

froglog

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Conservation news for the herpetological community

Volume 21, number 1

RECONNECTING WITH NATURE IN HAITI

*Photography inspired me to....
protect frogs*

Shedeline Louigene, age 15

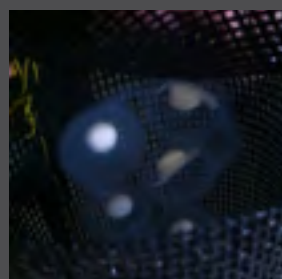


Education Special Edition

Photo: Robin Moore/iLCP.



**Latest News from
the ASA**



**Novel
Biotechnologies
Prove Winners in
Giant Salamander
Conservation**

froglog

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Editorial

Welcome to the first feature edition of *FrogLog*. With this issue, we wanted to highlight some of the amazing ways in which people are helping to spread the word about amphibian conservation and show what they are doing to assist with conservation efforts on the ground.

The success stories and the whole *feel-good factor* need greater exposure in the media. It's about giving the frogs and newts a leading role. It's about giving the people behind the conservation success stories center stage as well. Because there are a lot of success stories—we're just not hearing about them. And this is exactly what this edition is about.

Once people have been inspired, they will then want to do what they can do to help amphibians. This might just prove to be the most powerful conservation tool at our disposal. The organizations featured in this edition are doing just that and it is our honor and pleasure to share their work with our readers.

In this edition you will read many inspiring stories including an article from Avalon Theisen, an 11 year old hero of amphibians, who started her own charity to help raise awareness of amphibian conservation issues. There are a number of articles that look at new and innovative ways of engaging the wider community including articles from ARKive, Meet Your Neighbours and ASG's own Robin Moore who recently co-founded Frame of Mind, an initiative connecting people with their natural and cultural worlds through photography and visual storytelling. You will also see a few articles focused on the use of frogs in research and education.

As always we have also included a range of other updates from the Amphibian Conservation Community, including an article from the ASA announcing their new Executive Director, a call for submissions to *Alytes*, the latest news of the ASG/ARMI Seed Grant winners of 2012 and much, much more.

As the new Program Officer with the Amphibian Specialist Group, it is an honor for me to be writing the editorial for this edition of *FrogLog*. I bring to the ASG a bit of a different background than some might expect. I am not a biologist—I hold degrees in Geography and GIS. But having grown up in West Africa, Pakistan and India, as well as travelling throughout the world, I bring a lifelong passion for amphibians, creatures typically overlooked in mainstream media, to the ASG. I have taken every opportunity in life to inspire people to protect amphibians both in and beyond their own backyards. With this background, I look forward to assisting with the identification, development and management of communication strategies and media projects designed to build understanding and support for the ASG's strategic priorities.

We hope that stories and features in this edition will inspire and motivate you in one way or another. Keep us posted on what you are doing and let us know how we can help you get the word out.

Candace M. Hansen
ASG Program Officer



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ASA Partners with GWC to Drive Forward Global Collaborative Efforts to Save Amphibians

As co-chairs of the Amphibian Survival Alliance (ASA) board, we wanted to wish all of you a prosperous new year, especially with respect to amphibian conservation. The ASA has accomplished some significant activities in the past two years since it was launched under the leadership of Jaime Garcia-Moreno and Phil Bishop. The ASA was present at key International Meetings including last year's American Society of Ichthyologist and Herpetologist meeting and this year's World Congress of Herpetology as well as the World Conservation Congress in Jeju, where ASA was co-hosting a workshop on amphibian conservation—the only amphibian-focused event of the entire World Congress. Not only were these venues where we presented ASA broadly, but each was an excellent opportunity to network and to meet people and new potential partners interested in amphibian conservation. We have also established new alliances with two groups that are very relevant in advancing ASA's work. We signed a MoU with the Alliance for Zero Extinction (AZE)—a key group with which we expect to expand collaborations given the large number of amphibians that trigger the AZE criteria. We also managed to get ASA fully integrated into the IUCN SSC Freshwater Subcommittee. This should allow us to build bridges and work towards amphibian conservation from a freshwater perspective, and also to present amphibians as a group of organisms that help to integrate efforts of land and water resource management. Also, previous to the World Conservation Congress in Jeju, the ASA played an important role in getting a coalition of 15 institutions to sponsor a motion on amphibian conservation—which includes a request for support of the ASA's core activities; the motion was approved with proposed amendments to include attention to amphibian trade. Finally, we also successfully organized an event at the EU Parliament to call for attention on the amphibian crisis and to request the labeling of the chytrid fungus as an invasive species (to keep a handle on the European Biodiversity Strategy).

The future looks brighter for amphibians. Among ASA's priorities for 2013, we will focus on securing key amphibian habitats, the exploration and research of new species and their status, the establish-

ment and management of captive breeding programs and the development of a set of indicators to monitor the state of amphibians, inclusive of the IUCN Red List. As we move forward in our efforts to scale up our conservation impact and reach through the ASA, and building on one of the main recommendations of the recent board meeting to “refresh” and “rebrand” the ASA concept and structure, we are also pleased to announce a major new partnership for 2013. Global Wildlife Conservation and its president Don Church will bring some new blood to ASA and help in this critically important endeavor to save amphibians from extinction. Don Church and Global Wildlife Conservation have agreed to become major new partners of ASA, and to have Don Church take a new lead role within ASA. We feel that this allow us to rethink and update our ASA concept and structure, and Don will lead this discussion with Jaime and the rest of the ASA staff. Don started his new role on January 1st and took over from Jaime as ASA Executive Director at that point. We are also pleased to report that Jaime will remain with the ASA as Director of Conservation Programs. Don will engage with board members and staff and as we develop a new “refreshed” and “rebranded” ASA concept and structure. We are excited about this opportunity and are confident that this will continue to build on the past year's success. Please help us in welcoming Don Church and GWC to the ASA family.



Don Church president of Global Wildlife Conservation joins the ASA team as the new ASA Executive Director.

*Lena Linden and Claude Gascon
Co-Chairs of the Amphibian Survival Alliance*

Alytes: A Call for an Increased Batrachology Forum

By Gonçalo M. Rosa, Ché Weldon & Franco Andreone

The International Society for the Study and Conservation of Amphibians (ISSCA) is the only scientific society entirely devoted to the amphibians, and *Alytes* is the only scientific journal that exclusively publishes amphibian related manuscripts. An increase in the number of journals dealing with several aspects of biology and conservation (including that of amphibians) has an analogous effect of augmented competition for contributions.

In order to remain a player in this competitive arena, ISSCA recently underwent a metamorphosis of its board members as well as the whole editorial board of *Alytes*. This new team is seeking innovative approaches in terms of publishing format, access to

journal contents as well as appeal and visibility. Ultimately this will result in a new platform offering easy online submission and facilitating access to a data base comprising all published issues since the launch of the journal in February 1982. The journal is currently facing a process of accreditation as the first step towards attaining an Impact Factor, and is therefore investing in a faster revision and publication of manuscripts.

Given this context, *Alytes* should continue to be an outlet that publishes papers on all aspects of amphibian biology, but with increased focus on conservation and ecology. Concurrently, the journal will retain a sound tradition regarding taxonomic aspects. These aspects

have always been a core part of *Alytes*' rich heritage, and are becoming increasingly more important in the framework of biodiversity assessments. Moreover, *Alytes* always served as a forum for expressing new and original ideas and concepts. *Alytes* is therefore a unique journal with an originality that needs to be strongly safeguarded. As *Alytes* continues to evolve it should establish itself as a first option for publication in the field of batrachology.

In addition, the logistic and network strengths of the ISSCA and ASG offer unique opportunities for synergy that can accelerate research and conservation efforts focused on amphibians. Both platforms support a network of some 600+ amphibian experts across 40+ regions and countries and have access to a wide range of academic institutions and NGOs.

We are in the process of preparing special issues with thematic subjects. This has already been done for the special issue on conservation (see *FrogLog* 104) and with your collaboration (scientific community) this initiative intends to produce further important issues that could become landmarks for amphibian biology. Thus, we hereby invite further contributions that combine conservation with traditional fields of interest in order to increase the productivity and quality of the journal.

For more information about *Alytes*, please contact the ISSCA president, Franco Andreone at franco.andreone@gmail.com; for submitting manuscripts, please contact Gonçalo M. Rosa at goncalo.m.rosa@gmail.com (editor-in-chief) or Ché Weldon at che.weldon@nwu.ac.za (deputy editor).

The Amphibian Red List Authority is Re-structuring

By Ariadne Angulo

The International Union for Conservation of Nature (IUCN), of which the Amphibian Specialist Group (ASG) is a part of [through the Species Survival Commission (SSC)], held its quadrennial World Conservation Congress (WCC) in September 2012. Among the many WCC activities undertaken are also elections for key positions within the IUCN structure, including that of President, Treasurer, Commission Chairs and Regional Councilors. Dr Simon Stuart, the 2008-2012 Species Survival Commission (SSC) Chair, has been re-elected for the next IUCN quadrennium (2013-2016). The beginning of a new quadrennium gives Commissions the opportunity to review and modify their structure, processes and activities and adjust them as needed to ensure that the Commission's objectives are being addressed.

In this context, Specialist Groups (SGs) are also renewed, as are Red List Authorities (RLAs). The Amphibian Red List Authority (RLA), the group appointed by the SSC Chair to oversee maintenance of the IUCN Red List amphibian assessment database (formerly the Global Amphibian Assessment, <http://www.iucnredlist.org/initiatives/amphibians>), is also a part of this renewal process, and we are very excited about the changes planned for 2013-2016. Updating the Amphibians on the IUCN Red List database has been

challenging given changing priorities and shrinking resources in an uncertain global economic climate. This has prompted us to revisit our previous quadrennial strategy, and explore ways to maintain the database and at the same time de-centralize the process and strengthen capacity across regions and countries. We are now aiming to establish regional and national working groups who will be in a position to take stewardship of their respective regional/national assessments. We are currently in the process of identifying experts who are willing to act as focal points, establish and provide direction to working groups, and will be announcing a first list of regional/national working group focal points/Tier I RLAs through the ASG's different social media and through the renewed website. In the meantime, please do get in touch (ariadne.angulo@iucn.org) if you wish to become more involved in the assessment process for your region or country. The existence of the impressive resource that is the Amphibians on the IUCN Red List database is largely due to the collective knowledge and generosity of our amazing global herpetological and conservation communities, but its continued survival depends on these communities now more than ever. We hope that you will join us in our global effort of keeping the amphibian conservation database current and providing the most updated information to better guide conservation action.

AMPHIBIAN RED LIST AUTHORITY CALL FOR INTERNS FOR FIRST TERM OF 2013

The Amphibian Red List Authority is currently seeking three volunteer interns to work on different aspects pertaining to the IUCN Red List amphibian assessment database. Two of the internships are focused on the production of instructional videos: one on navigating and using the IUCN database system, the Species Information Service (SIS), and another one on performing basic operations in ArcGIS as pertaining to creating and modifying amphibian ranges for IUCN maps. Familiarity with screencasting software is an asset for both of these internships. For the GIS-based internship, knowledge of ESRI spatial software products is required, and knowledge of ArcGIS is an asset. The third internship is focused on helping update the amphibian database by assessing new species and/or helping reassess species that are already on the Red List. Please note that all three internships have a duration of six months part-time and can be carried

out remotely from your place of residence. The primary requirements for all internships are that interns have native or near native English language skills, that they have access to a good internet connection and are able to liaise with the Amphibian Red List Authority Coordinator or Assistant/Deputy Coordinator via Skype, and that they have a reliable computer with suitable audio and enough capacity for media development and storage (for the two video internships). Applications will be received until February 14, 2013. Please send applications to ariadne.angulo@iucn.org and Jennifer.LUEDTKE@iucn.org and include in your email a brief cover letter, résumé and contact details for two referees. Please indicate in the Subject heading which internship you are interested in (Amphibian RLA internship—GIS video or Amphibian RLA internship—SIS video or Amphibian RLA internship—assessments). We will be contacting selected candidates by the second week of March.

ASG/ARMI Seed Grant Winners 2012

We are pleased to announce the winners of the ASG/ARMI Seed Grants for 2012. We received more applications during the 2012 selection process than ever before so an especially big congratulations goes out to the six winners highlighted below.

ARMI Seed Grants are intended as one-time awards of between \$500 and \$2,000 for the support or initiation of research that furthers the Amphibian Specialists Group's mission to conserve biological diversity by stimulating, developing and executing practical

programs to conserve amphibians around the world, in addition to determining the nature, extent and causes of amphibian population declines.

The ASG/ARMI Seed Grant is managed by the Amphibian and Reptile Conservancy, a U.S.-based 501(c)(3) non-profit organization whose mission to assist in and develop amphibian and reptile conservation projects and partner efforts that support the mission and goals of Partners in Amphibian and Reptile Conservation (PARC).

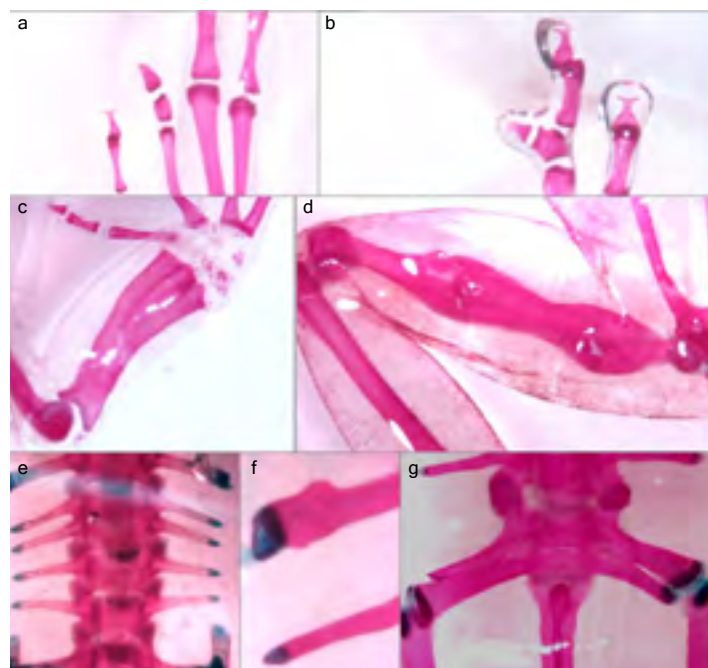
SKELETAL ABNORMALITIES IN TWO PUERTO RICAN *ELEUTHERODACTYLUS* SPECIES INCREASE WITH FOREST FRAGMENTATION: LINKING TEMPERATURE EFFECTS ON DEVELOPMENT WITH FIELD OBSERVATIONS

Zuania Colón

A study carried along a gradient of forest fragmentation in Puerto Rico revealed a high incidence of subtle skeletal abnormalities in two common species of frogs, namely *Eleutherodactylus coqui* and *E. antillensis*. The skeletal abnormalities were higher in fragmented than in continuous forests and included missing bones, as well as duplications, inversions and abnormal bones that were observed in cleared and stained specimens. Temperature is a key environmental factor that changes when fragmentation occurs. Temperature is also known to influence developmental time of amphibians and the incidence of abnormal embryos. I propose to examine the role of temperature under laboratory conditions as a likely environmental factor responsible for the observed skeletal abnormalities in tropical frogs without a tadpole stage.



E. coqui frog in Puerto Rico. Photo: Zuania Colón.



Skeletal abnormalities in *E. coqui* and *E. antillensis* frogs in Puerto Rico: a) phalanges absent and abnormally shaped, b) phalange duplication, c) radio-ulna duplicated, d) femur abnormally shaped, e) vertebrae fused, f) vertebra abnormally shaped, and g) vertebra inverted (left) and fused (right) (Restrepo *et al.*, in prep).

I am proposing the establishment of captive breeding colonies of each species at a satellite facility of the Animal Resources Facility of the University of Puerto Rico-Rio Piedras. These breeding colonies will provide a continuous source of individuals to study the effect of temperature on pre- and post-hatching skeletal development. Eggs, froglets and juveniles will be reared in two temperature regimes [20 °C to 25 °C control; 28 °C to 33 °C high temperature] to examine the role of increased temperatures on developmental rates, skeletal development and incidence of abnormalities. At each of 46 stages from embryonic to sub adult stages I will collect and measure morphological traits (head length, head width and snout-vent length), and subsequently I will euthanize, and clear and stain the specimens. This technique allows the study of cartilage and bone in place, including the identification of skeletal abnormalities. Studying skeletal development in these model species under laboratory conditions will be my first step towards understanding the incidence of abnormalities associated with forest fragmentation, a leading cause of amphibian population decline worldwide. The presence of abnormalities in the skeleton of these common species, which are adapted to different environments, is indicative of what may be happening to vulnerable species and perhaps with greater frequency.

The ASG/ARMI grant will allow me to buy the materials and equipments that are crucial for housing these animals in captivity.

DOES IMPLEMENTATION OF NO-TILL AGRICULTURE REDUCE LARVAL STRESS OF BLANCHARD'S CRICKET FROG (*ACRIS BLANCHARDI*)?

Melissa Youngquist

The goal of my project is to evaluate how different agricultural management practices affect juvenile recruitment of an at-risk species, Blanchard's cricket frog (*Acris blanchardi*). My study specifically focuses on conventional tillage vs. no-till agriculture. Cricket frogs, which are declining at the peripheries of their range, frequently breed in agricultural ponds. However, ponds in agricultural areas may be compromised as evidenced by generally having lower amphibian abundance and richness than ponds in natural areas. These agricultural ponds may be stressful environments for amphibian larvae; they are characterized by increased nutrient and pesticide contamination and increased turbidity from runoff. Farming techniques that minimize these stressful conditions could be beneficial to the long-term survival of cricket frogs and other amphibians. Implementation of no-till farming may be one such technique. No-till farming reduces soil erosion, increases water infiltration, and can reduce surface water runoff. These effects can, in turn, reduce aquatic inputs of sediment and chemical contaminants.

