here, we repeatedly measured metabolic rate (MR) in wintering Alpine newts, *Ichthyosaura alpestris*, to (1) confirm the consistent individual variation in this trait and (2) test whether the individual differences in MR affect body mass loss during overwintering. The individual identity of newts explained 72% of variation in mass-and-activity-corrected MR. Newts with a high MR lost a higher proportion of their initial body mass than individuals

with lower metabolic demands. We conclude that the consistent individual variation in MR during overwintering is an important predictor of spring body condition in newts. This provides a new perspective on intra-individual variation in metabolic rates as a mediator of winter climate change on the dynamics of ectotherm populations.

Kristín, L. Gvoždík, *J. Zool.* **294**: 99–103 (2014).

Diseases and Toxicology

Batrachochytrium dendrobatidis infection rates differ over short distances between natural lakes and artificial ponds in Minnesota, USA

Brian G. Wolff, Eric Wurm, Sarah M. Conway & Kathleen Kinzer

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is an emerging amphibian disease that is known to be widespread in North America. Unfortunately, little is known regarding the importance of spatial and temporal changes in infection rates resulting from anthropogenic impacts on chytrid transmission and pathogenicity. While anthropogenic ecological simplification is generally known to play a role in disease emergence, few studies have been published on this topic. We conducted a *Bd* survey in southeastern Minnesota (U.S.A). Samples were collected from Green frogs (*Lithobates clamitans*) and Leopard frogs (*L. pipiens*) taken from two artificial sites and two naturally occurring lakes. We found a significant difference in infection rates between the natural and artificial sites surveyed, as well as significant differences over small geographic distances (i.e., <5km). We cannot conclude from this study alone that ecological simplification is a driving force in the emergence of chytridiomycosis, but further study of this hypothesis appears warranted. Stormwater holding ponds, decorative ponds, and similar artificial structures may serve as important loci for *Bd* propagation.

B. G. Wolff, E. Wurm, S. M. Conway, K. Kinzer, *Herpetol. Rev.* **45**(2): 447–449 (2014).

Effects of pesticide exposure and the amphibian chytrid fungus on gray treefrog (*Hyla chrysoscelis*) metamorphosis

Kristina M. Gaietto, Samantha L. Rumschlag & Michelle D. Boone

Pesticides are detectable in most aquatic habitats and have the potential to alter host-pathogen interactions. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been associated with amphibian declines around the world.

However, *Bd*-associated declines are more prominent in some areas, despite nearly global distribution of *Bd*, suggesting other factors contribute to disease outbreaks. In a laboratory study, the authors examined the effects of six different isolates of *Bd* in the presence or absence of a pesticide (the insecticide carbaryl or the fungicide copper sulfate) to recently hatched Cope's gray treefrog (*Hyla chrysoscelis*) tadpoles reared through metamorphosis. The authors found the presence or absence of pesticides differentially altered the mass at metamorphosis of tadpoles exposed to different *Bd* isolates, suggesting that isolate could influence metamorphosis but not in ways expected based on origin of the isolate. Pesticide exposure had the strongest impact on metamorphosis of all treatment combinations. Whereas copper sulfate exposure reduced the length of the larval period, carbaryl exposure had apparent positive effects by increasing mass at metamorphosis and lengthening larval period, which adds to evidence that carbaryl can stimulate development in counterintuitive ways. The present study provides limited support to the hypothesis that pesticides can alter the response of tadpoles to isolates of *Bd* and that the insecticide carbaryl can alter developmental decisions.

K. M. Gaietto, S. L. Rumschlag, M. D. Boone, *Environ. Toxicol. Chem.* **33**:2358–2362 (2014).



Chorus frog (*Pseudacris maculata*) in an Iowa, USA wetland.

Photo: Rebecca Reeves, Iowa State University.

Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by agriculture

Kelly L. Smalling, Rebecca Reeves, Erin Muths, Mark Vandever, William A. Battaglin, Michelle L. Hladik & Clay L. Pierce

Habitat loss and exposure to pesticides are likely factors contributing to amphibian declines in agricultural landscapes. Conservation efforts have attempted to restore wetlands lost through landscape modifications. These efforts are designed to reduce contaminant loads in surface waters and provide quality habitats to wildlife. For amphibians, the benefits of this increased wetland area may be negated if habitat quality is insufficient to support persistent breeding populations. Scientists with the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI)

examined the presence of pesticides and nutrients in water and sediment as indicators of habitat quality and assessed the bioaccumulation of pesticides in the tissue of two resident amphibian species *Pseudacris maculata* (chorus frogs) and *Lithobates pipiens* (leopard frogs) at six wetlands (three restored and three reference) in Iowa, USA. The restored wetlands were positioned on the landscape to receive subsurface tile drainage water while the reference wetlands receive water from overland run-off and shallow groundwater sources. Results from this study indicated that the concentrations of the pesticides observed in water and sediment were not different between wetland types. The median concentration of atrazine in surface water (0.2 µg/L) was detected at similar levels where other studies have reported reproductive abnormalities in field caught leopard frogs. Concentrations of nutrients (i.e., nitrate) were higher in the restored wetlands but lower than concentrations considered lethal to frogs. Complex mixtures of pesticides including up to eight fungicides, some previously unreported in tissue, were detected with concentrations ranging from 0.08 to 1,500 µg/kg wet weight. Although not significant, pesticide concentrations tended to be higher in leopard frogs compared to chorus frogs which were hypothesized to be due to differences in life histories. Leopard frogs may be more susceptible to pesticides since they tend to overwinter in shallow wetland sediment deep enough to avoid freezing, whereas chorus frogs prefer logs and burrows away from the wetland. Our results provide information on habitat quality in restored wetlands that will assist state and federal agencies, landowners and resource managers in identifying and implementing conservation and management actions for these and similar wetlands in agriculturally dominated landscapes.

Smalling et al., *Sci. Total Environ.* **502**: 80 (2015).