To determine whether agricultural ponds negatively affect cricket frog recruitment and whether implementation of no-till farming mitigates this effect, I will rear tadpoles in *in situ* enclosures in conventionally tilled farm, no-till farm, and grassland ponds. I will use three replicates of each pond type, for a total of nine ponds; within each pond I will add three 1000 L enclosures containing 50 tadpoles. Enclosures will be constructed from fiberglass screening and untreated lumber. I will measure metamorph survival, time to metamorphosis, and mass at metamorphosis. Additionally, I will measure water quality indicators including algal abundance, dissolved oxygen, turbidity, and nitrogen and phosphorus concentrations throughout the study.



From left, Samantha Rumschlag, Myself, Ashley Gordon, and Tyler Hoskins. Photo: Michelle Boone.

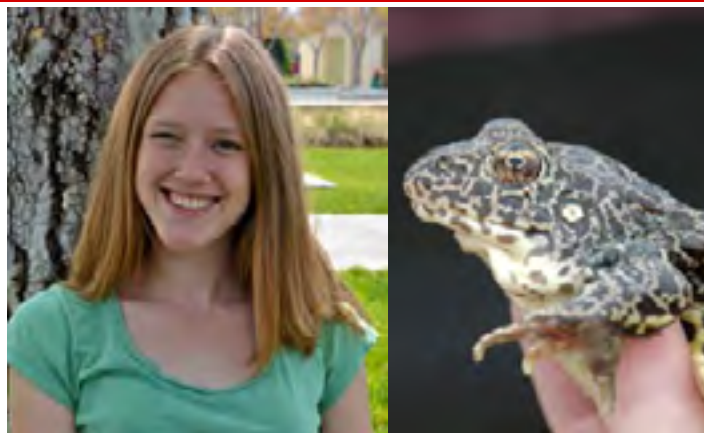
To determine if there are carry-over effects of the larval rearing environment, I will overwinter metamorphs from these ponds in terrestrial enclosures at Miami University's Ecology Research Center. I will add 10 frogs to 2 x 2 m outdoor enclosures immediately after metamorphosis; I will use six enclosures per pond type. Upon spring emergence, I will determine survival and growth of Cricket frogs.

Determining how agricultural ponds and soil management of agricultural plots affect Cricket frog recruitment will provide much needed information on factors contributing to the persistence of this at-risk species and other amphibians in general. My results will also inform management decisions regarding the creation and preservation of optimal breeding habitats to bolster amphibian recruitment. Funds from the ASG/ARMI seed grant will provide materials to construct *in situ* enclosures, conduct water quality analyses and travel between field sites.

CAN CAPTIVE-REARED TADPOLES RE-ESTABLISH CRAWFISH FROG POPULATIONS?

Rochelle Stiles & Michael Lannoo

Crawfish frogs (*Lithobates areolatus*) have experienced declines across large portions of their historic range and are classified as "Endangered" in Indiana and "Near Threatened" globally. The population bottleneck results from mortality in the larval stage. Kinney (2011, Thesis, Indiana State Univ.) has shown that > 1% of the larval population dies every day. The key to both maintaining the existing population and restoring or establishing new populations is reduced larval mortality. For example, Kinney reared Crawfish frog tadpoles in cages where they exhibited > 90% survivorship between hatching and metamorphosis. A successful large scale repatriation effort backed by a captive rearing program may reverse Crawfish frog declines to the point of long term population stability. In cooperation with Detroit Zoological Society we propose raising 15,000 larvae in captivity and releasing marked Gosner Stage 41 and 42 tadpoles at the state's largest breeding aggregation—Hillenbrand Fish and Wildlife Area—West in Green County, Indiana. During the 2013 breeding season, we will place captive-reared late stage tadpoles in breeding wetlands surrounded by drift fencing/pitfall trap arrays. If the hydroperiod is inadequate, we will supplement the wetlands with wading pools. We will calculate initial survivorship based on juveniles captured in traps upon dispersal from wet-



Rochelle Stiles (Photo: R. Stiles) and a Crawfish Frog (Photo: M. J. Lannoo).

lands. We will run drift fence surveys to monitor first time breeding males and a subset of breeding females in 2015 and the remaining first time breeding females and repeat breeders in 2016. As the first Crawfish frog repatriation attempt, this work will contribute to a better understanding of and guidelines for augmentation and re-establishment techniques for this Indiana endangered species locally and nationally.

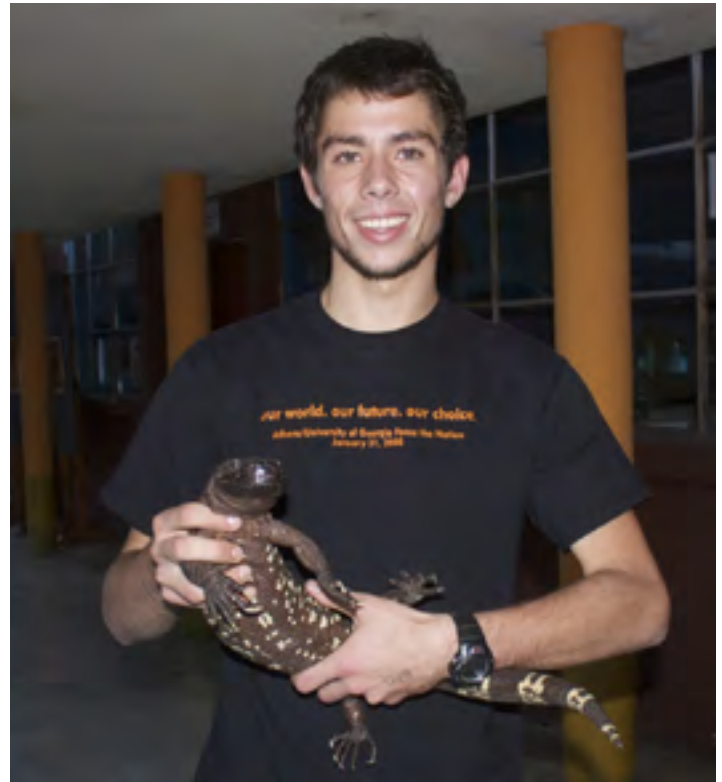
DEVELOPMENT OF A COMMUNITY-LEVEL eDNA PROBE FOR PLETHODONTIDAE

Todd Pierson

I will study and evaluate the potential of using environmental DNA (eDNA) for surveying and monitoring plethodontid salamanders in the Appalachians. This relatively new technique has been used to effectively survey for individual species of fish and amphibians in both lentic and lotic systems across the world, but its greatest potential appears to be at the community level. Rather than use specific probes to determine the presence of a single species, these more general assessments could potentially allow herpetologists to characterize an entire amphibian community.

Before testing the method in the field, I will test the sensitivity limits (e.g., salamander density, water quality, intraspecific vs. interspecific variation) in the laboratory. Once comfortable with the methodology, I will take the technique to the field. It will be tested at various sites across the Southern Appalachians where data for species-level occupancy and detection rate exists, including Coweeta LTER and the type locality of *Urspelerpes brucei*, to compare its effectiveness with that of traditional methods.

The ASG/ARMI Seed Grant will allow me to pay for transportation to and from my field site, purchase necessary sampling supplies, and purchase necessary laboratory supplies for the analysis of the eDNA samples.



Todd Pierson holding a captive *Heloderma horridum charlesbogerti* at the Museo de Historia Natural of the Universidad de San Carlos in Guatemala City. Photo: Carlos Vasquez.

FINDING A NEEDLE IN A HAYSTACK: DETECTION OF STATE-ENDANGERED *AMBYSTOMA LATERALE* IN THE PRESENCE OF UNISEXUAL "HYBRIDS"

Katherine Greenwald

I propose to test a new method, environmental DNA (eDNA) analysis, to aid in the detection of the Ohio state-endangered Blue-spotted salamander (*Ambystoma laterale*). Conservation of this species is difficult for two reasons: first, they spend the majority of the year underground, making it difficult to locate individuals

and populations outside of the breeding season. Second, they coexist with a lineage of unisexual (all-female) "hybrids," which can be nearly identical to the blue-spotteds and impossible to distinguish visually. Previous work has focused on trapping the salamanders in order to obtain DNA samples to identify the full blue-spotteds versus the unisexuals; however, this method is labor-intensive and has the potential to disrupt breeding or other important activities. Environmental DNA analysis is a new method which simply uses water samples from the salamanders' breeding ponds, in which DNA from present species can be detected at very low levels. I propose to test this method on a set of 30 ponds where we have already obtained larval samples for analysis. This will allow us to test the sensitivity of the eDNA approach (e.g., is the screen positive for sites where we know blue-spotteds are present?). It may also allow us to identify sites where blue-spotteds are present, but were not represented in our larval samples because of their low density relative to the unisexuals. This result would be particularly exciting, as blue-spotteds are only found at a handful of sites in Ohio. Any additional blue-spotted localities we could identify would be extremely important for conserving this state-endangered species at the southern edge of its range.



Katherine Greenwald (left). Photo: Kristin Stanford. Paul Anderson (right). Photo: Ohio Environmental Protection Agency, Wetland Ecology Group.

LIVESTOCK PONDS AS HABITAT REFUGES FOR DECLINING AMPHIBIANS: UNDERSTANDING THE IMPACTS OF CATTLE GRAZING TO ENHANCE MANAGEMENT PRACTICES

Daniel Preston

Our central aim is to enhance the capacity to manage livestock ponds as habitat for native amphibians in northern California. Our study region supports an assemblage of six pond-breeding amphibians, including two species that are listed on the Endangered Species Act (California red-legged frogs and California tiger salamanders). Much of the natural breeding habitat of these two species has been lost to development and agriculture, and they now reproduce in artificial livestock ponds in many regions. These livestock ponds can serve a valuable conservation purpose as habitat refuges; however, much debate exists over what levels of grazing intensity sustain useable amphibian habitat and whether separate amphibian species react differently to the presence of livestock. To address these issues, we will analyze an existing field survey dataset of >200 wetlands in northern California and we will conduct a long-term experiment to improve mechanistic understanding of observed field patterns. The survey sites experience a wide gradient of grazing intensity making them ideal to test hypotheses related to grazing and native amphibians. We will analyze these data using a combination of occupancy models to predict where native amphibians occur and an information theoretic approach to test which environmental variables are associated with increased abundance of particular species. In conjunction with field data analyses, we will initiate a field experiment at a property near Livermore, California where there are 89 livestock ponds that experience varying levels of grazing. Our experiment will involve 18 pre-selected ponds that will be divided into six treatments (each replicated three times). Two of the treatments will serve as grazed and ungrazed controls that will not be manipulated, allowing us to monitor background changes in amphibian populations. Two of the treatments will involve a



complete reversal of grazing practices (grazed to ungrazed and ungrazed to grazed, respectively) and two treatments will evaluate the efficacy of partially grazed ponds (completely grazed and completely ungrazed to partially grazed, respectively). Manipulations of grazing intensity will be accomplished by constructing (or removing) fences that exclude cattle from either the entire pond (ungrazed treatments) or half of the pond (partially grazed treatments). We will intensively survey all of the experimental sites before the manipulations, and for three consecutive seasons following the manipulations. Survey response variables will include amphibian occupancy and reproductive success, as well as environmental variables that are predicted to drive changes in amphibian populations (e.g., vegetation cover, water chemistry and pond hydroperiod).



Researchers from the University of Colorado surveying a lightly grazed pond (top right) and a heavily grazed pond (above) in Northern California. Livestock ponds provide a promising opportunity to conserve amphibian breeding habitat in many regions.



Tapping into Amphibian Conservation

Dear Friends, Colleagues and Supporters,

Welcome to the first TAP newsletter!

TAP is the newly formed Threatened Amphibian Program of the Endangered Wildlife Trust. It has been borne out of the need to bridge the gap between research and conservation action on the ground for threatened frog species in South Africa. The program aims to address a growing need in the country for the involvement of the Non-Governmental sector in frog conservation and fundraising. Jeanne Tarrant officially joined the EWT in September and is working toward securing sustainable funding, establishing relationships with relevant partners and stakeholders and spending time in the field.



Pickersgill’s reed frog from Mt Moreland. Photo: Sam Phillips.

TAP PROJECTS

The Threatened Amphibian Program has initially prioritized conservation projects for two of South Africa’s Critically Endangered frogs:

1) Pickersgill’s reed frog, *Hyperolius pickersgilli*, which is restricted to the KwaZulu–Natal coast, and whose wetland habitat is under increasing pressure from development and land transformation. TAP aims to implement the following specific conservation actions for Pickersgill’s Reed:

- Development of a Biodiversity Management Plan.
- Securing unprotected sites, through Biodiversity Stewardship agreements, where appropriate, land acquisition.
- Setting up a long-term monitoring programs and population estimates.
- Habitat restoration/rehabilitation of new habitat for future reintroduction of rescue/captive-bred animals.
- Providing advice and in-field assistance to Johannesburg Zoo and uShaka Marine World in developing captive assurance population of Pickersgill’s reed frog.



Amathole toad seepage habitat near Hogsback (Left). Photo: M. Cunningham. A male Amathole toad found in October 2012 (Right). Photo: J Tarrant.



"Frog Dog" taking part in Save Our Frogs Day 2012, Noordhoek Farm Village, Western Cape.

2) The Amathole toad, *Vandijkophrynus amatolicus*, is known only from upland grassland wetlands of the Amathole Mountains near Hogsback and Stutterheim in the Eastern Cape. The species has proved extremely elusive despite regular surveys in recent years. A single female and some tadpoles and eggs were found in September last year; the first sighting in 13 years. As part of this project, in October this year a single male was found by Michael Cunningham in a new locality on Elandsberg near Hogsback. I also met with various land-owners and key community members to discuss the project.

The project aims to:

- Survey the species range to establish distribution and population size.
- Investigate the threats, including possible impact of a Rana-virus infection.
- Establish relationships with forestry companies in the range to implement long-term management strategies.
- Provide recommendations to forestry companies for lessening their potential impact on this species.
- Work with ex-situ facilities to implement a captive breeding program.

SAVE OUR FROGS DAY 2012

TAP also aims to increase awareness about frogs through education and media, and on 1 December the first South African national awareness day for frogs, Save Our Frogs Day, was held. Ten events took place throughout the country in KwaZulu-Natal, Gauteng,

Potchefstroom, St Francis Bay and Cape Town. Many thanks to all of you who participated!

We would like to thank our donors, the Critical Ecosystem Partnership Fund, and Columbus Zoo for being the first to support this program and enable this crucial work to begin taking shape. We look forward to building many additional partnerships in 2013.

Wishing you all a green and safe festive season!

Have a Toadally Cool Christmas and Hoppy New Year!

Best regards,

Jeanne

To get involved or find out more, please contact: Jeanne Tarrant (Threatened Amphibian Program Manager) at: jeannet@ewt.org.za or 083 254 9563 or +27 31 (0) 7655471 <http://www.ewt.org.za/programs/ACP/acp.html>



Novel Biotechnologies Prove Winners in Giant Salamander Conservation

By Robert Browne, Sherri Reinsch, Heather Robertson, Vance Trudeau & Dale McGinnity

Novel biotechnologies are proving invaluable in the conservation of giant salamanders. The emerging fields of amphibian reproduction technology, populations genetics and state of the art zoo veterinary practice have provided Nashville Zoo with the first controlled breeding of North American giant salamanders (*Cryptobranchus alleganiensis*), and the first breeding of *C. alleganiensis* held in captivity for some time (1).

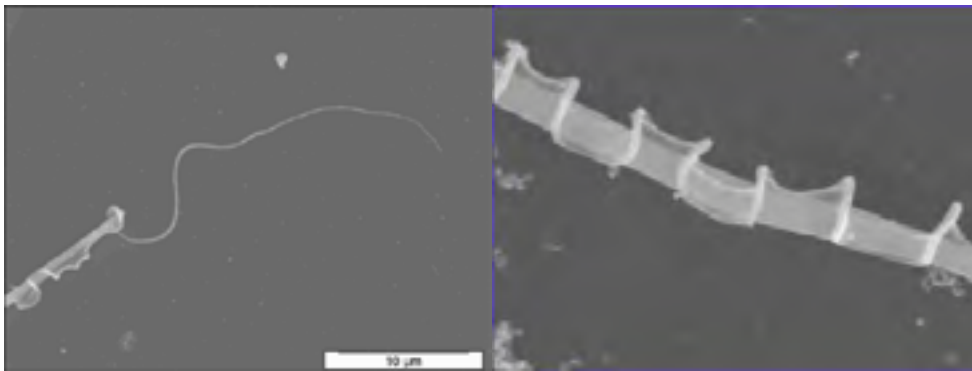
Giant salamanders lived long before the dinosaurs, but unlike dinosaurs survived the meteor impact 65 million years ago. Their survival can be partly attributed to cryptobranchids habitat of deep aquatic shelter, and their physiological characteristics of low metabolism, high longevity, and the ability to thrive in temperatures from near freezing to 28°C, and to tolerate temperatures several degrees higher.

Unfortunately, these species have declined. In the last 100 years, the Japanese giant salamander (*Andrias japonicus*) has experienced a slow decline, the Chinese giant salamander (*A. davidianus*) almost extinct in nature, and the North American giant salamander (*Cryptobranchus alleganiensis*) is now in rapid decline.

Some of the *C. alleganiensis* populations now only include old adults, suggesting problems with reproduction or larval survival. Dams have also resulted in isolated populations of *C. alleganiensis* in streams and rivers potentially creating inbreeding problems through a lack of gene flow between populations. Good habitats have been rendered unsuitable for *C. alleganiensis* by siltation and pollutants. While the role of disease is still being investigated, many populations are in decline for uncertain reasons.

Field conservation programs are being developed that include the supplementation of populations with larvae produced from locally sourced egg masses. This will provide valuable information on larval raising and the success of release programs. However, they cannot maintain the genetic variation of *C. alleganiensis*, due to eggs being rarely found and often from one nest, and therefore at a maximum only representing a few individuals of each sex. Release of genetically unscreened *C. alleganiensis* may also reduce the genetic variation in small populations by overwhelming the population's genetic pool with genes from only a few salamanders (2).