Detection of ranavirus in archival specimens

Roberta Muehlheim

This paper documents the earliest published record of ranavirus infection in wild Marbled salamanders in Ohio. This is an important record because, not only does it document ranavirus infection a decade before the first published report, it does so using viral DNA recovered from archival specimens.

The salamanders used in this study were raised from the eggs laid in the wild by a Marbled salamander (*Ambystoma opacum*) in 1999. Larvae were housed communally until transformation when they were separated and housed in pairs at different locations

isolated from other animals. Animals developed normally and appeared healthy and robust until approximately two years of age when disease symptoms manifested. Symptom onset was consistent among animals as were the disease features (i.e., lethargy, cessation of appetite, accelerated/excessive skin shedding, skin ulcerations and necrosis of the tissue surrounding digits that resulted in bone exposure). Illness progressed rapidly and ended in death between 7 and 12 days from the onset of symptoms.

The development of new techniques for extracting genetic information from formalin-fixed specimens initiated the testing of the specimens for ranavirus infection in 2011. Tissue samples from the liver, pancreas and kidneys were collected and tested for ranavirus and chytrid fungus, *Batrachochytrium dendrobatidis*. All animals tested positive for ranavirus and negative for chytrid fungus.

R. Muehlheim, *Herp. Rev.* 45: 441 (2014).



Sampling locations for *Batrachochytrium dendrobatidis* (Bd) in temperate North America with results of Bd detections from water samples. doi:10.1371/journal.pone.0106790.g001

Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America

Tara Chestnut, Chauncey W. Anderson, Radu Popa, Andrew R. Blaustein, Mary Voytek, Deanna H. Olson & Julie Kirshtein

Biodiversity losses are occurring worldwide due to a combination of stressors. For example, by one estimate, 40% of amphibian species are vulnerable to extinction, and disease is one threat to amphibian populations. The emerging infectious disease chytridiomycosis, caused by the aquatic fungus *Batrachochytrium dendrobatidis* (Bd), is a contributor to amphibian declines worldwide. Bd research has focused on the dynamics of the pathogen in its amphibian hosts, with little emphasis on investigating the dynamics of free-living Bd. Therefore, we investigated patterns of Bd occupancy and density in amphibian habitats using occupancy models, powerful tools for estimating site occupancy and

detection probability. Occupancy models have been used to investigate diseases where the focus was on pathogen occurrence in the host. We applied occupancy models to investigate free-living Bd in North American surface waters to determine Bd seasonality, relationships between Bd site occupancy and habitat attributes, and probability of detection from water samples as a function of the number of samples, sample volume, and water quality. We also report on the temporal patterns of Bd density from a 4-year case study of a Bd-positive wetland. We provide evidence that Bd occurs in the environment year-round. Bd exhibited temporal and spatial heterogeneity in density, but did not exhibit seasonality in occupancy. Bd was detected in all months, typically at less than 100 zoospores L⁻¹. The highest density observed was ~3 million zoospores L⁻¹. We detected Bd in 47% of sites sampled, but estimated that Bd occupied 61% of sites, highlighting the importance of accounting for imperfect detection. When Bd was present, there was a 95% chance of detecting it with four samples of 600 ml of water or five samples of 60 mL. Our findings provide important baseline information to advance the study of Bd disease ecology, and advance our understanding of amphibian exposure to free-living Bd in aquatic habitats over time. Importantly, these tools are not limited to only studying Bd. These same methods can be modified to quickly and affordably to provide early detection for other diseases, such as the recently described *B. salamandrivorans*, and to study other aquatic diseases that pose risks to the health of wildlife and humans alike.

T. Chestnut, C. Anderson C, R. Popa, A. R. Blaustein, M. Voytek, et al., *PLoS ONE* 9: 9 (2014).



Endemic Beysehir frogs (*Pelophylax caralitanus*). Photo: U.C. Erismis.

Survey of Turkey's endemic amphibians for chytrid fungus *Batrachochytrium dendrobatidis*

Ugur Cengiz Erismis, Muhsin Konuk, Taner Yoldas, Pinar Agyar, Dilay Yumuk & Safiye Elif Korcan

Chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), is an emerging infectious amphibian disease that has been associated with population declines in multiple regions worldwide. Both natural and anthropogenic causes can affect declines via emergence of Bd in amphibian populations. Of the

26 amphibian species found in Turkey, many are listed in the IUCN Red List (www.iucnredlist.org). Many of the causes of amphibian declines are still poorly understood in Turkey. Therefore, both endemic and non-endemic frog species need to be screened for Bd infection. The aim of our study was to screen for Bd in 2 different geographical regions of western Turkey. We report a new survey for Bd in Turkey. We swabbed 228 individuals of seven amphibian species (from five families) living in two locations (26-August National Park and the Turkish Lakes District) in the southwestern Anatolian region. The infection intensity of all the samples was determined using quantitative PCR. All four amphibian breeding sites and four amphibian species in 26-August National Park were infected by Bd, with the pre-valence at each site ranging from 8 to 29%. Only one species was sampled from Beysehir Lake near the conservation area Beysehir Natural Park, but these samples were notable for their high detection rates (prevalence of 32.11%). This study reports the first records of Bd infecting wild *Pelophylax ridibundus*, *Hyla orientalis*, *Pseudepidalea variabilis* and endemic Beysehir frogs *Pelophylax caralitanus*.

U. C. Erismis et al., *Diseases of Aquatic Organisms*, 111: 153–157 (2014).

Extremely low prevalence of *Batrachochytrium dendrobatidis* infection in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in southwest Virginia, USA

Evan A. Es skew, Brian D. Todd & William A. Hopkins

The Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a unique member of North America's salamander fauna and a focus of increasing conservation concern. Causes for population declines occurring across much of the species range are unclear, but studies have documented *Batrachochytrium dendrobatidis* (Bd) infection in both captive and wild hellbender populations. Although wild hellbenders do not seem to typically manifest clinical disease symptoms resulting from Bd infection (chytridiomycosis) or suffer disease-related mortality, there are emerging reports of hellbender mortality associated with chytridiomycosis. Bd infection might also negatively affect hellbender populations by allowing for detrimental co-infections or through interactions with other stressors. To address general deficiencies in our knowledge of Bd infection prevalence in Eastern hellbender populations, during 2011 and 2012, we sampled 14 different stream reaches across a 57 km expanse of a single stream within the Tennessee River Basin of

southwest Virginia, USA where hellbenders are known to be relatively abundant. A total of 230 unique individual hellbenders, 113 from 2011 (76 adults, 37 juveniles) and 117 from 2012 (103 adults, 14 juveniles), were captured and swabbed for *Bd* infection. Only a single hellbender collected in 2011 tested positive for *Bd*, resulting in a yearly prevalence of 0.88%. All hellbenders sampled in 2012 tested negative for *Bd* infection. Thus, our study suggests an extremely low prevalence of *Bd* infection within this Eastern hellbender population, which precludes us from making any strong general inferences about factors that might influence *Bd* prevalence. However, other researchers have hypothesized that their findings of low *Bd* prevalence in North American stream-associated amphibians might be driven by seasonally cold in-stream temperatures that limit *Bd* growth, and the low *Bd* prevalence in our study could partially reflect an inability of *Bd* to thrive in areas that experience suboptimal, cold winter temperatures. It remains unclear whether *Bd* infections regularly result in chytridiomycosis in wild hellbenders to a degree that negatively affects hellbender health, reproduction and survival. Broader spatial and temporal monitoring is needed to fully assess the risk that *Bd* poses to wild hellbender populations and to facilitate the early detection and mitigation of any chytridiomycosis threats to this unique freshwater amphibian, including the potential introduction of the recently described pathogenic fungus, *Batrachochytrium salamandrivorans* (*Bs*).

E. A. Eskew, B. D. Todd, W. A. Hopkins, *Herpetol. Rev.* 45: 425 (2014).



Photo: Tariq Stark.

Muerte en las nubes: Ranavirus asociado a mortalidad en el ensamblaje de anfibios del bosque nuboso de Nicaragua

Tariq Stark, Carlijn Laurijssens, Martijn Weterings, Annemarieke Spitzen-van der Sluijs, An Martel & Frank Pasmans

Las enfermedades de anfibios se conocen como contribuyentes importantes en el declive y extinción de especies de anfibios. Los principales culpables considerados actualmente son quitridiomycosis y Ranavirus. En América Central, especies altamente endémicas y restringidas geográficamente podrían estar en riesgo por esos males. Colectamos 49 larvas de

Agalychnis callidrias, una de *Lithobates forreri* y cinco larvas sin identificar en la Isla Ometepe de Nicaragua, todas muertas, y muestras de piel fueron tomadas. La presencia de Ranavirus fue determinada usando PCR. Ranavirus se encontró en 41 de 55 renacuajos. Cuarenta y un *Agalychnis callidrias*, una *Lithobates forreri* y otras cinco larvas sin identificar.

T. Stark, C. Laurijssens, M. Weterings, A. Spitzen-van der Sluijs, A. Martel, F. Pasmans. *Acta Herpetol.* 9: 125–127 (2014).

The effects of the amphibian chytrid fungus exposure to American toads in the presence of an insecticide

Rayona S. Wise, Samantha L. Rumschlag & Michelle D. Boone

Abiotic factors such as pesticides may alter the impact of a pathogen on hosts, which could have implications for host–pathogen interactions and may explain variation in disease outbreaks in nature. In the present laboratory experiment, American toad (*Anaxyrus americanus*) metamorphs were exposed to the amphibian chytrid fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and environmentally relevant concentrations of the insecticide malathion to determine whether malathion altered the effects of *Bd* exposure on growth and survival of toad metamorphs. Exposure to *Bd* significantly decreased survival over the 51 d of the experiment, suggesting that *Bd* could reduce recruitment into the terrestrial life stage when exposure occurs at metamorphosis. Malathion did not impact survival, but a 12 hr exposure at metamorphosis significantly reduced terrestrial growth. Toads that were exposed to both *Bd* and malathion showed a nonsignificant trend toward the smallest growth compared with other treatments. The present study suggests that *Bd* may pose a threat to American toads even though population declines have not been observed for this species; in addition, the presence of both the insecticide malathion and *Bd* could reduce terrestrial growth, which could have implications for lifetime fitness and suggests that environmental factors could play a role in pathogen impacts in nature.

R.S. Wise, S.L. Rumschlag, M.D. Boone, *Environ. Toxicol. Chem.* 33: 2541–2544 (2014).

Detection of the emerging amphibian pathogens *Batrachochytrium dendrobatidis* and ranavirus in Russia

Andrey N. Reshetnikov, Tara Chestnut, Jesse L. Brunner, Kaylene Charles, Emily E. Nebergall & Deanna H. Olson

In a population of the European common toad *Bufo bufo* from a rural pond in the region of Lake Glubokoe Regional Reserve in Moscow province, Russia, unexplained

mass mortality events involving larvae and metamorphs have been observed over a monitoring period of >20 yr. We tested toads from this and a nearby site for the emerging amphibian pathogens *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus (*Rv*). Both pathogens were detected, and at the rural pond site, with the above-noted losses and decline in toad breeding success, 40% of *B. bufo* metamorphs were *Bd* positive, 46% were *Rv* positive and 20% were co-infected with both pathogens. Toad metamorphs from a neighbouring water body were also *Bd* and *Rv* positive (25 and 55%, respectively). This is the first confirmation of these pathogens in Russia. Questions remain as to the origins of these pathogens in Russia and their roles in documented mass mortality events.

A. R. Reshetnikov, T. Chestnut, J. L. Brunner, K. Charles, E. E. Nebergall, et al. *Dis. Aquat. Organ.* 110: 235–240. (2014)..



Green-eyed treefrog (*Litoria serrata*). Photo: Elizabeth Roznik.