Evolutionary significant units (ESU) are defined by genetic differences and not by appearance. So what appeared to be one species may be many, and what appeared to be many may be one species. ESUs are the modern equivalent to species in terms of sustainable management and conservation (2). To maintain the genetic variation of a ESUs of *C. alleganiensis* up to 100 selected individuals would have to be kept. Therefore, to perpetuate the genetic variation of all the ESUs of *C. alleganiensis* in a captive breeding program would require 800 wild caught adults and large numbers of their offspring.



The flagellum of hellbender sperm showing the waves that propel the sperm and the unusual tail at the end. Photo: Dr. Dalen Agnew (Michigan State University).

Zoo curators have realized that the limiting factor for helping conserve species in captivity is space and valuable keeper time. This is especially true for species like hellbenders which require large aquariums with high quality water that requires constant monitoring and ad-

justment. However, even with the benefits of specialized scientifically based husbandry no ESU of *C. alleganiensis* had been previously bred in captivity prior to Nashville's Zoos success, except with recently captured individuals.

Without the support of reproduction technologies a captive breeding program for *C. alleganiensis* would be difficult to establish and maintain. It would require taking many females and males from Critically Endangered populations and then attempting to breed them in a controlled manner. Without controlled breeding the genetic variation of a captive population is lost over time. Controlled breeding without the support of reproduction technologies has not been achieved for *C. alleganiensis* despite many individuals being in captivity over decades.

But what if you could have females spawn when required, sample *C. alleganiensis* sperm in the field and preserve it for hundreds of years, and whenever you needed, use it to fertilize eggs. This technology would mean fewer disturbances of wild populations and individuals in captive breeding programs, better quarantine, lower cost, greater security and the perpetuation of genetic variation.

To achieve these goals Dale McGinnity, Curator of Ectotherms, Nashville Zoo, with other researchers and giant salamander conservationists, implemented a research program for the controlled reproduction of *C. alleganiensis*, and the cryopreservation of their sperm and its storage in a gene bank.



Research of hellbender reproduction showing ultrasound of testes and ovaries, hellbender eggs, and research staff Dale McGinnity (white tee shirt), Heather Robertson (Veterinary Officer) and Robert Browne. Photo: Christian Sperka.

Nashville Zoo achieved these goals within three years through institutional support, leadership, team effort, strategy, expert management and international collaboration. Veterinary oversight, scientific method and innovation guided the program. In the first sampling season in 2009 the Nashville Zoo team achieved the first cryopreservation of the sperm *C. alleganiensis* from both wild and captive males, in 2011 the induction of the first eggs and in 2012 the first controlled breeding of *C. alleganiensis*.

This conservation milestone is a product of a collaborative research project over the last six years by a team of international researchers using amphibian reproduction technologies. The applicability of the general techniques being developed means the reliable induction of amphibian reproduction, cryopreservation of their sperm and the production of genetically varied offspring (3, 4).

Many amphibian species have not been reproduced in captivity, or have only naturally reproduced from individuals captured that are already environmentally entrained for reproduction. Consequently, many amphibian captive breeding programs rely on hormones to induce breeding. However, hormones are expensive, exceptionally high doses are often required, and the techniques work efficiently only with some species of amphibians, sometime only at certain seasons (5).

Variations in the responses to hormones are dependent on species and the individual's reproductive condition. The high variation in these responses suggested that hormonal protocols would be spe-

cies specific and require a repetitive research effort for each species. This research is expensive and requires many experiments on hormone dosages and the timing of their administration. There may also be difficulty in obtaining experimental amphibians from highly threatened species.

In the example of *C. alleganiensis* at Nashville Zoo, only a very small group of three males and one female had been maintained for over five years in a laboratory setting by a dedicated amphibian specialist keeper, Sherri Reinsch. This small number of individuals to work with, and particularly only one female provided a further challenge to the project.

Using previous knowledge of the seasonal reproductive cycle of *C. alleganiensis*, and veterinary work including ultrasound by Dr. Heather Robertson, Nashville Zoo veterinarian and Dr. Sally Nofs, the Nashville Zoo team developed techniques to stimulate the reproductive condition of *C. alleganiensis* through temperature and photoperiod cycling.

The *C. alleganiensis* were monitored over the last four years with portable ultrasound equipment to determine the status and development of their reproductive organs throughout each breeding season. For the first two years in captivity, the female produced ovarian follicles and the males developed enlarged testes, but no sperm or eggs were produced. The first trials of the use of only hormones to induce *C. alleganiensis* resulted in no eggs.



The eggs (Photo: Sherri Reinsch, Nashville Zoo) and larvae (Photo: Amiee Stubbs, Nashville Zoo) of the first hellbender breeding using reproduction technologies.

In 2010 Dr. Vance Trudeau, a Canadian endocrinologist and his team, published a research discovery that contributed to the successful breeding in 2012 of *C. alleganiensis* at Nashville Zoo (6). Metaclopramide, a drug that blocks dopamine in the brain, in combination with a hormone proved very successful for the induction of spawning and fertilization of eggs in four frog species. The formulation is called Amphiplex, which is derived from the combination of the words amphibian and amplexus. The dose of hormones needed is far lower than generally used and therefore is safer for repeated administration and more economical to use.

In 2011 Amphiplex injection was based on ultrasound determination of gonadal development and milt and eggs were collected from *C. alleganiensis* for the first time, but no eggs developed. In 2012, with fine tuning of fertilization and egg incubation techniques the first larval *C. alleganiensis* were produced.

Once assisted reproduction technologies are fully developed, they can be utilized to increase the genetic diversity in small isolated populations and repopulate extinct populations with genetically fit stock. One of the best ways to perpetuate genetic variation is through the use of cryopreserved sperm. It can almost eliminate the need to keep captive males and greatly reduce the number of required captive females. Cryopreserved sperm in milt can be used to fertilize eggs anytime in the foreseeable future as it can remain viable for thousands of years (3). Dr. Robert Browne, an Australian cryobiologist from Antwerp Zoo, worked with the Nashville Zoo team and global researchers to develop general cryopreservation techniques for amphibian sperm. Through applying this knowledge the thawed sperm of *C. alleganiensis* from our first trial in 2009 was shown to be highly viable by testing at Michigan State University. This has enabled Nashville Zoo to establish a gene bank of frozen *C. alleganiensis* sperm collected during field surveys (4).

After further refinement of protocols in 2010 a gene bank for *C. alleganiensis* has now been established at Nashville Zoo from milt with sperm collected during field surveys. This work was sup-

ported by Bill Reeves, with the Tennessee Wildlife Resources Agency (TWRA), who have also collaborated on and helped fund the project, along with statewide surveys, gene banking, disease testing and genetic work on Tennessee's *C. alleganiensis* through a (SWG) State Wildlife Grant. It has taken five years to develop the techniques to reliably collect milt and eggs from captive *C. alleganiensis* in a laboratory setting. Further refinement of incubation and fertilization techniques over the next few years will enable *C. alleganiensis*, and other cryp-

tobranchid species to be reliably reproduced in captivity, and genetically fit offspring produced to suit various conservation needs, including their eventual release to conserve natural populations. The larval *C. alleganiensis* in this program were produced through good management and collaboration, and benefited by the high standards of oversight by veterinary medicine, novel reproduction technologies, and the implementation of scientifically based husbandry techniques applied by dedicated husbandry staff.

The use of biotechnologies in conservation breeding programs, including amphibian reproduction technology, populations genetics, and good veterinary practice, promises the long term conservation of cryptobranchids and eventually all threatened amphibians (3).

Valuable collaborators on this project not included in the main text are Joe Greathouse; Dr. Michael Freake; Dr. Brian Miller; Dr. Dalen Agnew; and Dr. Carla Carleton.

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FrogLog is made possible by the kind support of the following partners



THANK YOU



Hello Neighbour! Why Conservation Begins at Home

By Clay Bolt

Have we grown numb and weary from the reports that our world isn't doing so well, that the environment has been degraded and we're to blame? It's a lot to take in at times, isn't it? I consider myself a conservationist, someone who spends every day trying to learn more about what it is that is happening to our planet, and what might be done to correct our course. Even for the interested there are days when ...well. At some point, we can only take in so much bad news, so much guilt and grief. This realization has weighed on me for some time, because I understand that we can't be numb and tuned out if things are going to turn around, that species—including our own—will retain a decent place to live.

One of the things that occurred to me while mulling all of this over was that we've lost sight of the natural world around us. Yes, an obvious statement, except that I mean this literally: I believe that most people have forgotten how to "see" the wildlife that they come in contact with on a daily basis. Of course, no one can see or understand everything that they encounter, but a few generations ago, people depended directly on the cycles of nature, and at the very least had some idea of humanity's roots in the natural world.

One of the issues that "modern" humanity faces is that as we have moved into a more mechanized society, we have ordained certain locations in the world as "nature important" while somehow completely overlooking, and undervaluing, the treasures that surround us each day. Even an elementary school student can tell you that the equatorial rainforests have more biodiversity than anywhere else on the planet, but that shouldn't always be equated with more

important or more beautiful. And yet, isn't that the commonly shared perception?

It took a trip to Australia in 2001 for my own recognition to materialize, regarding just how beautiful the birds of America are. I had just returned home after *oohing* and *ahhing* over Rainbow lorikeets and Crimson rosellas for weeks when a Northern cardinal landed just outside my kitchen window. Still in the mindset of my trip, I became elated by the sight of this familiar bird that I had seen

and ignored countless times in my life up to that point. As is often the case, I had become blind to my surroundings, and this lack of vision led to a loss of recognition and ultimately a broken link with my wild neighbors.

OUT OF SIGHT, OUT OF MIND

Imagine flipping on the TV one evening and a news report comes on. The anchor describes a terrible house fire that has left a family homeless. They've lost everything and their lives will never be the same again. If you're like most people, you will watch with some feeling of remorse. Perhaps you'll even make a comment to your significant other about the unfortunate victims.

Their tears will move us, but if we're honest, it won't be long before the moment has passed, the channel is changed or something else has captured our attention.

Now envision a second scenario. This time, imagine flipping on the television, which instantly bursts in flames. The fire begins to spread to your walls, your furniture and possessions, and it isn't long before your entire home—the culmination of a lifetime of hard work and the cradle of your most precious memories—is a charred,



In early 2012, Meet Your Neighbours co-founded a children's program called Backyard Naturalists. MYN co-founded a children's program called Backyard Naturalists, whose mission is to inspire a lifelong appreciation of the natural world in children through educational programming that integrates science, art and technology. The project, which is just wrapping up its 2nd session in North Carolina, recently held its first international class in Canberra, ACT Australia under the leadership of MYN contributor David Wong. Photo: Clay Bolt.



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From Top, Left to Right:

Limnodynastes dumerilii. Photo: J. Tiddy.

Teratohyla albomaculata. Photo: A. Lario.

Megophrys nasuta. Photo: J. van Alphen.

Rana temporaria. Photo: M. Masterl.

Gastrophryne carolinensis. Photo: P. Marcellini.

Rhacophorus bipunctatus. Photo: S. Kadur.

Rhinophrynus dorsalis. Photo: S. Patterson.

smoking shell of ash and cinder. At that moment, the devastation, which has been laid out before you is undeniable and all consuming. Nothing is more important. You will do what it takes to repair the situation. There is simply no other option.

I've shared this rather melodramatic example because I believe that it parallels how many of us respond to the issues facing the natural world today. It's all too easy to react with the "not on my doorstep, not my problem" response. We already have too many plates to spin within our immediate vicinity: bills, raising children, marriage and on it goes. In order for us to combat this apathy, a (re) connection with the natural world must begin to occur in the places where we live, not just in the most biologically rich areas, but also on bustling city sidewalks. Nature can't just be "allowed" or assumed to live only in national parks and tropical forests—it also needs to be allowed to once again be an acknowledged part of our communities too.

Going a step further, it seems apparent that this introduction is best made to children who are only just forming their opinion of what a successful life looks like. Adults, including yours truly, have grown to expect a certain level of comfort and routine. It can often be difficult for us to make the big decisions that need to be made to protect wild places and wild species because we are concerned with what may need to be sacrificed in return.

SEE THE WORLD, BY KNEE!

So what can be done to re-establish this reconnection? Well, the good news is that regardless of where one lives, we are surrounded by amazing, overlooked wildlife, with behaviors as fascinating as anyone could ever dream. What's more, as Piotr Naskrecki points out in his book *"The Smaller Majority,"* with over 99% of life on Earth being smaller than the human finger, you don't have to go far to find something worth getting excited over. By slowing down and taking the time to look—to really attempt to see—the process will begin immediately. I often tell people that travel to some of the most exotic locations in the world can only be made via the hands and knees.

Thankfully, we are wired to interact with nature, much more so than for texting, or sitting in cubicles. A willing individual simply needs to be plugged in, and the mind will do the rest. Children receive so many of their cues about how to respond to wildlife and wild places from their parents. I've seen it with my own boys, who are allowed more freedom to explore than some of their classmates. For a very, very long time, survival was ensured, at least in part, from an understanding of the comings and goings of plants, animals and the seasons. If you ask me, it still is.

In 2009 I co-founded a project, along with Scottish wildlife photographer [Niall Benvie](#), called [Meet Your Neighbours](#). Our mission at MYN is to help people connect with the wildlife in their own communities. Currently, photographers working in over 30 locations around the world have joined together to support this effort. By photographing our subjects against a brilliantly lit white background, commonly found species that have often been taken for granted are revealed in a new way, encouraging a second glance or renewed interest from the viewer. (The details of this technique are described [in great detail here](#).) Our contributors strive to present their images to the people who live where the photos were made,

during lectures, exhibitions and workshops in hope that a connection back to the wild will be made. One example, in particular, stands out in my mind. Carl Battreall, who is based out of Anchorage, Alaska, recounted the story of a family who came up to him after a presentation. They were new to the area, and shared that each time Carl posted a new MYN image on his website, they would go out on an adventure seeking the species in the species in the wild for themselves.

Amphibians rank high in the species that our photographers showcase as part of their outreach efforts. In particular, frogs are wonderful ambassadors for smaller wildlife because people of all ages tend to find them fascinating. A contributor who has already done so much for amphibian conservation prior to joining the ranks is Robin Moore. A few years ago Robin co-founded a wonderful project called [Frame of Mind](#), which aims to help children in developing countries connect with nature through photography. He recently returned from a trip to Haiti where he spent time photographing some of the nation's remaining amphibians in hopes that the people of Haiti might learn what they have before it's too late. In a similar fashion, and in coordination with the Highlands Biological Foundation, MYN co-founded a children's program called [Backyard Naturalists](#) in early 2012, whose mission is to inspire a lifelong appreciation of the natural world in children through educational programming that integrates science, art, and technology. All around the globe—from the United States, to South and Central America, the UK, Europe, Africa, India, Southeast Asia and Australia—photographers who are driven by a passion for working in their own "backyards" are sharing their knowledge of local wildlife because they recognize the changes—and joy—that this awareness can bring.

PROACTIVE CONSERVATION

I like to call Meet Your Neighbours a "proactive" conservation project. Tremendous amounts of money, time and energy are spent each year in an effort to rescue species on the brink of extinction from slipping away forever. However, through simple actions such as creating shelter and growing native plants in your own backyard, you offer hope to commonly found species like the Green frog (*Rana clamitans melanota*), which are often displaced by the pressures of development. We hope that the introductions that we are facilitating with MYN will offer one way that this process can begin. And this is important, because as I've already stated, conservation and a love of biodiversity must begin at home. As Robert Michael Pyle once said *"What is the extinction of a condor to a child who has never seen a wren?"*

Finally, consider this: Many of the rare species that live in our world today were once quite common. These days, people often feel a sense of desperation at the state of our world and wonder if anything that they do will even matter, and yet here is a concrete opportunity to make a lasting impact for good in our world. If neighborhoods banded together to create green spaces for these wild neighbors, or if parents spent more time in the outdoors with their children, imagine the positive impact that could be made! Yet, none of this will happen if we haven't had the pleasure of an introduction to whom it is we might be sharing our space with.

To learn more about Meet Your Neighbours, visit our website at: www.meetyourneighbours.net



The Sticky Tongue Project Sticking Amphibians To Your Screen

By Candace M. Hansen

We all know that amphibians are in serious trouble around the globe. With so many amphibian species being threatened with extinction, addressing this crisis is clearly one of the biggest conservation challenges we face today. To make matters worse, positive news stories about amphibian successes are usually overshadowed by the prevailing doom-and-gloom news stories.

Unfortunately, as a result of this, people seem to be giving up hope. Why care and even bother trying to help if they just appear to be disappearing anyways? Doom-and-gloom messages are clearly not effective. If they were, we wouldn't be continuing to lose so many amphibians.

Something needs to change. We have to replace these depressing extinction messages with something that is more positive, fun and engaging. Efforts that focus solely on loss and extinction are merely creating an atmosphere of apathy and inaction.

Encouraging a fascination with these species might just be the most effective way to reach out to the public about the amphibian extinction crisis. We should be educating and motivating the public instead of scaring and depressing them. We can do this using a variety of creative and upbeat approaches that reach as wide of a public audience as possible. The more people that know, the more people there are that can help.

The Sticky Tongue Project (TSTP) was created by myself (Candace M. Hansen) and Iwan Hendriks as a simple way to share biodiversity and conservation issues/news, with a special focus on reptiles and amphibians. By sharing all of these species with an online audi-

Candace M. Hansen is the Project Developer and Manager of The Sticky Tongue Project, passionate spokeswoman for underappreciated species, an aspiring wildlife documentary filmmaker and Editor for *FrogLog*. The Sticky Tongue Project: <http://www.TheStickyTongueProject.com>



Screen capture of Wood frog (*Lithobates sylvaticus*). Filmed by The Sticky Tongue Project.

ence, we tried to inform and inspire a large audience to appreciate the biodiversity around the world. We hoped this would encourage people to help preserve it, both in, and beyond their own backyards.

Within a couple of months of being launched, the website was ranked as the #1 Biodiversity site by the renowned Dr. Stuart Pimm of Duke University. In his review he stated that, “The Sticky Tongue is a quirky, imaginative approach to informing and educating about biodiversity and conservation.”