Host-specific thermal profiles affect fitness of a widespread pathogen

Lisa A. Stevenson, Elizabeth A. Roznik, Ross A. Alford & David A. Pike

Host behavior can interact with environmental context to influence outcomes of pathogen exposure and the impact of disease on species and populations. Determining whether the thermal behaviors of individual species influence susceptibility to disease can help enhance our ability to explain and predict how and when disease outbreaks are likely to occur. The widespread disease chytridiomycosis (caused by the fungal pathogen *Batrachochytrium dendrobatidis*, *Bd*) often has species-specific impacts on amphibian communities; some host species are asymptomatic, whereas others experience mass mortalities and population extirpation. We determined whether the average natural thermal regimes experienced by sympatric frog species in nature, in and of themselves, can account for differences in vulnerability to disease. We did this by growing *Bd* under temperatures mimicking those experienced by frogs in the wild. At low and high elevations, the rainforest frogs *Litoria nannotis*, *L. rheocola*, and *L. serrata* maintained mean

thermal regimes within the optimal range for pathogen growth (15–25 °C). Thermal regimes for *L. serrata*, which has recovered from *Bd*-related declines, resulted in slower pathogen growth than the cooler and less variable thermal regimes for the other two species, which have experienced more long-lasting declines. For *L. rheocola* and *L. serrata*, pathogen growth was faster in thermal regimes corresponding to high elevations than in those corresponding to low elevations, where temperatures were warmer. For *L. nannotis*, which prefers moist and thermally stable microenvironments, pathogen growth was fastest for low-elevation thermal regimes. All of the thermal regimes we tested resulted in pathogen growth rates equivalent to, or significantly faster than, rates expected from constant-temperature experiments. The effects of host body temperature on *Bd* can explain many of the broad ecological patterns of population declines in our focal species, via direct effects on pathogen fitness. Understanding the functional response of pathogens to conditions experienced by the host is important for determining the ecological drivers of disease outbreaks.

L. A. Stevenson *et al.* *Ecol. Evol.* 4: 4053–4064 (2014). <http://onlinelibrary.wiley.com/doi/10.1002/ece3.1271/abstract>

The effects of the amphibian chytrid fungus, insecticide exposure, and temperature on larval anuran development and survival

Samantha L. Rumschlag, Michelle D. Boone & Gary M. Fellers

Chytridiomycosis, a disease caused by *Batrachochytrium dendrobatidis* (*Bd*), has been implicated as a cause of amphibian declines. Susceptibility may be influenced by environmental factors that suppress the immune response. The authors conducted a laboratory study to examine the effect of temperature, insecticide exposure, and *Bd* exposure during larval anuran development. The authors examined the consequences of exposure to *Bd*, an insecticide (carbaryl or malathion) and static or fluctuating temperature (15 °C, 20 °C, 25 °C, or 15 °C to 25 °C 72 hr flux) on larval development through metamorphosis of the Pacific treefrog (*Pseudacris regilla*). High and fluctuating temperature had negative effects on survival in the presence of *Bd*. Insecticides inhibited the effects of *Bd*; time to tail resorption of Pacific treefrogs decreased when tadpoles were exposed to carbaryl. The present study indicates that abiotic factors may play a role in the host-pathogen interactions in this system.

S. L. Rumschlag, M. D. Boone, G. M. Fellers, *Environ. Toxicol. Chem.* 33:2545–2550 (2014).



Amphibian communities threatened by emerging lineage of *Ranavirus*. Photo: S. J. Price.

Collapse of amphibian communities due to an introduced *Ranavirus*

Stephen J. Price, Trenton W.J. Garner, Richard A. Nichols, François Balloux, César Ayres, Amparo Mora-Cabello de Alba & Jaime Bosch

The emergence of infectious diseases with a broad host range can have a dramatic impact on entire communities and has become one of the main threats to biodiversity. Here, we report the simultaneous exploitation of entire communities of potential hosts with associated severe declines following invasion by a novel viral pathogen. We found two phylogenetically related, highly virulent viruses (genus *Ranavirus*, family Iridoviridae) causing mass mortality in multiple, diverse amphibian hosts in northern Spain, as well as a third, relatively avirulent virus. We document host declines in multiple species at multiple sites in the region. Our work reveals a group of pathogens that seem to have preexisting capacity to infect and evade immunity in multiple diverse and novel hosts and that are exerting massive impacts on host communities. This report provides an exceptional record of host population trends being tracked in real time following emergence of a wildlife disease and a striking example of a novel, generalist pathogen repeatedly crossing the species barrier with catastrophic consequences at the level of host communities.

S. J. Price, T. W. Garner, R. A. Nichols, F. Balloux, C. Ayres, *et al.* *Current Biology* 24: 2586–2591 (2014).



Infected individual of Browns' leopard frog (*Lithobates brownorum*) for which *Bd* had not been previously confirmed in previous researches. Photo: Roberto García-Roa.

First record of *Batrachochytrium dendrobatidis* in Nicaragua

Roberto García-Roa, Javier Sunyer, Andrés Fernández-Loras & Jaime Bosch

The infectious disease chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is considered one of the main culprits causing major amphibian declines and extinctions worldwide. It is known to occur on every continent except Antarctica and is particularly damaging to amphibian tropical populations. We studied 18 different amphibian species from three different localities in Nicaragua. Our results confirm the presence of *Bd* for first time in Nicaragua and involve ten amphibian species corresponding to Bufonidae, Craugastoridae, Hylidae and Ranidae. We additionally record *Bd* for the first time in four amphibian species: *Craugastor lauraster*, *Dendropsophus microcephalus*, *Smilisca baudinii* and *Lithobates brownorum*.