And then it hit us. Maybe there was more we could do more to grab people’s attention ... and keep it. Everyone knows that a picture is worth more than a thousand words. But what about 25 pictures a second? What about pictures with sound?

Online videos are quickly becoming some of the most powerful conservation tools available. Not only do they let audiences know that action needs to be taken for these species, but they can also show that efforts are working. They touch people emotionally and this in turn, stimulates action.

And that’s exactly why we started to use videos to help get the message out about species that are typically overlooked, under the pseudonym: [The Sticky Tongue Project](#).

Our [online video series](#) allows the audience to follow our adventures across the globe as we showcase the amazing diversity of reptiles and amphibians, small or large, harmless or deadly, within their natural habitats in a fun, educational and non-threatening manner.

We showcase most species in-situ. This is an approach that differentiates us from other reptile and amphibian themed video series where the focus is often on drama, danger, adrenaline rushes and man-handling of these animals. And it is an approach that we hope others will follow—to simply sit back and enjoy these animals in their natural settings.

One of the funniest moments we’ve experienced in the field occurred this past summer. We were filming Eastern spotted newts (*Notophthalmus viridescens*) along the Niagara Escarpment in Ontario, Canada. It was quite

the hike up the Escarpment along the Bruce Trail but once you finally made it to the top, you were instantly greeted by dozens of efts boldly stumbling around and attempting to scale the cliff face. I say attempting to scale because none were successful—they kept falling down and trying again. It was a rather interesting and yet incredibly hilarious scene. We knew then that this would make a great video sequence so the cameras started rolling.



Green frog (*Lithobates clamitans*). Photo: The Sticky Tongue Project.



Screen capture of Yellow-bellied toad (*Bombina variegata*). Filmed by The Sticky Tongue Project.

As soon as we started filming, a hiker came up and asked what we were filming. He apparently couldn't see the bright red newts everywhere. So we told him that we were filming newts which he thought was great. Then there was a dramatic pause before he asked the greatest question of all: what are newts? As if on cue, [the newt fell in shock](#).

And it was all caught on film which we promptly turned into a short funny little video.

Through our videos we have changed people's views of reptiles and amphibians from one based on fear and repulsion to one based on admiration, interest and respect. After watching a video, people tell

us that they want to discover more about the species we shared with them, as well as how they can help them. Mission accomplished!

When people do want to learn more about these animals, they can easily navigate to our [StickyPedia](#) section the website. This is our growing multimedia collection featuring some of the different reptiles and amphibians featured on our website as well as in our video series such as the Yellow-bellied toad (*Bombina variegata*) in Europe to the Jefferson salamander (*Ambystoma jeffersonianum*) in North America. By clicking on a species name, visitors can learn more about that species' natural habitat, range, current threats, interesting facts and more about them. There is also a growing library of photos and videos to accompany these sections.

We then took things to the next level with the creation of a new interactive amphibian project that is geared towards everyday people, and not necessarily just herpetologists or biologists. At the end of the day, people are the ones who will ultimately help fund and generate excitement for conservation efforts. They can actually get a lot more accomplished on the ground at the neighborhood level without all the red tape many bigger groups have to contend with.

In 2012, we launched our [iFrogography](#) project: cell phones versus frogs (and toads). This project encourages people to go out and photograph amphibians that they can then share with others online.

Taking pictures of frogs (and toads) with a regular camera can be hard enough sometimes as they tend to be rather ... well ... hoppy. But we wanted to give people a fun challenge to get them even more excited about amphibians. We wanted to see how close people could get to them with their camera phones. This takes skill, persistence, creativity and plenty of time observing a frog's behavior.



iFrogography in action with a Wood Frog (*Lithobates sylvaticus*) and an iPhone. Photo: The Sticky Tongue Project.

We set forth some basic rules. Frogs are not to be touched, captured, posed, manipulated or harmed in any way. We also tell people to not stray off trails or paths to take their photos because this could destroy the frog's natural habitat. We just want people to spot the frogs and photograph/film them—that's it.

The quality of the photo does not matter. Everyone can participate—you don't have to be a professional photographer. It's simply about getting people to appreciate and enjoy the species that live within their own neighborhoods, as well as encouraging them to learn more about the species they are finding and what they can do to further protect them.

And it's working. The feedback that we have been getting is amazing. The stories that people are sharing with us are great. People are having fun ... learning more about the world around them ... and doing what they can to help protect the habitats they are finding these amphibians in. We even have an 80+ year old lady who now checks her swimming pool every morning to rescue all toads and frogs that may have hopped in by mistake. She then heads down to her pond to say good morning to all the frogs that live there and also counts all the frogs (as best as her vision allows) to make sure everyone is "safe" and accounted for. Her enthusiasm is contagious and spreading to others. We couldn't ask for anything better to come out of the project than this!

The iFrogography project is now being expanded to have people attempt to film frogs/toads as well as spot and document all species of amphibians (such as newts and salamanders). In the spring of 2013 we hope to release our iFrogography video special. We are

also looking at ways we can integrate iFrogography with various citizen science programs to see how it might further assist amphibian conservation efforts.

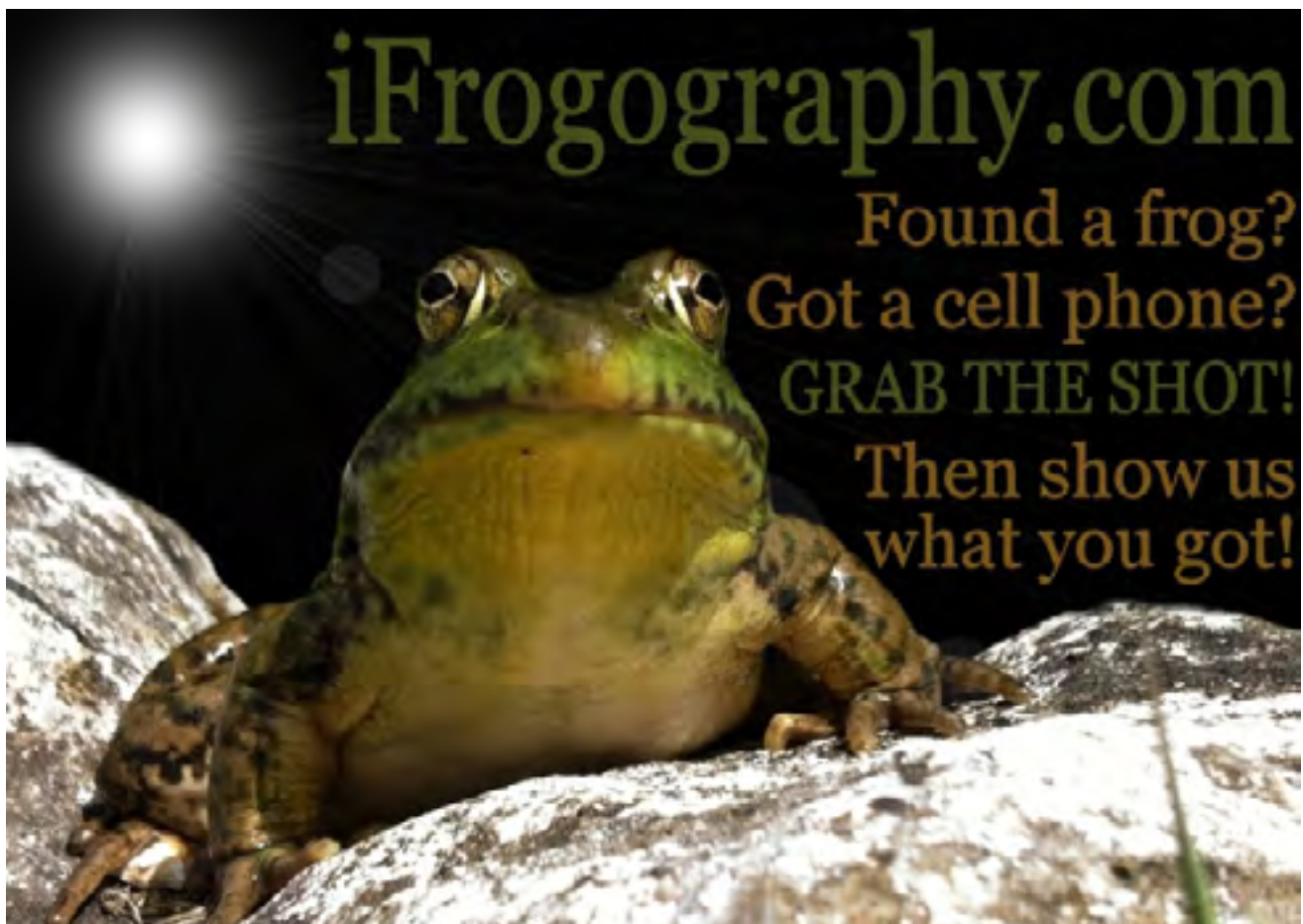
So what is next for TSTP? What are we going to do in order to help take amphibian conservation to the next level?

With the creation of our own production company (TSTP.tv), we can now shine the spotlight on other groups and organizations that are behind some of the most successful and inspirational conservation initiatives, yet lesser known ones. By doing this, we can combine a positive message with a call to action that motivates people to get involved.

One of these video projects is [24 episode yearlong education—out-reach](#) starring Whit Gibbons (Herpetologist and Author) that we have jointly produced with The Amphibian and Reptile Conservancy. We have also released [a feature video](#) on a successful reptile conservation initiative by the Long Point Basin Land Trust that will also help reduce amphibian mortality rates as well.

Inspiring a love for and understanding of these amphibians will help create a whole army of conservationists who are ready to take action. It does not take a lot of time, money and effort to make a difference. We want people to realize the power of the individual to make a change for the better: we can all help.

You can learn more about The Sticky Tongue Project by visiting the website: <http://www.TheStickyTongueProject.com>.



Biodiversity PEEK

Photography, Educating and Empowering Kids and their Communities



Jhonny Vaca, age 13, proudly shows off his fine macro photography skills in the Jama Coaque Ecological Reserve. Photo: Paul Hamilton.

By Stephanie Bowman

The Biodiversity Group (TBG) is a US-based non-profit made up of a diverse, international group of professionals and lay-persons with a mission to use photography to discover, document and help preserve life overlooked, with our primary focus being on amphibians and reptiles. We believe that effective conservation of species and their habitats depends on the evolution of peoples' concept of science changing from something that others do to something that we do as a society. With that in mind, we designed a citizen-science-via-photography educational program that is for anyone, anywhere, anytime. We call it Biodiversity PEEK.

Through Biodiversity PEEK we provide equipment and train local adult mentors to work with children to use digital cameras as a means to explore and digitally document the threatened flora and fauna around them. The program strives to increase the rate of literacy of both computers and the written word, as well as feelings of empowerment, and regenerative lifestyles, all while raising awareness and appreciation of local conservation concerns in the often impoverished communities where we work. And, Biodiversity PEEK is scalable and adaptable to other communities and amphibian-focused organizations around the globe.

Biodiversity PEEK is a hands-on, place-based program that satisfies our outreach objectives:

- To collect data in the field at our research sites year-round; as opposed to just every few months when we visit on research trips.
- To give real, concrete assistance to the educationally, economically and technologically impoverished communities at our study sites; knowing that people can only afford to take care of their wild places if their own human needs are met first.
- To help develop a relationship between local people and their neighboring ecological reserves; run by organizations with whom we partner in the field.
- To be both place-based and international in function; something carried out on a local level around the world, extending to places where we do not have a physical presence.

TBG has had remarkable success with the program this past year where it was launched in the Manabí Province of coastal Ecuador. This study area is home to the richly biodiverse yet greatly imperiled coastal forests; a notably understudied and under-protected region compared to the country's well known Amazon rainforest and Galapagos Islands. Two nature reserves in the area are Jama Coaque (near the community of Camarones) and Lalo Loor (near the community of Tabuga). These small rural communities have a 70% illiteracy rate, and an average of 6.7 children per family with most mothers bearing their first child around the age of 15.



A hug Biodiversity PEEK style at San Jose de Payamino (Left). Photo: Ross Maynard. The camouflage of a Masked tree frog (*Smilisca phaeota*) is not enough to avoid notice from Tabuga PEEK kids (Top right). Photo: Ariana Patrón. Local Tabuga mentor, Geomaira Patrón Alava, and her PEEK kids freeing tangled Loggerhead sea turtle (*Caretta caretta*) (Lower right). Photo: Geomaira Aracely Patrón Alava.

Most men work as day labors earning about \$7.00 per day on large farms created from clear-cutting, on mangrove-destroying shrimp farms, or via unsustainable fishing—including wild-caught shrimp—where, among other by-catch, sea turtles may be bludgeoned to death when caught in nets. Over the past century citizens here have lost much of their deep connection to the forest and its wildlife. The reserves are spoken of as something other, separate, *adentro*. People generally do not go into the woods, especially at night, much less allow their children to explore there. These many challenges led TBG to launch Biodiversity PEEK in Manabí Province in 2012.

Here is how it worked in Manabí: Before the launch, TBG identified and trained a handful of local adults as Biodiversity PEEK mentors. Then we introduced the program with a small party, hosted by our partners, in each community. Residents were treated to slides of some of the stunning photos we had previously taken of their local, often overlooked wildlife. We caught insects and helped parents and kids take macro-shots of them that we then projected for them to see. By the end of the parties the kids were eager to work with their adult mentor and take their own photos of life in their neighborhood as well as in the reserve.

Thanks to two grants we were able to give financial compensation to these mentors; hoping to send the message that there can be a direct, personal benefit to working as a local naturalist. With the help of our partners on the ground, the mentors each began meeting with their group of 3-10 kids each week. After the children learned basic field safety and macro-camera techniques they be-

gan to visit the reserves armed with their water-proof, shock-proof, GPS-enabled cameras we provided to them and their mentors.

So far, the kids of Camarones and Tabuga have collectively taken and uploaded thousands of scientifically identifiable photos of amphibians, reptiles, insects and more. Not only is there the great possibility of one of the mentors and their kids discovering a new species or range extension but they could even co-author a scientific paper on the discovery! And, best of all, with every Biodiversity PEEK photo taken as data, there is the addition to the conservation currency used by our partners to expand the nature reserves. Finally, both reserves are happily reporting that they now

have many more local visitors, indicating a growing interest and appreciation for local wildlife by nearby residents.

With these successes there have come challenges. First, neither community currently has Internet access . . . yet. But, it is soon to come and the kids will then be able to upload their own photos to iNaturalist, the online citizen science photo voucher program, and explore what other Biodiversity PEEK folks are sharing from around the world. Also, because we are paying people of a country whose labor laws are ambiguous to us and where we ourselves are lacking support staff in the field, we are relying on our valued local partners to help distribute salaries appropriately.

Next year we hope to expand the program with other potential partners in the neo-tropics and to our study areas in Vietnam and Mexico. We invite you, your group, and your community to take part in Biodiversity PEEK to help grow awareness of amphibian biodiversity at risk. Please visit our website and download our free, on-line, Biodiversity [PEEK tool-kit](#) to get started. We also have a version especially designed for educators that employs the current US National Science Standards.

Acknowledgements

Warm thanks go out for financial support from two family foundations, including the McGrory Family Foundation, as well as ongoing support and collaboration in the field from our partnering organizations; Third Millenium Alliance and Ceiba Foundation for Tropical Ecology, and for our generous individual donors.



© Edwin Giesbers / naturepl.com

By Helen Roddis

Golden frog (*Mantella aurantiaca*). Photo: Edwin Geisbers/naturepl.com

AMPHIBIANS IN PERIL

Despite having survived on Earth for millennia and having weathered several mass extinction events in the process, amphibians today face myriad threats which have already devastated whole populations and wiped entire species off the face of the planet.

The causes of these catastrophic declines are many, with habitat destruction and fragmentation, the introduction of exotic and invasive species, commercial exploitation, pollution, climate change and disease all emerging as major contributors to the alarming disappearance of amphibian species worldwide.

THE CONSERVATION CHALLENGE

Combating the amphibian extinction crisis is one of the greatest challenges faced by the conservation community. Given that between one-third and one-half of all amphibian species are currently threatened with extinction, and with that number expected to rise in the near future, it has never been more important for scientists, conservationists and communities to work together in a coordinated bid to protect the countless amphibian species currently perched on the very edge of existence.

Addressing the crisis and putting a halt to the barrage of threats currently endangering amphibians will require conservation organizations and their partners to come up with innovative solutions, both in the field and in captive breeding programs.

With species extinctions now occurring at a faster rate than at any time in Earth's history, raising public awareness of the world's threatened amphibian species and the need for their conservation is also increasingly crucial. Sitting alongside practical conservation efforts, communication, education and public engagement initiatives will have ever-more vital roles to play in the struggle to prevent an irreversible loss of the world's amphibian biodiversity.

A UNIQUE INITIATIVE

ARKive, a not-for-profit initiative of the UK-based charity Wildscreen, is leading the "virtual" conservation effort. Working with the world's very best wildlife and environmental filmmakers, photographers, conservationists and scientists, ARKive is an award-winning educational platform that is harnessing the power of outstanding wildlife imagery to create the ultimate free, online guide to the natural world. This truly unique educational resource is being made freely available to all via the ARKive website, www.arkive.org

With around one-third of the world's population now having access to the Internet, freely accessible online media resources are an increasingly influential conservation tool. Through its astounding free collection of films, photographs, fact files and educational resources, ARKive is connecting people to nature; helping to bring conservation to the forefront of public consciousness across the globe.

The importance of gathering together imagery of the world's threatened species—including images of the world's most threatened frogs, toads, salamanders and caecilians and preserving them for

future generations should not be underestimated. Photography is a powerful tool in conservation, inspiring greater appreciation and care for the Earth's incredibly rich biodiversity by engaging people with nature and showcasing the intricacies of the natural world around them.

USING THE POWER OF WILDLIFE IMAGERY

Found on every continent except Antarctica, amphibians—frogs, toads, newts, salamanders and caecilians—are amazingly diverse, displaying incredible variety in their life histories, from unusual body shapes to unique behaviors, extraordinary adaptations and, in some cases, striking coloration. With over 5,500 amphibian images, the ARKive collection shines a spotlight on this remarkable group of animals, revealing what they look like, where they are found, and why we should care about them.

The team at ARKive are privileged in having strong relationships with many individuals and organizations working at the forefront of amphibian conservation. With unprecedented access to a fantastic selection of amphibian imagery, ARKive is one of the largest collections of audio-visual resources available to the herpetological community.

To date, ARKive has profiled nearly 1,400 amphibians—a fifth of the world's known amphibian species—but there is still a long way to go in sourcing images and footage of some of the rare, enigmatic, and lesser-known species that make up this astonishing class of vertebrates. If you have any hidden gems among your collection and would like them to feature on the ARKive website, get in touch!

INSPIRING THE CONSERVATIONISTS OF TOMORROW

Wildlife imagery is a great way to excite, entertain and engage the conservationists and decision makers of tomorrow.



With over 100,000 amazing photos and videos, more than 15,000 threatened species fact files, exciting educational games and activities, and an expanding selection of curriculum—linked teaching resources for use in classrooms globally, ARKive is an invaluable educational tool for teachers and students around the world.

Our thousands of wildlife video clips and images allow students to visually engage with some of nature's greatest wonders, while ARKive's stunning collection can also be used by students, teachers and lecturers to illustrate a wide range of science topics, from animal behavior to adaptation, variation and movement. All of ARKive's formal education resources are actively designed to engage students in their local environments and encourage greater understanding of the world's wild plants and animals, with a particular focus on threatened species and their habitats.

ARKive's EXCITING EDUCATION RESOURCES

ARKive has over 40 formal education resources which cover a range of cross-curricular topics for use in schools worldwide, in addition to a whole host of "fun stuff" activities and topic pages to inspire people to learn about the natural world. And we've not forgotten the amphibians either!

From our demonic poison frog symmetry resource, to our Barbour's forest tree frog origami activity, frogs, toads, salamanders and caecilians feature in many of our educa-

tion resources, helping to promote the urgent need for their conservation. Amphibians have even made it into our fast-paced gaming app, Survival, with the golden frog included among our cohort of colorful characters. Go to: www.arkive.org/apps/survival to find out more about how you can tap, drag, scroll, swipe and pinch your way through a series of quick-fire mini-games to reveal the identity of some of the world's most threatened animals.



To achieve our ambitious goals, ARKive depends on the generous support of people and organizations from all over the world. There are many ways to help, whether it's through the contribution of media, the authentication of species fact files, downloading our education resources, or merely by spreading the word so that more people become aware of the project, and the vital conservation message it promotes.

For further information or to find out how to get involved visit the website at: www.arkive.org



Photography inspired me to....

protect frogs

Shedeline Louigéne, age 15



RECONNECTING WITH NATURE IN HAITI

Frame of Mind is an initiative born to empower youth to connect with their natural and cultural worlds through photography and visual storytelling. This is the story of how it all began.

By Robin Moore





Few places reflect the mirrored fortunes of the environment and people as poignantly as Haiti, where thin topsoil washes into the ocean in dirty red plumes from hillsides once cloaked in verdant forest. I first visited the country in 2007 after it was identified by the Alliance for Zero Extinction as containing the highest levels of amphibian endemism in the world. The Massif de la Hotte, a mountain range in the southwest of the island, harboring a staggering 13 Critically Endangered and Endangered frog species found nowhere else. This figure now stands at 15 and is set to increase further. **A sobering 92% of Haiti's 50 frog species are threatened with extinction—placing Haiti as a top global priority for immediate conservation action!**

But how so you go about trying to protect the last remnants of cloud forest when people go to bed hungry every night, and rely on the forests for fuel? While we are all concerned about the plight of amphibians, the reality is that the root cause behind the continued loss of amphibian habitat needs to be addressed. This will involve changing attitudes and behaviors.

In 2010, we embarked on a three-year project supported by the John D. and Catherine T. MacArthur Foundation to work with local partners to protect the last forest remnants in Haiti, adopting amphibians as a focal group. After more than half a dozen trips to

Haiti it started to dawn on me that, while each and every Haitian suffers the repercussions of environmental degradation in some form, whether through infertile soil, drought, floods and landslides, very few people—from government officials to farmers—have an intimate relationship or concern for the forests or the species that call them home.

I started to wonder, what would happen if Haitians were empowered to connect with nature and see their forests and its unique inhabitants through different eyes? It was with good fortune that, during a workshop convened by the MacArthur Foundation for its grantees in Haiti in early 2011, I met Indi McLymont-Lafayette, the regional director of Community, Media & Environment at [Panos Caribbean](#). This organization was working locally to empower youth to tell their stories, but was keen to integrate conservation into its workshops and open to the idea of using photography as the medium. I brought in our existing partner, Société Audubon Haiti, and we started planning.

Six months later, armed with 20 digital cameras and as many promising Haitian youths, we embarked on a crash course on biodiversity, conservation and hands-on training in photography and visual storytelling, before sending the kids out to capture and compile photo essays on themes of their choosing. They chose charcoal, water, plants and life in Haiti. I was excited by the concept but truly blown away by the results. Through the kids, I learned more about local perceptions of wildlife and conservation than I had in my previous trips to Haiti. I found an incredible openness to learn and an infectious enthusiasm and sense of pride—as well as a fascination with my passion for amphibians, which earned me the nickname «Dr Krapo» after the local Creole name for “frog.”

Photographs by Robin Moore/iLCP. Cover image: Shedeline, one of the workshop participants, holds up a board indicating what photography has inspired her to do. Page 30: A potentially new species of *Eleutherodactylus* frog from the Massif de la Hotte. Page 31 upper image: Mist-shrouded pine forest in La Visite National Park. Page 31 lower image: Some of the last remaining broadleaf forest in Haiti on the Massif de la Hotte. Page 32: A Hispaniola giant tree frog, *Osteopilus vastus*. Page 33: Radechine, one of the workshop participants, in La Visite National Park.







The most rewarding experience was taking the kids into Parc La Visite—the closest national park to their coastal hometown of Jacmel and home to some endemic amphibians—for the first time. “Before coming here, I thought it was a very ugly area,” said Wolnique, a 15-year-old student, after the visit “but it’s actually very beautiful.” This sentiment was echoed by many of the kids, who seemed energized by the beauty of the park, giddy with excitement at the images they were capturing in the mist-shrouded forest and thrilled by the night time search for frogs. The connections between the health of the forest and the quality of their lives downstream were brought out in images and stories that captured the good, the bad and the ugly sides of the park. They appeared genuinely saddened and concerned about the continued loss of trees and the species within them.

And their images and stories turned out to be a surprisingly powerful conduit for an important message. A photo exhibit and book launch at the municipal library attracted a diverse audience as the kids turned out in their Sunday best to lead proud parents around their creations. When Lovely, a bright and energetic 11-year-old, challenged the region’s minister of environment on his reforestation policy for Parc La Visite, it forced answers to some of the most difficult—but important—questions about the future of Haiti’s forests. The next generation was given a rare voice in the future of its country.

And so, the initiative Frame of Mind was born, to empower youth to connect with their natural and cultural worlds through photography and visual storytelling. With a workshop in the Massif de la Hotte in the pipeline, the initiative is set to continue and to grow. We will try to get as many people into the forest as we can; as for everyone else, we will bring the forest to them through images, video and stories told through the eyes of their children and peers.

The frogs of Haiti rely on the same forests as we do for survival. As people become increasingly disconnected from the natural world, I believe it is our responsibility to rekindle a meaningful bond that will energize people to fight for the protection of a dwindling treasure and ensure a brighter future for Lovely, Wolnique and the next generation of optimistic Haitians.

To find out more about this initiative please visit: www.frameofmind.org

Producing the Field Guides to the Yachana Reserve, in the Ecuadorian Amazon “Volunteering to Inspire Near and Far”

By Andrew Whitworth & Christopher Beirne

In 2009 we began working for Global Vision International (GVI - www.gvi.co.uk), a volunteer organization working in Amazonian Ecuador, situated in the Yachana Reserve on the banks of the Rio Napo (77°13'43.9"W 0°50'45.281"S; 300-350 m altitude). GVI were working to help support the maintenance and protection of the 1,000 ha private reserve, catalogue the species present and provide baseline biodiversity data to the Ecuadorian Museum of Natural Sciences (MECN).

Despite both being budding herpetologists at the very birth of our careers in herpetology, we quickly became overwhelmed by the diversity of species present and frustrated by the limited information and detailed descriptions that were presently available. Photographs of species were from only one angle, if included at all. If we were finding it hard to identify what was present, how could we ever hope to inspire the volunteers we worked with and the surrounding local communities to engage with wildlife and conservation?

It became quickly apparent that with over fifteen, super enthusiastic, camera laden volunteers at our disposal each expedition; we could steadily amass an incredible photographic database of local herpetofauna. So we decided to make a field guide using the volunteers, their photos and time to help us!

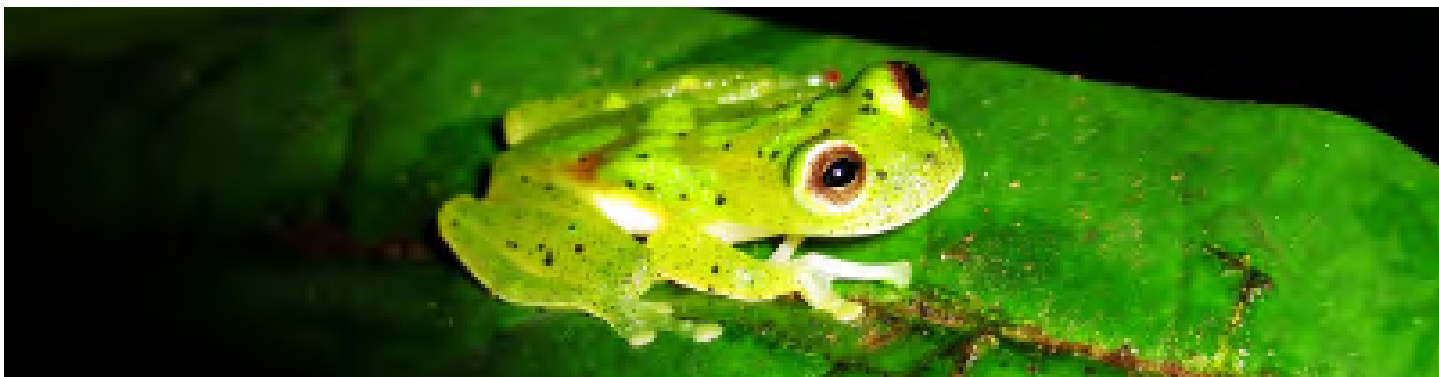
Andrew is currently working for The crees Foundation in the Manu Biosphere Reserve, SE Peru (www.crees-manu.org/) and studying for his Ph.D at the University of Glasgow (<http://www.gla.ac.uk/researchinstitutes/bahcm/staff/postgraduates/andrewwhitworth/>). Chris is a Ph.D student at the Center for Ecology and Conservation, University of Exeter (<http://www.animalsocieties.org/Chris.html>).



Front cover shot of “Frogs of The Yachana Reserve,” with a picture of stream loving frog *Osteocephalus cabrerai*.



Stunning lateral view of the Monkey frog, *Phyllomedusa tomopterna*. Photo GVI staff and volunteers.



The rarely encountered Glass frog, *Hyalinobatrachium iaspidiense*, originally only thought to occur in Venezuela; populations were found in Northern Peru and Ecuador, at the Yachana Reserve. Photo: GVI staff and volunteers.



From Top, left to Right: A male poison dart frog (*Ameerega bilinguis*) guarding his eggs. A mating pair of *Trachycephalus venulosus*. Staff and volunteers on a nocturnal stream walk. Volunteers searching the leaf litter. A highly arboreal *Trachycephalus coriaceus*, encountered for the first time after four years of surveying, in the field camp. One of GVI's volunteers and Yachana High School Students using the guide in the field. Photos: GVI staff and volunteers.



Using the guide in the field to identify a Peruvian rainbow boa (*Epicrates cenchria gaigei*). Photo: GVI staff and volunteers.

THE BEGINNING

The first step was to get out into each forest habitat, find as many species as we could and begin to correctly identify them. The volunteers helped us to conduct nocturnal line transects, stream transects and dig over 16 pitfall arrays, which were comprised of over 64 25 liter buckets—not a simple feat in a tropical forest. Volunteers were not just the physical man power in searching the forest both day and night, digging in buckets and then checking them daily but they were also the photographers and the gatherers of information from a variety of books, guides, articles and on-line resources. With the help of the volunteers, GVI field staff whom had preceded us, the expertise of the MECN scientists and countless hours looking at frogs in the lab, we could finally confidently identify the majority of the reserve's species.

TAKING SHAPE

Eighteen months on, a female sand-fly handed Chris the chance to begin piecing together the information and the photographs that we had collected since the start of the project. The three-week period in which he was receiving treatment for leishmaniasis, gave Chris ample time in the city of Quito, seated in front of a computer to produce the first draft of our field guide—the “Frogs of the Yachana Reserve.” We included at least four photographs of each species from multiple angles in order to cover all the characters crucial for identification. We also included both sexes for dimorphic species and juveniles when available. However, simple pictorial guides are not sufficient for identification either. So we also included detailed descriptions of each species in terms of their morphology, similar species, ecology, distribution in the reserve and their Red List status as listed by IUCN. We also included global distribution maps provided by IUCN which are available for use as long as you cite them and don't use the final product for commercial gain.

Despite GVI having worked at the Yachana Reserve for over five years, new species kept popping up and allowing us to add to the guide

time and time again. The current edition of the guide documents 50 amphibian species that can be found within the Yachana Reserve. Delighted with how the project was progressing we also gathered a wealth of information and photographs of various reptiles, a group of animals that are particularly understudied and lacking in information—especially in the Amazon region. A few months following the production of the amphibian guide we were able to produce further detailed, pictorial and bilingual guide to the “Reptiles of the Yachana Reserve.” Containing 59 species consisting of snakes, lizards, tortoise and caiman we could then begin to spread our gathered knowledge of a second group of amazing animals far and wide.

GETTING IT “OUT THERE”

One of the most important things to consider when you are making a field guide is getting it into the hands of the people who need it most. On our project we needed to make sure that the guide was usable not only for English speakers but also for National Spanish speakers. This led us to ensure that the guide was fully bilingual with all sections translated by two of our GVI volunteers, Edwin Vaca and Jaime Villacampa. This meant that not only was the guide useful to western researchers and tourists but also to National guides, researchers and students working in the local region.

We made sure that the guides became freely available to view and download online (see: www.cadwizz.net/frogs/). Since going live, the website has over 1800 hits from 1100 unique users. When the guide is located through search engines, the search terms are in Spanish 90% of the time—a clear demonstration that translation into the local language is of upmost importance. After the success of the guide electronically GVI saw fit to support the efforts of all of its volunteers and partners by self-funding the printing of 300 hard copies of the amphibian guide, and distributing them throughout Ecuador to various Universities, students, guides and lodges working in the Amazon.

THE BENEFITS

This project shows clearly how the efforts of volunteering can help to inspire outcomes to help raise the awareness and diversity of small private protected areas as well as National Parks. The project has enabled not only future researchers and visitors to have a base knowledge of what they can find in the local area but also has allowed local guides, biologists and students to build upon their knowledge and understanding of the species of amphibians and reptiles they can find in and around their own homes. Being able to identify something is the first step in feeling connected with it; hopefully an understanding of the threats which affect such species will follow.

We both encourage anybody looking to volunteer or to do research to seek out such projects that are attempting to provide tangible results not only for the wider scientific world but also to help inspire local people and partners to be proud and protect their own environments. A sense of pride and ownership helps to inspire action in us all.



Front cover shot of 'Reptiles of The Yachana Reserve', with a picture of a Big-headed Snail-eating Snake (*Dipsas indica*). Photo: GVI staff and volunteers.

Froglife—Engaging Diverse Audiences in Amphibian Conservation

By Sam Taylor

UK-based charity Froglife has been working for over 20 years on conservation projects for reptiles and amphibians. As commented in *FrogLog* 101 in March 2012, species diversity in the UK is limited, but there are crucial issues to be addressed none-the-less. Nearly every Froglife project involves volunteers, often including students, retired people, trainee ecologists, herpetologists and those with a general interest in habitat management, surveying and monitoring. The organization also takes a lead in coordinating the national “Toads on Roads” project, acting in a supportive and advisory role to networks of volunteers across the UK rescuing common toads *Bufo bufo* and other species in amphibian mortality hotspots.

Since 2004 Froglife has taken this public engagement work further through an active program involving community groups not typically reached through conservation volunteering. So far 12 conservation learning projects have been run with a strong emphasis on social inclusion, reaching 15,000 people as of the end of November 2012—with further work in development. Audiences have included multicultural groups, young offenders, those at risk of offending, vulnerable and disadvantaged young people, people with mental health issues or learning difficulties, communities suffering urban deprivation, vulnerably housed and homeless people and those out of education, employment or training. Consultation has taken place for increasing access to green spaces, with the aim of tackling some of the boundaries at practical and intellectual levels, widen-



“There is no such thing as a pure environmental project. A so called pure environmental project is one that has neglected its social, cultural, and economic context.” Judy Ling Wong, Director UK. Black Environment Network. (1)

ing participation in conservation activities. This has been as simple as providing free transport or wet weather gear, and more complex in providing resources for different learning styles or working on the organization’s overall communication style.



Mural on underpass in Peterborough designed and created by young people taking part in the Green Pathway Scheme. Photo: Rebecca Neal.



Pond restoration work at Railway World, Peterborough undertaken by people taking part in the Wildlife Ambassador Project (Top Left Photo: Laura Brady, Top Right Photo: Jodie Coomber). Pond creation at the Green Backyard, Peterborough (Lower Left). Photo: Laura Brady. Children attending the Life Under the Surface exhibition that won first prize in National Science and Engineering Week (Lower Right). Photo: Sam Taylor.