R. García-Roa, J. Sunyer, A. Fernández-Loras, J. Bosch, *Herpetol. J.* 24: 65–68 (2014).

Call for recent publication abstracts

If you would like to include an abstract summary from a recent publication in this section of *FrogLog* (FL) please follow proper formatting conventions and style for your submission (e.g., note the most recent issue of FL available) email: froglog@amphibians.org. We also encourage all authors of recent publications to inform Professor Tim Halliday (formerly DAPTF International Director) (tim.r.halliday@gmail.com) of their publication in order for it to be referenced on the AmphibiaWeb latest papers page. The full list of latest papers from AmphibiaWeb is also included in every edition of *FrogLog* following the recent publications abstract section.

General Announcements

Events

The following information can be found at:
<http://www.amphibians.org/meetings>

March 2014

30 – April 2 The Association for Tropical Biology and Conservation – Asian Chapter, Phnom Penh

April 2014

11–12 Amphibian Conservation Research Symposium 2015, University of Cambridge

May 2014

30 May–1 June The Third International Symposium on Ranaviruses, University of Florida in Gainesville, FL, USA

July 2014

13–16 The Association for Tropical Biology and Conservation – International, Honolulu, Hawaii

August 2014

2–6 The Society for Conservation Biology (and the International Congress for Conservation Biology), Montepelier, France

September 2014

7–12 Brazilian Herpetological Congress 2015, Fundacao de apoio da Universidade do Rio Grande Do Sul

Internships & Employment

Native and Invasive Reptile Data Management Intern (Multiple)

Department of Wildlife Ecology and Conservation at the University of Florida Fort Lauderdale Research and Education Center, Fort Lauderdale, FL (Posted to PARC 01/08/15, Deadline for applications is 6 weeks before corresponding start date (Multiple))

Rock Iguana Volunteer Field Assistants

Hispaniola (Posted to PARC 12/26/14, Open Until Filled)

Human Dimensions of Fisheries and Wildlife Assistant Professor, Tenure Track

Corvallis, OR (Posted to PARC 12/26/14, Closing 01/15/15)

M.S. Research Assistantship - Amphibian and Reptile Conservation

Clarksville, TN (Posted to PARC 12/17/14, Closing 02/09/15)

Amphibian Research Technicians, Student

Laurel, MD (Posted to PARC 12/17/14, Closing 01/31/15)

Post-doctoral fellowship, Atlantic Forest Phylogeny

Sao Paulo, Brazil (Posted to PARC 12/17/14, Closing 01/18/15 or until filled)

Amphibian and Reptile Monitoring Internship (With Stipend)

American Conservation Experience, Everglades National Park, FL (Posted to PARC 12/09/14, Anticipated Start Date 01/19/15)

M.S. Research Assistantship

Austin Peay State University, Clarksville, TN (Posted to PARC 12/05/14, Closing 01/15/15)

Full-Time Amphibian Department Zookeeper

Detroit, MI (Posted to PARC 12/02/14, No Closing Date Provided)

OPS F&W Biological Scientist II

Gainesville, FL (Posted to PARC 12/02/14, Closing 12/02/14)

MS Graduate Opportunity: SUNY-ESF

Syracuse, NY (Posted to PARC 11/17/14, 12/15/14)

Chief of the Grand Canyon Monitoring and Research Center

Flagstaff, AZ (Posted to PARC 11/17/14, 12/3/14)

Graduate Assistantship on Bog Turtle Conservation Planning, Virginia Tech Department of Fisheries and Wildlife Sciences

Blacksburg, VA (Posted to PARC 11/10/14, No Closing Date Provided)

Ph.D. graduate assistantship available in population dynamics, ecology, and behavior of amphibians in a fire-dependent system

Blacksburg, VA (Posted to PARC 11/10/14, No Closing Date Provided)

Research Manager, Davidson College Herpetology Laboratory

Davidson, NC (Posted to PARC 11/10/14, Open Until Filled)

Zilla Marketing Brand Manager

Franklin, WI (Posted to PARC 11/04/14, Open Until Filled)

Wildlife Technician, Senior

Coeur d'Alene River Wildlife Management Area (WMA) near Harrison, ID (Posted to PARC 11/04/14, Closing November 18, 2014)

Stream Ecologist, Assistant Professor, Tenure Track

Corvallis, OR (Posted to PARC 10/23/14, Closing December 3, 2014)

M.S. Assistantship, Amphibian Genetics

Western Kentucky University, Bowling Green, KY (Posted to PARC 10/03/14, Open Until Filled)

Supervisory Biologist, Patuxent Wildlife Research Center

Laurel, Maryland (Posted to PARC 10/03/14, Closing November 3, 2014)

Research Manager, Brown Treesnake Research Project

Guam, Mariana Islands (Posted to PARC 06/05/14, Open Until Filled)

Funding Opportunities

The Amphibian Survival Alliance is pleased to announce an open call for seed grant applications. Seed grants are normally provided in amounts ranging from USD \$500-\$1,000 and are designed to help kick start projects or allow teams to try new innovative approaches to address conservation, research and education challenges. [Link](#)

The Leapfrog Conservation Fund has been created specifically to support the creation of new reserves for important amphibian habitat, or the expansion of existing reserves through local organizations. If your organization is working toward the protection of critical habitat for threatened amphibian species, we would love to hear from you. [Link](#)

The following information is kindly provided by the Terra Viva Grants Directory, for more information please visit: <http://www.terravivagrants.org/>

German Academic Exchange Service (DAAD) -- Postdoctoral Researchers International Mobility Experience. DAAD is partnering with the EC's Marie Curie Program to fund the new program, Postdoctoral Researchers International Mobility Experience (PRIME), for outstanding postdoctoral researchers across all disciplines and nationalities. Funding is provided for 18 months, of which 12 months are spent abroad and 6 months at a German university. The funding consists of salaries instead of scholarships. DAAD's announcement includes a list of participating German universities. The deadline for applications is 01 February 2015. [Link](#)

Association of Avian Veterinarians (AAV) -- Research Grants 2015. The AAV makes grants for research addressing clinical aspects of exotic and wild birds -- including diagnostic tests, drug doses, practice management, and conservation. Grants are up to US\$10 thousand for individual projects of one year. There are no nationality restrictions. The deadline for pre-proposals is 01 February 2015. [Link](#)