There has been a growing recognition during this time of the benefits to people who take interest in nature, or simply spend time outdoors. This highlights one aspect of the value in tackling barriers and a need to make activities as accessible as possible to all audiences. For example, Richard Louv's book on Nature Deficit Disorder in young people *Last Child in the Woods*(2) published in 2005, mental health charity Mind's *Ecotherapy* report published in 2007 (3) and the National Trust's *Natural Childhood* report in 2012 (4) all present evidence of improved mental or physical health and improved social skills through outdoor activities, potentially leading to increased opportunities and positive changes in lifestyle. However, Froglife's public engagement projects have a double purpose—to benefit participants whilst also helping conservation targets. This is done through bringing new audiences and greater people-power into the sector, as well as working on specific practical conservation aims. Many projects have the specific aim of linking local people more strongly with the habitats on their doorstep and encourage them to take an active interest in their conservation and management. In terms of impact on local landscapes, projects have built new ponds, created wildlife gardens and outdoor classrooms and helped with the maintenance of such areas. The level of potential people to help with practical work can be very high—for example, all young offenders in Peterborough have come to Froglife since 2006 for their community service hours, with this project delivering an average of 1,300 hours of activities in a year. The current learning projects aim to restore or create another 27 habitats by the

end of March 2013. One key finding throughout all the projects has been that many participants who have missed earlier education opportunities and/or found themselves on the wrong side of the law have strong practical interests and skills. These skills have perhaps been overlooked or not fully catered for by the formal education system—yet when given the opportunity to do something hands-on many participants in Froglife projects have been seen to flourish. They can then also go on to further volunteering and employment within the sector, as a number of participants have done so far.

There are challenges within this work, including the fact that habitats being created or managed by learners may progress quite slowly. There are other factors at play in the participants' lives than access to conservation activities, and those cannot be resolved through this work in isolation. Conservation staff also need support in working with these issues. There is the potential of distancing more traditional audiences through being perceived as “dumbing down” messages. Evaluation also remains a challenge; there are a number of tools that can and have been used by Froglife and other organizations to monitor the success of outreach projects. However, some individuals from the demographics targeted are quite itinerant, or lose touch with support services when they hit a certain age or move either physically or demographically. Combined with project funding being short term this means it can be difficult to evaluate precisely the long term impact. Monitoring the success of the conservation outputs is more straightforward, but



YMCA staff member attending a training course provided by Froglife to YMCA staff and volunteers on delivering conservation focused educational activities. Photo: .

again depends on investment generally beyond the short term nature of project funding. Part of this work is recognizing that not every project participant will undergo a profound transformation, and that habitats need continual community involvement. Despite these issues, the work remains meaningful and important for both the organization and the people involved. The most successful outcomes involve partnerships, with social organizations able to support participants beyond the scope of a conservation organization and Froglife providing positive activities, new skills and increased ownership of local wild places.

In order to make sure that projects remain strongly linked to conservation rather than social inclusion alone, Froglife created its own curriculum in 2010 with Knowledge, Appreciation and Action aims on which activities are based. Increasing appreciation is important to help protect amphibians and reptiles—tackling negative stereotypes of the animals and therefore lessen chances of persecution, as well as increasing understanding of the importance of preserving native species. As such, some activities may not appear to have direct conservation outputs, yet have Froglife’s conservation aims at their heart. These more general activities follow the principle that people will not act to conserve things that they do not appreciate and understand, and Froglife has found that creative activities are a great entry point to further learning. Creative events take every March as part of National Science and Engineering week, with Froglife’s “Life Under the Surface” event winning Best Science Event nationally in 2010. Young people created a giant “pop up” art installation of creatures found below the surface of a pond which was then used as a venue for sessions teaching about biodiversity, attended by 288 people.

Froglife very much sees this as a growth area for the organization, and will continue to explore new ways of bringing together learning and participation with conservation aims in projects, as well as looking for opportunities to increase the scientific rigour of evaluation and monitoring. The organization believes this work is crucial in continuing conservation of species and habitats into the future.

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Children splashing in puddles on Hampton Nature Reserve managed by Froglife on behalf of O&H Hampton Ltd. Photo: Sam Taylor.

Conserve It Forward

By Avalon Theisen

Social networking, email, environmental frog booths, giving presentations, hosting nature videos, all of these things are part of my ordinary life. I am 11 years old and the founder of a non-profit organization called Conserve It Forward. Amphibian conservation is our main focus. I am going to share some of my story with you.

I always loved amphibians and reptiles, and in 2009, I started taking classes with a herpetologist named Mr. George L. Heinrich. In early 2010, I also started attending frog listening workshops in Tampa, Florida, learning to identify frogs by sight and sound. I had always thought frogs were really cute, but I was learning they are also an important indicator species that let us know about the health of the environment. Unfortunately, they are disappearing quickly.

Also that same year, I took citizen science classes at Camp Bayou Outdoor Learning Center, one of my favorite local nature preserves, and was invited to pick my favorite topic to share with people at an event. I chose frogs and frog listening, borrowed a frog from my backyard, and had my very first booth for amphibian conservation. Frogs, like other amphibians, are facing a lot of problems, but there are many small things that ordinary people, especially other children, can do to help. After talking with families at that first booth, I knew I could be a voice for the environment through frogs.

These things are some of what inspired my amphibian conservation project. Now called Conserve It Forward, Inc., in spring 2012, my project became a Florida non-profit organization which has now applied for a 501(c)(3) charitable designation. We offer environmental frog booths, presentations and events that are educational, interactive and fun. Some of the places we have been are preserves, zoos, classrooms, conferences, retirement homes and festivals. Our audience includes all ages, and this is important because we strive to offer opportunities for all people to get involved.

Avalon Theisen, founder of ConserveItForward.org, has been recognized internationally for her conservation efforts, most recently as a recipient of the Gloria Barron Prize for Young Heroes for people who have made a significant positive difference to people and our planet. With a goal of pursuing a conservation career when she grows up, her hobbies include traveling abroad, animal handling and archery.



Knowledge is great, but action is even better! Whenever we talk about challenges, we also talk about solutions. I want people to walk away from us with frog-saving solutions they can put to use, and many of which do not cost a dime! Some of those ways are keeping pet cats indoors, reducing chemical use in their yards, and picking up trash in natural areas. A really fun way that involves

reusing things that may otherwise end up in landfills is turning old pvc pipes and storage containers into frog habitat.

One really cool and effective solution for saving frogs that I like to talk about is digital frog dissection. No horrible smell, no mess and frogs that can be dissected again and again (again, don't try that with a real frog!). Companies like Digital Frog International and Frogguts, Inc. offer fun software for home and school use. Digital Frog International even offers a 25% discount code to our Conserve It Forward

fans with the case-sensitive code CONSERVEFROG. All this software is really fun, kind of like playing a video game!

Many people have swimming pools, and as you probably know, frogs and other small animals can get trapped and drown in them. Products like Critter Skimmer offer a way out, and this is something I share often because pools are very popular where I live. One game I created puts this frog saving tool in the middle of a kiddie pool, and guests send frogs (fake frogs, that is!), through the air with a goal of landing them on the Critter Skimmer.



Avalon runs a Conserve It Forward frog booth at Tampa's Lowry Park Zoo. Photo: Deborah Theisen.



Avalon shows off the magnificent eyes of the Eastern spadefoot toad during a night hike. Photo: Deborah Theisen.



Avalon leads a frog listening hike using her laminated paper frogs and handheld recorder. Photo: Deborah Theisen.

In spring 2012, we created a really fun, interactive project called [Florida's Largest Human Frog Chorus](#). 327 people, 16 foot tall frog (that would be our mascot, Clover) and one happy dog croaked for a video to raise awareness for amphibian conservation. Now people can take the Human Frog Chorus Challenge and create their own chorus. This free activity will work great for all kinds of groups, like classrooms, parties or just friends and family having fun! Conserve It Forward will even supply ribbons for participants to wear, so they can proudly show they croaked in the chorus!

One exciting event that I am planning right now is the 2013 Tampa area Save the Frogs Day. I ran this event in 2011 and 2012 at Camp Bayou Outdoor Learning Center. This world-wide celebration of amphibian conservation and awareness was started by Save the Frogs, a non-profit organization that has supported my efforts. They awarded me their Nate the Newt Award for Amphibian Conservation in 2011, and a Save the Frogs Day Award in 2012. Both awards helped fund our free, educational day which is full of froggy fun and prizes for everyone, as guests play games, attend frog talks and go on frog listening walks.

Speaking of frog listening walks, I have found a unique way to do mine when there is limited time and the audience cannot get to a good frog listening location. I simply choose a couple samples of arboreal, terrestrial and aquatic frogs. I print their pictures and basic information, laminate and hide them on an indoor or outdoor route. As I lead the group and we approach a frog spot, I play a recording of the frog's call on a handheld recorder. We then stop and talk about what we are hearing. Of course, a hike at the right time of day and year in nature is preferred for frog listening, but sometimes it is helpful to be a little creative!

Conserve It Forward is my way to help people and the environment get more connected. When people better understand frogs, they are more likely to help them. This understanding is very important, because when we save frogs, we save the environment! Find a project you love, and then do something to help, even if it is a small thing, because if we all do small acts, then together we will make big changes!

During 2012, we have increased our internet presence. Our website is now more interactive. We offer ways for people to get involved, like our guest blogger program and environmental projects. Website visitors can color digital frogs that show their promise to help save frogs and the environment. This activity is based on the paper frogs that guests color to go on large banners at our booths. Video is a great way to share my adventures in nature with others, and I do this on the Conserve It Forward YouTube channel.

We have started Conserve It Forward social media accounts this year to help broaden our audience. Facebook has especially helped us connect with other organizations and people. We have met many of these wonderful and kind people during our travels this past year.

Frog Education at the Student Level

By George Sellers

The current declining status of frogs on this planet has been thoroughly documented in the literature, so this article will focus on an educational program in a public school. You could say that I am an experienced biology teacher after being an educator for over 30 years, and I have witnessed a lot of good and bad educational practices in that time period. National and state biology teaching standards seem to be here to stay, but one thing that I have noticed on a state level is the absence of conservation standards in the South Carolina biology teaching standards. It has been my goal to educate my students on the rapidly demise of frogs across the world by getting students involved in numerous projects that I hope will have a lasting effect on helping frogs. I am convinced that because of my efforts these students will continue to help frogs for a long time after high school graduation.

Ware Shoals High School, located in Ware Shoals, SC, is where I am presently teaching. Five years ago I started a National Association of Biology Teachers BioClub at the school. The students meet after school to work on a variety of biological conservation projects (1). A couple of years ago the students got actively involved in Save The Frogs (<http://www.savethefrogs.com/>), and this was a big turning point for them. Save The Frogs of which I am a member of its Advisory Board—is an outstanding source of information and ideas for getting anyone involved in frog conservation efforts. The organization sponsors frog art contests, poetry contests; help with building frog ponds, and a variety of other really good helpful projects. They will send you laminated cards to distribute that contain all kinds of current frog information for the general public and other types of material to give away. On an international level they sponsor a Save the Frogs Day celebration every year. Last year (April 28, 2012) there were over 200 Save the frog events in 39 countries and 34 states. The next Save the Frogs Day event will be April 27, 2013 and it is hoped that it will be celebrated in over 300 events in 50 countries! This would be a perfect time for you, the reader, to organize an event where you live. It is easy to do, and it is an excellent way to get the word out about frogs!

Two years ago my BioClub hosted their first Save the Frogs Day event at the Greenwood Public Library. The students set up a table in the library that contained a lot of information about frogs. For example, they displayed information on why frogs are declining



Save The Frogs Day at Greenwood Public Library. Photo: George Sellers.

ing, what people can do to help the frogs and they collected petition signatures to help stop the use of Atrazine in the USA. Dr. Tyrone Hayes has shown that low concentrations of atrazine can disrupt sexual development in amphibians even to the point of causing sex reversal in frogs (<http://newscenter.berkeley.edu/2010/03/01/frogs/>). The students also collected donations that were sent to Save The Frogs, and gave presentations to the public on the plight of frogs. In addition they had games for the kids to play and frog face painting for anyone who wanted to have a frog depicted on their face. The little kids enjoyed participating in a game to see who could eat the most flies (the flies were raisins) off a paper plate in a given amount of time. The winners were given Save The Frogs wristbands as prizes. In April 2012 the students set up a Save The Frogs table (Fig. 1: Save The Frogs Day at Greenwood Public Library) in the Greenwood Mall where they had similar types of activities going on, plus they read frog books to the kids. One of the most interesting things that happened at the mall was the number of adults that participated in the “eat a fly” contest! Some adults came back more than twice to try their luck again.

The students are really interested in frog conservation projects. Last school year they came up with the idea of petitioning SC Governor Nikki Haley to issue a state proclamation to recognize Frog Awareness Day. They researched proclamations, and they composed one that they sent to Governor Haley. Along with their proclamation they sent a petition with several hundred signatures that they had collected to show that people are interested in frog conservation in SC. The Governor’s Office was impressed with what the students had done, and Governor Haley did issue and sign the proclamation (Fig. 2: Frog Awareness Day Proclamation by SC Governor Nikki Haley) recognizing April 28, 2012 as the official Frog Awareness Day in SC. The students were very thrilled that their efforts did not

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go unnoticed. This year the students are setting their sites a little higher and are working on petitioning the President of the United States to officially recognize April 27, 2013 as Frog Awareness Day across the United States. Who knows what that might lead to!

Another program that my students are getting involved in is FrogWatch (<http://www.aza.org/frogwatch/>) developed by the Association of Zoos and Aquariums as their flagship citizen science program. The FrogWatch chapter in this area of SC is sponsored by the Greenville, SC Zoo. In this program the students attend a training session, they learn to recognize the calls of local frogs (they have to pass a test on the frog calls), and then they go to designated areas on multiple nights to record the calls that they hear. This is a great program that students can participate in to help gather useful data for frog conservation. At the end of the reporting season the Greenville Zoo's FrogWatch chapter hosts a wonderful dinner with awards presented to the participants.

Last year a group of Professors from The University of North Carolina Greensboro, Elon University and The University of North Carolina Pembroke received a NSF grant to educate rural living NC citizens about herpetology. They call their program HERPS which stands for Herpetology Education in Rural Places and Spaces. Their first meeting for the public was on May 12, 2012 from 10-2 at Cedarrock Park, NC. It was well attended, and I hosted an information table for Save The Frogs along with a NC student who is very much interested in frogs. One exciting aspect of the HERPS program is the opportunity for high school and middle school students to attend extended residential programs during the summer to learn more about NC amphibians and reptiles. This type of student outreach endeavor should serve as a model for the rest of the states. The contact person for HERPS is Dr. Catherine E. Matthews: cmatthews@uncg.edu

State of South Carolina

Governor's Proclamation

WHEREAS, with the exception of Antarctica and some ocean islands, frogs can be found in their natural habitats throughout the world, with the greatest diversity of species occurring in tropical areas where water is readily available; and

WHEREAS, in addition to serving as biological indicators of the health of our environment, frogs make important contributions to our ecosystem by eating algae and helping keep our waterways clean during the tadpole stage, providing a source of food for other animals, consuming significant numbers of ticks, mosquitoes, and other disease-transmitting pests, and eating harmful bugs that damage crops; and

WHEREAS, frogs have played an important role in research, leading to significant discoveries and advancements in the field of medicine; and

WHEREAS, from pollution, pesticides, and habitat destruction to disease, invasive species, and over-harvesting for the pet, food, and bait industries, frogs and other amphibians face a variety of threats to their existence.

NOW, THEREFORE, I, Nikki R. Haley, Governor of the Great State of South Carolina, do hereby proclaim April 28, 2012, as

FROG AWARENESS DAY

throughout the state and encourage all South Carolinians to recognize the importance of frogs to a healthy environment and the preservation of our ecosystem.



Nikki R. Haley

NIKKI R. HALEY
GOVERNOR
STATE OF SOUTH CAROLINA

Frog Awareness Day Proclamation by SC Governor Nikki Haley.

As you can see, I think that it is extremely important to get the youth involved in frog conservation activities. It is easy to do, but it does require a lot of planning. The rewards are everlasting, and the students will be better citizens not to mention good stewards for the environment. I give a lot of frog presentations during the year, but the most important ones involve students. If you are a teacher get your students involved; if you are not a teacher then get yourself involved. Please feel free to contact me for any help that you might need.

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Building Educator Capacity for Amphibian Conservation—The Houston Toad Teacher Workshop

By Rachel E. Rommel

It's Murphy's Law. Something is going to go horribly awry. Partners have spent over a year meticulously planning, developing and collating curricula, tirelessly contacting school districts and educators across a five county region, purchasing countless supplies, and pre-loading buckets full of flash drives for this one momentous event—the first ever Houston Toad Educator Workshop to take place in Bastrop, Texas on September 29th, 2012. In a drought ravaged state, and in a city that witnessed one of the most horrific and catastrophic fires known in United States history just a little over a year ago—we are now seeing torrential down pours and street flooding. There are newscasters warning of terrible driving conditions on the eve, and the morning of, our much awaited event.

Nonetheless, I am immediately reassured as we pull out of the hotel driveway at 6:00 am in route to the workshop venue and must stop to help a few Southern leopard frogs (*Lithobates sphenoccephala*) cross the road before even exiting the parking lot. The frogs are telling me all is going to be fine—it's their day to shine, and the teachers will come. And they did. Close to 60 teachers, the majority from current toad counties, braved the buckets of rain, with hot coffee and huge smiles on their faces, in addition to some even driving in from cities, like Austin and Houston. The majority of them even dutifully filled out my obnoxious pre-workshop evaluations, without any protests!