World Wildlife Fund U.S. -- Grants for Conservation Workshops in 2015. WWF-US offers up to US\$7,500 for workshops that train communities, stakeholders, park guards, and others on conservation issues. Applying organizations must have an established presence in an eligible country (countries are listed in the announcement). The workshops should focus on practical skills and field activities to increase local learning and build capacity. The application deadlines in 2015 are 01 February; 01 May; 01 August; and 01 November. [Link](#)

Fondation Ensemble -- Sustainable Development and Conservation 2015. Fondation Ensemble supports field projects in sustainable development and conservation. The Foundation's focus areas are sustainable agriculture, sustainable fishing, biodiversity conservation, and sustainable technologies. The eligible countries for projects are Cambodia, Ecuador, Laos, Mozambique, and Peru. For projects in biodiversity conservation and sustainable fishing, activities can also be undertaken in the coastal areas of some West African countries (Mauritania, Senegal, and Gambia for year 2015). Grants are a maximum of €50 thousand per year for two to four years. Grants cannot be over 50% of a project's total budget. The deadline for concept notes is 02 February 2015. [Link](#)

Sonoran Joint Venture -- Mexico-USA Bird Conservation 2015. Sonoran Joint Venture makes grants for bird conservation and research in the border region of the southwestern USA and northwestern Mexico. The priority is for projects that focus on habitat, monitoring, or planning. Proposals should demonstrate bi-national cooperation (USA and Mexico) and a partnerships orientation. Grants are up to US\$10 thousand. The application deadline is 02 February 2015. [Link](#)

Climate Co-Lab -- Local Knowledge to Anticipate Climate Change in the Pamir Mountains. The Climate Co-Lab invites team projects that focus on "calendars of the human body" (i.e., traditional ecological knowledge related to weather and seasons) to help anticipate and respond to climate change at the scale of villages in the Pamir Mountains of the Afghan-Tajik border region. The winning team will be awarded up to US\$2 thousand to develop collaboration opportunities. The application deadline is 06 February 2015. [Link](#)

Fondation Nature & Decouvertes -- Nature Protection in France and Africa. The foundation supports projects for nature protection, education, and public awareness in France and Francophone Africa. Applications for small grants ("coup de main") from €500 to €3 thousand can be submitted throughout the year. The application deadline for major projects (from €3 thousand to €10 thousand) is 15 February. [Link](#)

World Academy of Sciences (TWAS) -- Science Prizes 2015. Each year, TWAS awards eight prizes of US\$15 thousand each to individual scientists who have been working and living in a developing country for at least 10 years. The disciplinary fields include agricultural sciences, biology, and six others. Nominations of women scientists are particularly encouraged. The deadline for nominations is 15 February 2015. [Link](#)

International Tropical Timber Organization (ITTO) -- Freezailah Fellowships, First Cycle 2015. ITTO makes grants through the Freezailah Fellowship Fund for training opportunities, demonstration tours, participation in conferences and workshops, preparation of technical papers, and post-graduate degrees. Grants up to US\$10 thousand are in support of sustainable tropical forest management. Applicants are young and mid-career professionals in ITTO's member countries; most grants are to individuals in the developing countries. The deadline for the first application cycle in 2015 is 20 February 2015. [Link](#)

Association of Commonwealth Universities -- Mobility Grants for Early-Career Academics. The Association of Commonwealth Universities offers staff at ACU member universities the opportunity to attend a relevant conference in a Commonwealth country outside their home region. The scheme is aimed at early-career academics who have not yet had the opportunity to work, study, or travel outside their own country. Grants are up to £2 thousand. The closing date for the current round of applications is 22 February 2015. [Link](#)

French Institute for Development Research (IRD) -- GUYAMAZON 2014-2015. France's IRD collaborates with CIRAD, the French Embassy in Brazil, and three Brazilian states to sponsor GUYAMAZON for collaborative Amazonian research. Research themes include biodiversity, terrestrial and forest ecosystems, aquatic ecosystems, climate and health, agro-ecology and subsistence farming, renewable energy, and social sciences. Projects link researchers in France (especially the department of French Guyana) and the Brazilian states of Amazonas, Amapá, and Maranhão. The application deadline is 28 February 2015. [Link](#)

NEPAD (African Union) -- Climate Change Fund, 2nd Call. With financial support from Germany, NEPAD announces the second call for proposals for its Climate Change Fund. Focus areas are adaptation of agriculture to climate change; biodiversity; access and benefit sharing; development and implementation support to National Adaptation Plans (NAPs); and mainstreaming of climate change into National Agricultural Investment Plans (NAIPs). The Fund supports training, capacity development, and awareness creation. It also supports policy advice and technical support. Applications are invited from African government institutions; regional economic communities; and regional and national coalitions of civil society organizations. The application deadline is 28 February 2015. [Link](#)

Rainforest Alliance -- Kleinhans Fellowship for Research in Community Forestry.

The Rainforest Alliance is interested in helping communities and small- and medium-sized enterprises to harvest and manufacture forest and non-timber forest products in a sustainable manner, and market these goods to responsible businesses and consumers. The Kleinhans Fellowship provides US\$20 thousand over two years for research on this topic in Latin America, with preference for sites where

Rainforest Alliance currently supports community forestry. Applicants must have at least a master's degree in forestry, ecology, botany, environmental science, or an appropriate related field. The deadline for applications is 28 February 2015. [Link](#)

March 2015

American Society of Mammalogy (ASM) -- Support to African Graduate Students 2015. The ASM's African Graduate Student Research Fund makes awards to African nationals pursuing graduate degrees in mammalogy. Each of two awardees will receive US\$1,500 and an online membership to the Society. The application deadline is 01 March 2015. [Link](#)

John Ball Zoological Society -- Wildlife Conservation 2015. The John Ball Zoological Society makes grants to conserve wild animals and their habitats; to improve the management of captive animals; and to develop education programs related to these objectives. Most funded projects are in developing countries. Applicants can be of any nationality, and they should be associated with a recognized zoo, educational institution, conservation organization, etc. Grants generally range from US\$500 to US\$2,500. The deadline for applications is 01 March 2015. [Link](#)

Chinese Academy of Sciences (CAS) and World Academy of Sciences (TWAS) -- PhD Studies in China. The CAS-TWAS President's PhD Fellowship Programme will support up to 200 students worldwide for PhD studies in China. The areas of concentration include agricultural sciences; biological systems; and several others. The maximum age for applicants is 35 years. Funding support is for up to four years. The application deadline is 31 March 2015. [Link](#)

April 2015

Fresno Chaffee Zoo -- Grants for Wildlife Conservation. The Fresno Chaffee Zoo Wildlife Conservation Fund makes grants for wildlife conservation and research that focuses on rare, threatened, and endangered animals and their habitats. The priority is for in situ conservation projects. Most grants range from US\$1 thousand to US\$2 thousand. The principal investigator must be associated with a recognized institution. The application period is 01 February through 01 April of each year. [Link](#)

Program for the Endorsement of Forest Certification (PEFC) -- Collaboration Fund 2015. PEFC offers competitive grants in support of its mission to improve

forest management and expand forest certification around the world. The grants program is open to PEFC's members and partner organizations, both public and private. PEFC will consider proposals up to CHF 40 thousand for projects up to two years. Grantees must provide at least 35% co-funding. The deadline for applications is 04 April 2015. [Link](#)

American Society of Mammalogy (ASM) -- Research in Latin America. The ASM offers the Latin American Student Field Research award for field projects by Latin American graduate students. Each of five grant recipients receives US\$1,500. The ASM also offers the Oliver P. Pearson Award to young professional mammalogists of any nationality who hold academic or curatorial positions in Latin America. The Pearson research grant is US\$5,000 plus a travel grant of US\$2,000 to attend the ASM annual meeting following the award. The application deadline for both types of awards is 15 April of each year. [Link](#)

Indian National Academy of Sciences -- Fellowships for Visiting Scientists from Developing Countries. India's National Science Academy administers the JRD-Tata Fellowships to support visiting scientists from the developing world and promote South-South cooperation. The program is open to applicants from developing countries (except India) younger than age 45, and who possess doctorate or masters degrees in science or equivalent degrees in engineering/medicine. Past participants have included several in agriculture, biological sciences, and geo sciences. The fellowship is for three months and includes transportation, maintenance allowance, accommodation, and contingencies. The application deadlines are 30 April and 31 October of each year. [Link](#)

Patagonia -- Environmental Grants for Native Fish Populations. Patagonia makes grants to support nonprofit organizations for campaigns to preserve and protect the environment. Patagonia's grant-making priority is protecting and restoring native fish populations and the habitat on which they depend. Eligible countries include Argentina and Chile, among others. Grants are a maximum of US\$12 thousand. Proposals are submitted through Patagonia's retail stores at any time of the year, or online before 30 April and 31 August of each year. [Link](#)

United Nations University, Institute for Natural Resources in Africa (UNU-INRA) -- Visiting Scholars Program 2015-2016. The UNU-INRA invites applications for its visiting scholars program in the theme

"Unleashing the Potential of African Rural Economies through Green Growth". Up to ten visiting scholars will be selected to spend 3-4 months carrying out research at UNU-INRA headquarters in Ghana or at one of its operating units based in Cameroon, Cote d'Ivoire, Namibia, Senegal, and Zambia. Applications are accepted from faculty members and researchers at African universities and other research institutions on the African continent, and who have a PhD degree in the relevant fields. The application deadline is 30 April 2015. [Link](#)

May 2015

IUCN-Netherlands -- Purchase of Nature 2015. The World Conservation Union in the Netherlands (IUCN-Netherlands) manages the Purchase of Nature program, funded by the Dutch Postal Code Lottery. The program provides grants of up to €85 thousand for the purchase and protection of threatened wildlife habitats and vulnerable ecosystems in Asia-Pacific countries, Africa, and Latin America. The grants are to qualified and experienced local conservation organizations. The deadline for pre-proposals is 01 May 2015. [Link](#)

Northeastern Wisconsin Zoo -- Conservation Grant Fund. The zoo's Conservation Grant Fund accepts proposals from individuals and organizations for one-year grants in conservation field projects and research. There are no restrictions by nationality or residence, and there is no minimum or maximum grant size. Most grants range from US\$250 to US\$1,000. Applications are accepted from 01 March through 01 May. [Link](#)

Trust for Mutual Understanding -- Environmental Collaboration USA with Russia, Central Asia, and Eastern and Southern Europe. The Trust for Mutual Understanding (TMU) makes grants to nonprofit organizations in the USA for environmental projects in collaboration with partners in Russia, Central Asia, and Eastern and Southern Europe. TMU supports professional exchanges and capacity building, together with jointly managed research and field projects. The deadline for the next cycle of initial inquiries is 01 May 2015. [Link](#)

EC Research and Innovation (Horizon 2020) -- Funding for International Cooperation 2015. The EC's research program Horizon 2020 includes a component of international cooperation (INCO) with key partner countries and regions. Proposals are invited from consortia consisting of balanced

partnerships between European and international partners in Australia, Brazil, China, Russia, South Africa, Ukraine, and USA. The deadline for proposals is 12 May 2015. [Link](#)

ARCOS Network -- Biodiversity

Conservation in Africa. The ARCOS Small Grants enhance collaboration to develop sustainable solutions for biodiversity and people, focusing on critical landscapes and watersheds of the Albertine Rift, the Great Lakes Region, and the African mountains. The grants program is open to civil society organizations. Governments and for-profit organizations may participate as project partners, or in coalitions where a civil society organization is the main applicant. The maximum grant is US\$5 thousand. Applications (English, French) are invited during two periods each year: 01 April to 15 May, and 01 October to 15 November. [Link](#)