My point? If you create it, they will come. Educators care, and they want to help. They want to connect with you, hear your stories and expertise. Many of them are hungry for new information, material and resources, they want the knowledge and tools to be able to integrate lessons about native wildlife (and in our case, amphibians) in their own respective learning settings, whether the subject is biology, ecology, environmental studies, outdoor recreation, culture, art, history, music or writing. *Who are educators?* Educators are described for this purpose as either *formal*, your K-12 school teacher, or *non-formal*, which might include your city, state or national



Promotional flyer for the Houston toad educator workshop.

park interpreters, urban environmental educators, citizen science volunteers, nature center staff, community naturalists, camp instructors, zoo and aquarium educators, etc. All of these groups have the collective potential to reach thousands (if not millions) of children, and adult audiences each year through their programming. In many circumstances, as with the Houston toad, information and resources can be limited, or not readily available for educators. If available, many times they don't know where to find the information, or they don't know how to apply it. It is incredibly important, as conservation practitioners, that we consider reaching out to this invaluable resource and help build their capacity to use amphibians as a channel for exploring, understanding and valuing our natural resources, and endangered amphibians sitting on the precipice—like the Houston toad.

The Houston toad (*Anaxyrus houstonensis*), is an endemic to the sandy soils of east central Texas, and was the first amphibian to be

placed on the Endangered Species Act in the early 1970's. The Houston toad was extirpated from "Houston" by the 1960s and is currently only known to exist in nine counties in Texas, in highly fragmented, isolated and small populations. The largest population of Houston toads is believed to be in Bastrop County and biologists have estimated the population here to be between 100 and 200 individuals—that was, before the fire. The 2011 Bastrop fires were devastating; they burned too long and too hot, due to increased fuel loads resulting from over a century of fire suppression. The fire devastated up to 40% of the toads remaining habitat, killed millions of the iconic Lost Pines trees—canopy cover essential for the toads survival. Only time will tell how the toads fared.

Historically, focus on community support with the toad has been with private landowners and little attention has been given to educating the general public, teachers and students throughout their range. In a recent assessment of public knowledge and support for recovery of the Houston toad in Bastrop, Texas, (Jones *et al.*, 2012) researchers note the city's population is rapidly growing and that new residents may have zero to limited knowledge of this species. Although results from the study find that attitudes towards

the toad are generally favorable, few people made the connection between toads having value in the local ecosystem and having value to them personally. In addition, significant results found that in the 18-30 age class, all respondents did not believe the toad benefitted them in any way. This is a troubling prospect, that youth in the area may be even more apathetic to the existence of this endemic and highly endangered species than their predecessors. This age class represents our future land stewards. If the Houston toad has any hope of long term persistence in Texas, this needs to change, and quick.

In 2011, some of the partners working in the conservation and management of the toad formed a very informal Houston Toad Education Working Group—members including the Texas Parks and Wildlife state herpetologist, interpreters and resource managers from Bastrop State Park, and conservation and education staff from the Houston Zoo. To date, significant deliverables from this collaboration include 1) an *Educator's Guide to Houston Toads*, 2) A toad trunk loan program that any educator can check out free of charge, and 3) a mixed media and resource flash-drive for Houston toads, thanks in part to the hard work of collaborating institutions, and a small local grant from the Jacob and Terese Hershey Foundation. All of these materials were distributed for the first time at our first Houston Toad Educators Workshop.

The workshop included enthusiastic presentations and multi-media, led by partner educators, biologists and natural resource managers—and focused on general topics that should not just be confined to herpetological undergraduate students, such as an overview of amphibian diversity, natural history and biology, ecosystem function, habitat health and human connections to these natural processes. Global amphibian declines and causes were covered, and tangible things individuals can do on a local scale were explored. Focus was then geared regionally towards the Houston toad, its endemism, uniqueness, decline and conservation, why endangered species matter, what they might be telling us and why we should listen. After the morning presentations, the educators were rotated through break out activity stations which were designed to share resources and train in hands on activities that can be used in the classroom, or adapted for nonformal education settings. Activity stations included 1) a life cycle maze, including threats and conservation measures for various stages of the life cycle in aquatic and terrestrial environments 2) amphibian adaptations and activities for lessons on pollution and wetland health 3) a frog call activity from the TPWD Texas Amphibian Watch citizen science program 4) an advanced role play activity about habitat loss and fragmentation. Educators were given the resource flash drive

(the most significant cost of the workshop) including the educators guide, mixed media resources such as video and photographs, frog call files and activities from the workshop, supplemental resources, and contact information, etc. And perhaps the most fun

of all, the participants got to meet the live Ambassador Toads from the Houston Zoo. Results from the post workshop survey found that 96% of participants “strongly agreed” (majority at 74%) or “agreed” (22%) with the statement that they care more about wild Houston toads after meeting the live toads from the Houston Zoo. An optional field visit and interpreter guided hike to a Bastrop State Park burn site and a historic Houston toad breeding pond was available to teachers following the workshop, in which a large percentage of participants attended. A highlight for the hike was a chorus of



Workshop participants listen to presentations from partner biologists and educators at the LCRA McKinney Roughs Nature Park. Photo: Rachel Rommel.

chuckling and cackling *Lithobates sphenoccephala*, a very common species in this area, which set my mind at ease at the dawn of the day's event. For this particular workshop, logistics and weather did not lend to a large component taking place in the field, however, common sense and personal experience tells me the more you can get participants out to field sites or a local pond to contextualize information and provide direct experience with amphibians, the more of an impact you will have.

As for our evaluation, a pre and post survey was conducted to gauge participant's level of enthusiasm, values, knowledge, current practices and intent to utilize information and resources gained from the workshop in their respective education settings. Short and long term results of the workshop will be submitted for publication at a later date, however, preliminary results show success in increasing capacity and intent for utilizing the Houston toad in participants education activities. For example, there was a significant change in pre and post workshop responses in regards to whether or not educators believed they had the resources needed to teach about the Houston toad ($\chi^2=68.57$, 4df, $n=44$, $p < 0.001$). In addition, a combined ninety-seven percent (97%) of participants either “strongly agreed” or “agreed” that they intend to use information from the workshop in their educational activities. In collecting qualitative data, one district Assistant Superintendent commented on her post workshop survey in regards to what she believed was most helpful, “*The abundance of knowledge provided in reference to the Houston toads... All information provided was useful and will be utilized to introduce new programming opportunities to our target area zones.*” We had numerous comments such as this one, a potentially notable impact for just a four hour workshop. A follow up survey in the summer of 2013 will tell us if their new found knowledge and resource availability were utilized. There has been little research to determine the short and long term effective-



Educators take an afternoon interpreter led hike to a Bastrop State Park Houston toad breeding pond and burn site. Photo: Katie Raney.

ness of educator capacity building programs for an endangered species, and the factors influencing the long term use of resources by participating educators in their respective settings. We hope to provide insight through this case study with the Houston toad, into some of the factors which may influence use, and may better inform conservation practitioners wishing to implement similar educator programs.

In conclusion, and on a personal note—after the workshop, and over pizza, a natural resource manager and fire-fighter from Texas Parks and Wildlife said to me “*you guys served these teachers real*

well today.” I believe that was one of the nicest things anyone has ever said to me, and the truth is, those experiences serve us as well, and I have hope, in turn, those educators will help us serve amphibians too. Right after the workshop, a teacher followed up with a landowner who has been doing active habitat restoration for the toad. The teacher wants her kindergarten class to make native bunch grass seed balls to broadcast on her property, for the benefit of the toad, so they will have protection, and lots of bugs to eat. It’s small, local accomplishments and collaborations like these that give me hope. We must continue to build bridges between conservation biologists and educators, and those that want to learn, and help. We need an army of amphibian advocates on our side.

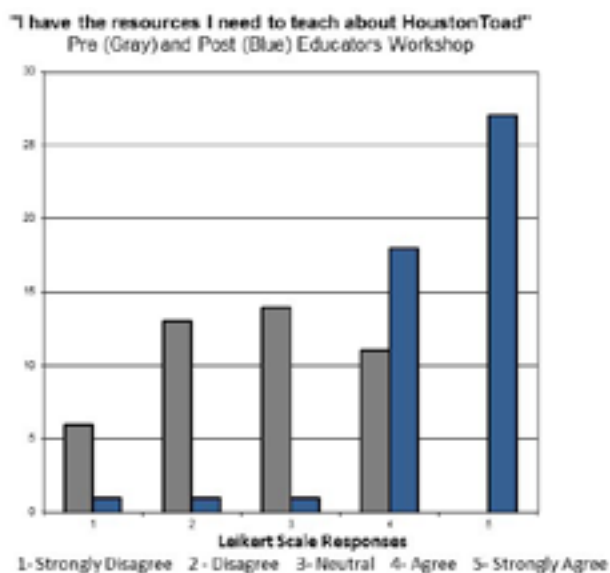
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Partners: Texas Parks and Wildlife Department, Houston Zoo, Inc., Lower Colorado River Authority, Amphibian Ark, Jacob & Terese Hershey Foundation

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Amphibian and Reptile Groups of the UK

By ARG UK

The Amphibian and Reptile Groups of the UK (ARG UK) are unique. They are a network of local, volunteer groups dedicated to the conservation of amphibians and reptiles in the United Kingdom (strictly speaking the British Isles, as there are links with the Channel Islands and the Republic of Ireland). Most groups are based on counties (historic or administrative regions), the first being established in Surrey and Sussex in 1987. Two years later a national umbrella group was established, the Herpetofauna Groups of Britain, later expanding to include Ireland and finally changing its name to the Amphibian and Reptile Groups of the UK in 2005.

Amphibian and Reptile Groups (ARGs) within the network vary greatly, their nature being determined by the available pool of volunteers, their particular interests and local conservation priorities. Other than a passion for amphibians and reptiles perhaps the only other common factor is recording—mapping the locations where herpetofauna is found. Some groups have even published county herpetofauna atlases (1, 2).

ARGs are nodes of local expertise. In a geographic area with a long interest in natural history but with only a very limited herpetofauna (a dozen or so native species and a similar number of established non-natives), and where large numbers of people can tell one small brown bird species from another, at a great distance, it is aston-

ishing how many cannot identify our few amphibians and reptiles. ARGs are often involved in education and training, running courses for professionals and amateurs. Some carry out site visits, advising garden pond owners about amphibians or in some cases making an input to the management plans of larger areas managed for nature conservation.

Some groups carry out practical work, for example managing or creating amphibian ponds. Many groups are involved with toad crossings—helping migrating Common toads *Bufo bufo* across roads.

ARG UK is the umbrella for the local groups. It is governed by a panel, originally intended to facilitate communication between the central body and the constituent groups. This function has been overtaken by developments in information technology and now the panel's role comprises essential administration and support of the network. This includes providing an insurance scheme, a newsletter, website (www.arguk.org), advice notes and administering two small grants schemes, one aimed at research, the other at funding conservation projects. Of the latter amphibian pond creation and restoration have been the most commonly funded projects.

A significant change within ARGs over the years has been the nature of membership. Local group members are volunteers. Initially



A Smooth newt being swab sampled by volunteers taking part in the national chytrid screening programme. Photo: Chris Allen.



(Top Left) Hampshire ARG chytrid swab sampling. Photo: Rachel Hardy.
(Lower Left) ARGs are often involved in education and training. Photo: Darren Rowley. (Above) HART Atlas.

most group members pursued their interest in amphibian and reptile conservation as a pastime. But more recently the consultancy sector has expanded and many “hobby-herpers” have become professional, carrying out survey work driven by the demands of legislation, mostly related to building development. So although ARG members are volunteers, the backbone of many local groups now comprises professional ecological consultants who, unpaid and in their own time, provide support to the local groups.

The strength and weakness of ARG UK is that it is a network. It is difficult to determine a majority view on a specific issue and local groups are generally keen to maintain their individual identity. Nevertheless, linking local groups through a network allows information exchange and provides a framework for national projects. ARG UK has a working agreement with Amphibian and Reptile Conservation (www.arc-trust.org), the professional organization with a national remit. The network of local voluntary groups and the national professional body have complementary roles. They work together on national recording projects, and are currently developing an online recording system, the Record Pool (www.arguk.org/recording). They also share responsibility for organizing the Herpetofauna Workers Meeting, a weekend event of presentations and workshops. This is not a research conference but instead caters for those involved in practical herpetofauna conservation in the region. Hence it is attended by a mixture of academics, con-

sultants, volunteers and public sector employees, creating a forum for information exchange and, equally importantly, a networking opportunity.

ARG UK has also collaborated with the Institute of Zoology (based at London Zoo), providing the volunteer network to carry out a national program of chytrid screening. In 2008 and 2011 almost 9,000 amphibians were captured and swab sampled from 185 sites across the UK with volunteers contributing more than 3,000 hours of time (Freya Smith, pers. comm.), facilitating what must be the world’s most comprehensive chytrid screening program to date. This is an excellent example of citizen science, linking Amphibian and Reptile Groups with a local interest to a national project which is in turn addressing one of the global issues of amphibian conservation.

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Scientific School Work of Amphibian Monitoring in Patagonia



Watching an adult male of *Alsodes coppingeri* in Villa O'Higgins' forests. Photo: Javiera Cisternas.

By Javiera Cisternas

During 2011 the NGO “Aumen o el eco de los montes” developed the project “Investigating shapes, colors and songs of our amphibians” in four schools of Patagonia, Chile. The main objective of this project was to improve children’s knowledge of their local amphibian species. This was achieved by presenting purpose—designed posters, books and compact discs of amphibian’s calls in each working day, conducting field activities of watching tadpoles and adult amphibians near their schools and visits from amphibian experts (university teachers and doctoral students) to their classrooms.

The impact of the project was so successful that a group of four children from the school “Pioneros del Sur” at Villa O’ Higgins locality, decided to research into specific issues of the amphibians that occur nearby. Through e-mail exchanges between the manager of the school and the NGO, the children were presented with a range of different topics for them to investigate. Finally, they decided to separate into two groups to investigate two important issues; “Fungal and bacteriological effects of skin secretions from *Alsodes coppingeri*” and “*Alsodes coppingeri* the strange frog that didn’t sing.” The results of both projects were presented in the Regional Scientific Congress Aysen Region awarding First prize on Primary Education and First prize “Forjadores Ambientales” respectively.



Watching a tadpole of *Alsodes coppingeri* in Villa O’ Higgins. Photo: Javiera Cisternas.

With these awards they were able to compete at the National Scientific Congress from Chile obtaining First Prize on Quality of Presentation.

Furthermore, the children maintain constant visits to frog breeding sites surrounding their school and make natural history observations, which are well-known for some species, but also unpublished for others. An example of this were the unprecedented observations collected for *Alsodes coppingeri* such as the recording of mating calls, description of egg-laying and determination of time of reproduction for this species in this locality.

Furthermore, they extended the geographic distribution of *Pleurodema bufoninum* which was thought to only live on steppe environments.

Given the success of this experience, Aumen NGO decided to initiate a joint monitoring program with “Pioneros del Sur” school, achieving the first step on the evaluation of appropriate indicators to monitor *Alsodes coppingeri* populations on a long term.

South Africa's First National Awareness Day for Frogs: 1st December 2012

By Jeanne Tarrant

The 1st of December 2012 saw the first of what is hopefully to become an annual event on the South African conservation calendar. SAVE OUR FROGS DAY was organised by the Endangered Wildlife Trust's new Threatened Amphibian Program (TAP) and implemented by various interest groups, institutions and zoos throughout South Africa. The aim of the day was to highlight the importance of frogs and include the general public in some froggy activities.



A school group enjoying the frog stand at NZG, Pretoria. Photo: Michael Adams.

Ten events took place around South Africa. In KwaZulu-Natal talks and outings were held at uShaka Marine World and Mt Moreland Conservancy. Alfred Park in New Germany hosted its usual "Froggy Operatics Evening" and the Honorary Officers at Vernon Crookes Nature Reserve spent the evening searching the dams on the reserve.

In the Western Cape the Western Leopard Toad Committee organized a family day out at Noordhoek Farm Village with music, face painting, Lenny the Leopard toad, a farmers market and kids tombola.



Western leopard toad face-painting at Noordhoek Farm Village for Save Our Frogs Day in the Cape. Photos: Alison Faraday, South Peninsula Outstanding Toad Savers.



The National Zoological Gardens Reptile Park in Pretoria set up two educational gazebos and two poster stands. This included two terrariums, one with Painted reed frogs and one with a Banded rubber frog to help showcase some of our South African frogs!