African Network of Scientific and Technical Institutions (ANSTI) -- Post-Graduate Fellowships 2015. The German Academic Exchange Service (DAAD) collaborates with ANSTI by offering financial support for masters and Ph.D degrees at ANSTI's institutions in Sub-Saharan Africa. Fellowships are for studies

outside the applicants' home countries. The program is open to participants at ANSTI's member institutions who are less than 36 years old at the time of application. The application deadline is 31 May 2015. [Link](#)

Rolex SA -- Rolex Awards for Enterprise 2016. Rolex SA announces that it is accepting applications for the 2016 Rolex Awards for Enterprise. Eligibility extends to anyone over 18 years of age, of any nationality, whose exceptional project expands the knowledge of our world and improves the quality of life on our planet. Projects are grouped in science and health; exploration and discovery; environment; and cultural heritage. Projects are assessed on the basis of originality, potential for impact, feasibility, and especially spirit of enterprise. Each Laureate will receive CHF 100 thousand. Each Young Laureate (between ages 18 and 30) will receive CHF 50 thousand. The deadline for pre-applications is 31 May 2015. [Link](#)

United Nations University, Institute for Natural Resources in Africa (UNU-INRA) -- PhD Internships 2015-2016. With funding from Canada's International Development Research Centre, UNU-INRA is undertaking a three-year project

entitled "Unleashing the Potential of African Rural Economies through Green Growth". The project will select 13 PhD interns to help provide evidence on how green growth strategies can influence employment, income generation, competitiveness, and social inclusion in rural Africa. Applicants should be African nationals who are enrolled in doctoral studies, most preferably at the later stages of their PhD programs. UNU-INRA accepts both Anglophone and Francophone applicants. The application deadline is 31 May 2015. [Link](#)

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INSTRUCTIONS TO AUTHORS

Background

FrogLog has been one of the leading amphibian conservation community newsletters since the early 1990's. Over the years it has been affiliated with different groups but has always strived to help inform the community. In 2005 *FrogLog* became the official newsletter of the IUCN SSC Amphibian Specialist Group and is produced on a quarterly basis.

As the ASG's newsletter members are encouraged to contribute to *FrogLog*'s content and direction. To aid in this process each edition of *FrogLog* focuses on one of the six broad geographical zones identified by the ASG. The publication schedule is as follows:

- January—Special Topical Edition
- April—The Americas
- July—Africa, West Asia, Madagascar, Mediterranean and Europe
- October—Asia, Russia and Oceania

FrogLog invites contributions of research, reviews on current management and conservation issues, methods or techniques papers and, editorials. We also actively encourage submissions describing the current activities relating to projects and academic institutions in order to help inform the community as to the general state of current research and conservation activities.

PUBLICATION

FrogLog is published online at: www.amphibians.org and is Open Access.

REVIEW

All contributions should ideally be channeled through Regional ASG Chairs, the details for which can be found at <http://www.amphibians.org/asg/members/>. If for some reason this cannot be done, contributions will be reviewed by at least one individual within the ASG. *FrogLog* is not a peer-reviewed publication and the onus for submitting accurate information remains with the authors.

PRODUCTION EDITOR

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EDITORIAL COMMITTEE

Candace M. Hansen-Hendrikk
Craig Hassapakis
Lindsay Renick Mayer

Additional reviewers will be requested as required.

SUBMISSION OF MANUSCRIPTS

Manuscripts can only be received as electronic files. Text should be submitted in MS Word format and may contain tables, but figures should be sent as a separate attachment where possible. All documents should be sent to froglog@amphibians.org. Each file should be labeled in a style that illustrates clear association, i.e., authors_name_ms and authors_name_figure1.

GUIDELINES FOR AUTHORS

All manuscripts must be written in Standard US English. For example, "colour" should be spelled "color."

TITLE

Titles should ideally be no more than 15 words.

AUTHORS

Authors names should be written in full as follows: By James P. Lewis & Robin D. Moore

MAIN BODY OF TEXT

Use Georgia 11-point font. Genus and species names should be in italics as should the abbreviation for *Batrachochytrium dendrobatidis*, *Bd*. Suggested headings include Acknowledgements, Author Details and References and Notes.

AUTHOR DETAILS

Author details may be provided, including affiliations and contact details.

FIGURES

Figures should be numbered and include brief, concise legends. Where photographs or illustrations are used please state whom the image should be credited to, e.g., Photo: James P. Lewis. Graphics should preferably be submitted in tiff or jpeg format in the highest possible quality. Resolution should be at least 300 dpi at the final size.

TABLES

Tables may be included within the text file and should be numbered and include brief, precise legends.

CITATION OF LITERATURE

FrogLog uses a numbering system for references and notes. This allows explanatory or more detailed notes to be included with the references. Journal names are abbreviated using common abbreviations to save space.

Journals/Periodicals

1. E. Recuero, J. Cruzado-Cortés, G. Parra-Olea, K. R. Zamundio, *Ann. Zool. Fenn.* 47, 223 (2010).

Books

2. J. Gupta, N. van der Grijp, Eds., *Mainstreaming Climate Change in Development Cooperation* (Cambridge Univ. Press, Cambridge, UK, 2010).

Technical reports

3. G.B. Shaw, *Practical uses of litmus paper in Möbius strips* (Tech. Rep. CUCS-29-82, Columbia Univ., New York, 1982).

Paper presented at a meeting

4. M. Konishi, paper presented at the 14th Annual Meeting of the Society for Neuroscience, Anaheim, CA, 10 October 1984.

Published Online Only

5. N. H. Sleep, *Geochem. Geophys. Geosyst.*, 10, Q11010 (2009); DOI:10.1029/2009GC002702.

Web site

6. National Oceanic and Atmospheric Administration, Beaufort Wind Scale, <http://www.spc.noaa.gov/faq/tornado/beaufort.html> (2012).

SPECIAL NOTE: Use only one space after all punctuation marks (this includes only one space after "periods" at the end of sentences).

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April 2015

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