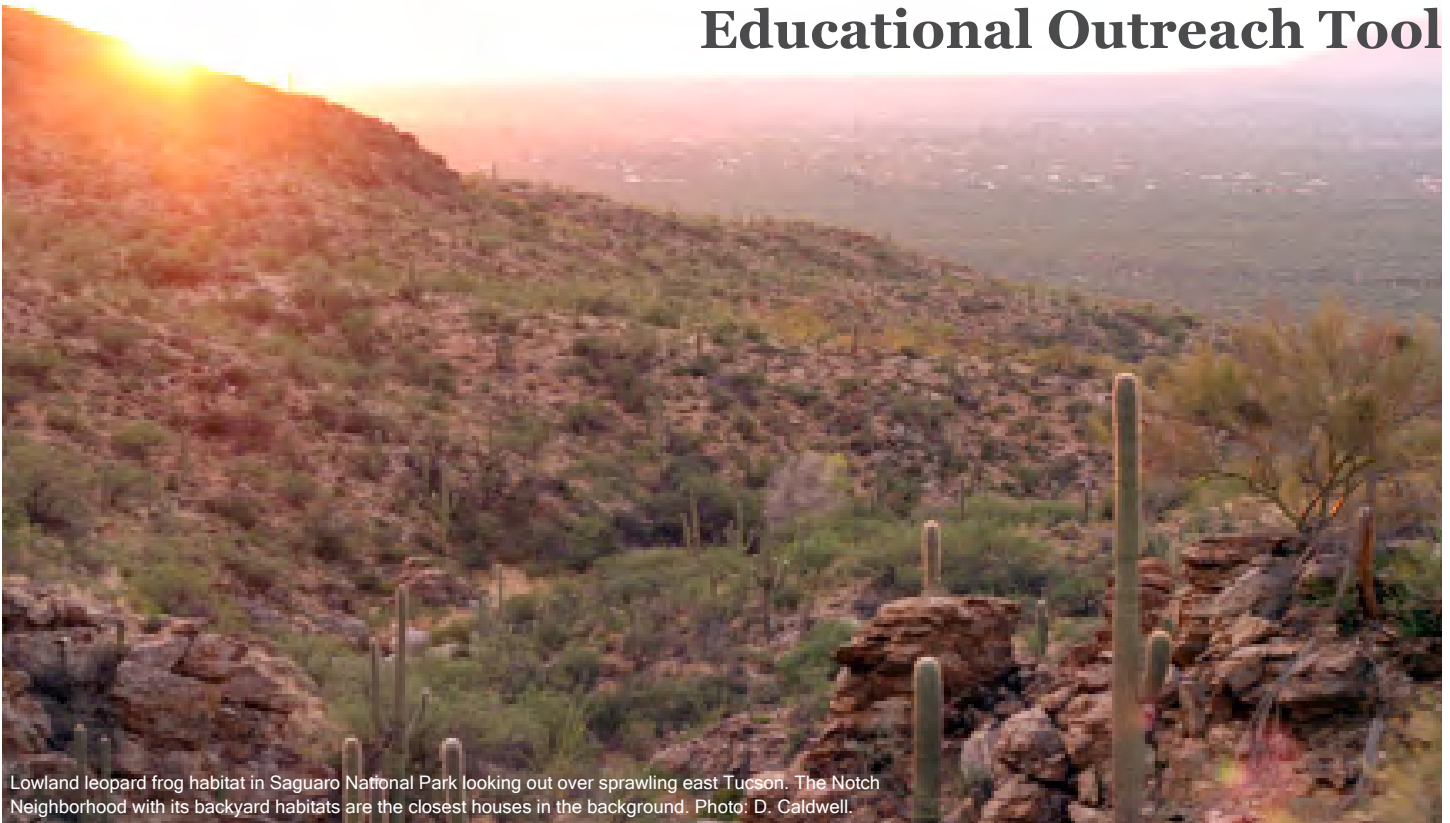
Other events took place at Johannesburg Zoo; in the Eastern Cape at the St. Francis Bay Community Gardens; and in the North-West Province, Potchefstroom. The African Amphibian Conservation Research Group from North-West University put on a great show in Potchefstroom with fact-filled presentations, demonstrations on chytrid swabbing, pit-tagging and bioacoustic monitoring followed by an outing to the Botanical Gardens.

In total about 100 people learned more about frogs-a number we can certainly improve on! A big thank you to all those who took part in making the day a success. Some valuable lessons have been learned and will be put toward making the next SAVE OUR FROGS day a bigger success! For more information on the EWT-TAP please contact: jeannet@ewt.org.za



Releasing a toad at Mount Moreland, Durban on Save Our Frogs Day, South Africa. Photo: Angie Wilken, Mt. Moreland Conservancy.

Arizona Leopard Frog Conservation as an Educational Outreach Tool



Lowland leopard frog habitat in Saguaro National Park looking out over sprawling east Tucson. The Notch Neighborhood with its backyard habitats are the closest houses in the background. Photo: D. Caldwell.

By Dennis Caldwell

Over the past 12 years I've been involved in many conservation outreach projects involving amphibians of Arizona, primarily leopard frogs. Thanks to the editors of the *FrogLog*, I have the opportunity to reflect on some projects that have had, sometimes, surprising results. Though many of the conservation needs outlined here are unique to this region, the Desert Southwest, most of our techniques are fairly basic and will be applicable elsewhere and are surely already being implemented by others on a grander scale.

Based in Tucson, Arizona, the primary species of leopard frogs I work with are the Lowland leopard frog, *Lithobates yavapaiensis*, a low elevation desert stream frog and the federally Threatened Chiricahua leopard frog, *L. chiricahuensis*, a higher elevation frog from our grassland cienegas and mountain streams.

The primary conservation concern regarding aquatic species in the desert southwest is water. Many of our natural streams and wetlands have dried due to over pumping groundwater for human needs. Additionally, climate change predictions point to future droughts. Of the perennial aquatic habitat remaining, most have been converted to recreational sport fisheries, stocked with non-native predatory fish, which are incompatible with native frogs.

Problems with invasive species compound when urban areas interface with natural areas. For example, private ornamental ponds and golf course waters often harbor breeding populations of non-

native American bullfrogs (*Lithobates catesbeianus*). Young bullfrogs disperse during the summer rainy season and travel amazing distances to find new habitats. This creates conservation challenges in natural areas nearby and many of our projects focus on buffer zones; the interface of private property and key leopard frog conservation areas like Saguaro National Park and Las Cienegas National Conservation Area.

FROG CONSERVATION PUBLIC OUTREACH

About ten years ago I was asked to help manage a project that was a collaborative effort between Arizona Game and Fish Department



A backyard frog habitat constructed in east Tucson. Photo: D. Caldwell.

FROG Conservation Project, Frog & Fish Restoration Outreach Group

and Saguaro National Park in Tucson. The goal was to work with a neighborhood bordering Saguaro National Park to develop backyard habitat for the lowland leopard frogs of the Park. Initially this was a simple way to bank a precarious lowland leopard frog population against problems like drought, fire-induced erosion, and disease. As the project developed, we soon learned that the educational aspects of this project far outreached our initial conservation goals.

At the start of the project, many residents didn't know that their neighboring "desert park" had any perennial water, let alone native frogs. It was hard to convince them that bullfrogs from near-by golf courses or neighboring backyard ponds could be a threat to wild populations of native leopard frogs in the high canyons above their neighborhood.

The neighborhood newsletter intently followed the progress of the project. Due to a drought, the team initially had problems finding a single egg mass or tadpole from the Park canyons to introduce to the backyard habitats. As the neighborhood residents learned about leopard frogs, they also learned about other threats to native frogs like drought and a deadly amphibian disease (caused by a chytrid fungus, *Batrachochytrium dendrobatidis*). While bullfrog immigrations into the ponds threatened the project, it also raised community awareness of the problem.

- These families developed an appreciation for this species plight through:
- Learning about drought impacts on aquatic species
- Learning about leopard frogs
- Learning about amphibian diseases
- Learning about invasive species
- Learning about habitat needs of leopard frogs
- Participating in hands-on conservation
- Passing along knowledge to friends and neighbors
- Putting pressure on neighborhood bullfrog sources
- Advocating leopard frog conservation

At Las Cienegas National Conservation Area south of Tucson, I have been part of a team of biologist working on a National Fish and Wildlife Foundation funded project to recover the Chiricahua leopard frog population in Cienega Creek and the surrounding watershed. Perhaps our biggest challenge is to keep invasive aquatic

species out of the valley. The valley is populated with a few scattered ranches and a small but sprawling, low-density community sitting at the top of the watershed on the headwaters of Cienega Creek, the primary perennial stream of the recovery area. A growing number of these properties have ponds with established bullfrog populations. Outreach to this community is a huge priority.

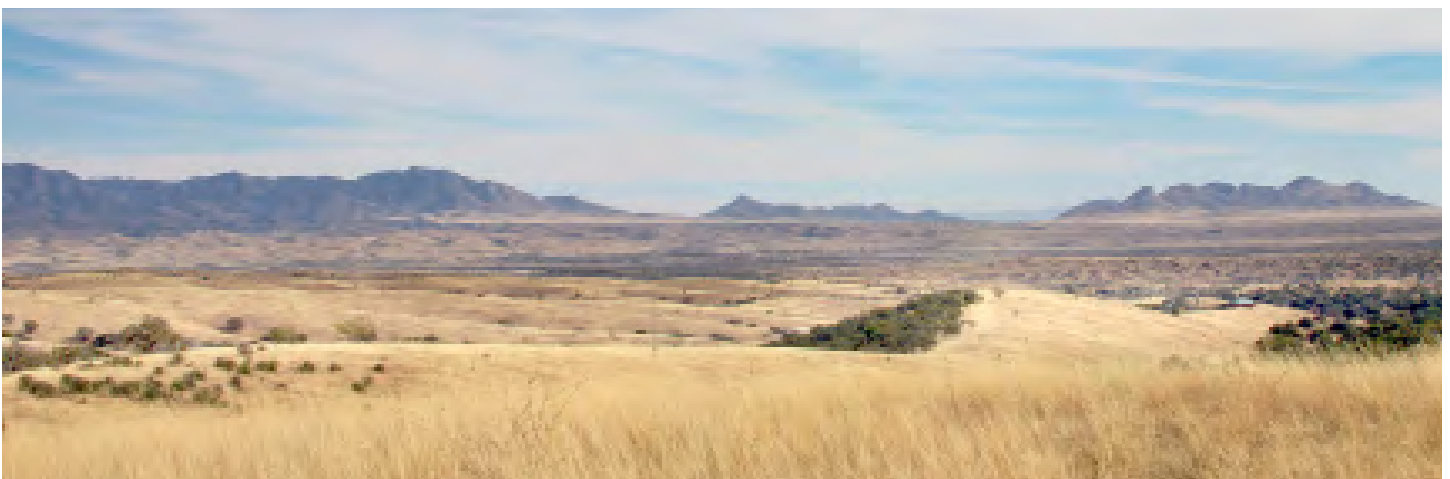
Since the Chirichaua leopard frog is protected federally, a "Backyard Habitat Program" has proved to be impossible to implement due to permitting and management burdens of such a large-scale, long-term effort. So we set up field trip activities for local school students where we can develop awareness of aquatic species and habitat conservation. In the process they learn about the invasive species problems in their community and can help spread the word.

Working with a local high school environmental education class we set up a survey protocol for visiting habitats on the Conservation Area. Every other month the class visits 1 to 2 sites performing a site survey. They record the results of each survey in a database to perform comparisons and develop profiles for each site. A scaled down version of these activities is also used for one-time-only field trips from other classes and other schools.

Invertebrate sampling provides fun, hands-on learning with low impact to the aquatic community. This was a work-around to avoid direct stress to the frogs and tadpoles with the added benefit of monitoring the macro invertebrate dynamics of each site.

Survey sites need to be coordinated with BLM and FROG Project. At least one biologist with a State and Federal Chiricahua leopard frog permit needs to be supervising any dip netting. Avoid disturbing any egg masses, dumping testing solutions near water and contaminating habitat with equipment used at other sites (disease control).

The Youth Engaged Stewardship Program, funded through the Arizona Game and Fish Department's Heritage Fund, is designed to encourage youth to participate in on-the-ground conservation by managing a conservation project on federal land. The students were awarded the grant money managed by an NGO and had to decide amongst themselves, from three different conservation projects, which site they would steward.



Las Cienegas National Conservation Area where efforts to recover the Chiricahua leopard frog have provided great learning opportunities for local schools. Photo: D. Caldwell.

After an introductory field trip they spent most of a day thoroughly evaluating each project to determine which would have the greatest impact. They chose the leopard frog project. As their site they selected one of eight live-stock ponds in the process of being converted to Chiricahua leopard frog and native fish habitat on Las Cienegas National Conservation Area.

The conservation area includes a working ranch with an active grazing operation. As an effort to share drought-proof, ranch managed waters with aquatic species and cattle, our team and the land managing agency, the Bureau of Land Management, have worked out a conservation relationship with the rancher that presents many mutual benefits and a few challenges that the students will need to figure out.

The students will prioritize their budget to get the most conservation bang-for-the-buck. They will work on habitat improvements like plantings and rock structures as well as installation of a cattle proof welded pipe fence. They identified the importance of public outreach and will create an interpretive sign at the site. They plan to launch a website/blog to try and engage other youth to follow their efforts and to help carry the project forward to future student stewards of this site.

Although the project is in its infancy, I think this is going to be an incredible tool for developing deep conservation awareness among youth as well as their families and communities.

CONCLUSIONS

Frogs and tadpoles have potential to be great teaching tools for kids of all ages. They offer a mechanism to engage the public in real, hands-on conservation especially when political and economic climates make many feel powerless to make a difference.

Many of the projects involving high schools strive to use real science to develop useful data but my experience is that this is impractical. The time frames necessary to develop skilled observers are too long, the repetition and structure of data gathering can become tedious and boring. We want the students to have fun seeing and learning new things in the short amount of time we have with them.

Too many of the outreach projects I've been involved in are small in scope and don't reach enough people or schools. Habitat sites are too sensitive for repeated mass field trips and are usually remote and full of hazards. Field trip transportation is always a problem for schools. Classroom learning is much more practical on a large scale and might be an alternative.

Perhaps exemptions to Endangered Species Act protections could be developed for classroom outreach projects where students could raise tadpoles in aquariums. Classes could be linked to students raising frogs at other schools through social media to communicate better husbandry techniques or to drive competition. Frogs that are successfully raised could be used in reintroduction projects. This could be a way to offer a lot of students the experience of contribut-



High School students counting aquatic invertebrates at Las Cienegas National Conservation Area. (Top), The Youth Engaged Stewardship group learning about Chiricahua leopard frog conservation efforts at Las Cienegas National Conservation Area. (Lower). Photo: D. Caldwell.

ing to the recovery of a sensitive species. Risks could be mitigated so the rewards greatly outweigh them.

I'm hoping there are a lot of creative outreach projects utilizing frog conservation and that *FrogLog* will be a venue for others to share their ideas. As conservation biologists, we need to facilitate skilled educators to give them the right tools to engage the public.

Western Toads as Wildlife Ambassadors

By Howard O. Clark, Jr.

Chances are, most kids are being raised in an urban setting, and wildlife appreciation is likely not a top priority. Competition from other attention-grabbing activities pushes this important pastime down to the bottom of the list. However, there are ways to pique a child's curiosity toward the wild side. I currently reside in such an urban setting, but I do not allow such a disposition to hold back on natural explorations. Surprisingly, our yard each evening comes alive with a rather ubiquitous and adaptable amphibian, the Western toad (*Anaxyrus boreas*). During the day the toads hide under garden rocks, decorative railroad ties and other items that provide sufficient cover and moisture. After the sun sets, hoards of toads hop out of their hiding places in search of insects and invertebrates. I do not use pesticides so there are plenty of choices for the toads. My compost pile provides a source of food too—from beetle grubs to worms.

My daughter has taken a special interest in our toad population. I began showing her the toads in our yard a few years ago in order to get her acclimated to something typically considered "slimy and gross." Now she asks to see the toads and several times a week before bath time we head out on a toad hunt. Sometimes she picks up the toads herself, but oftentimes asks me to do the initial grabbing. She is quick to take the toad from me, being careful to watch out for the emptying of the bladder toads are well-known for. She brings the catch into the light and carefully examines it. She recently no-



Western toad (*Anaxyrus boreas*) in a Fresno County, CA, backyard. Photo: Howard O. Clark, Jr.

ticed that they have little hands that look astonishing similar to human hands. I instruct her to hold the toad very carefully and not squeeze too hard. She is thrilled that some toads squeak (the males) and some don't (the females).

At five years old, my daughter is gaining an appreciation of nature which will hopefully stick with her for the rest of her life. During her 5th birthday party, we invited her pre-school friends and neighbors for a day of celebration. After the initial festivities some of the children ran off to the garden. I took this opportunity to catch a toad and explain the difference between a toad and frog, two species oftentimes confused for one another. The children were thrilled at the discovery and I believe repeated positive experiences such as these will enhance their understanding of nature and wildlife.

These sorts of activities no doubt provide a high level of enrichment crucial to developing young minds. I encourage everyone with young children or grandchildren to find similar activities so that nature can be an important part of their lives. Too often I see parents overreact when their kids try to get closer looks at nature, but with a little education on the parents' part, and with good judgment, natural exploration can be fun and exciting.



Certified Wildlife Biologist Howard Clark showing a Western toad (*Anaxyrus boreas*) to a group of children. Photo: Becky Chase.

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FrogLog Schedule

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



Robin Moore / iLCP

Educative and Participative Amphibian Monitoring For a Wider and More Effective Conservation



Observing young fish that coexists with amphibians in upstream habitats after wet years. Photo: W. de Vries.

By Wouter de Vries, Adolfo Marco & Edo Goverse

Sierra Norte Natural Park in the Biosphere Reserve “Dehesas de Sierra Morena” in Southwestern Spain (respectively 177,484 ha and 424,200 ha), has a diverse amphibian community with metapopulations of 12 species (1). Since 2005 data was recorded on ecology, phenology and habitats, while an overall decline of biodiversity was indicated by naturalists and land users in the region. In 2011 an educative amphibian monitoring program was developed and initiated to define and hold the scale of loss of biodiversity and to improve and direct conservation actions. In the region of Sierra Norte Natural Park (80 km north of Seville) few people have affection with nature and there are no finances for monitoring by professionals. As in other regions with an actual high conservation value there is a low accessibility and (local) volunteers will not easily commit to a longer term monitoring. However there is a large pool of interested students and herpetologists for whom it is a pleasure and adventure to participate a weekend in sampling amphibians together with local naturalists, managers and land-owners and users in a nature reserve. In Sierra Norte Natural Park the commitment for the monitoring is made by the local NGO AMBOR while every year experienced and other interested persons, including local naturalists, land users and owners are involved. An

important element of the educative and participative monitoring is that the organizers are based in the region and have a continuous close relation and exchange with both naturalists and land owners. These persons are actually the ones who influence most on the conservation of the amphibian community and metapopulations via their local acting. The monitoring weekends create an environment in which there is an important exchange between the locals that understand about land management and the functioning of the ecosystems, and scientists and other herpetologists with their knowledge on amphibian recognition, ecology and conservation requirements.

OBJECTIVES

The main goal of the educative monitoring in Sierra Norte Natural Park is to reach an overall improvement of the conservation status of amphibians. The objectives include monitoring of both populations and habitats throughout the region with a quality guarantee of the results. The methods were developed such that part of the data can be directly incorporated in the Spanish National Amphibians Monitoring Program (SARE). At the same time the monitoring allows participants to learn on species recognition, detection methods and increase awareness on ecology and conservation.

METHODS

Out of 400 aquatic sites with data on amphibians from the period 2005 to 2010 (W. de Vries and Association AMBOR), 121 sites

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were selected for monitoring throughout the park so that the species distribution can be mapped over the entire park territory (24 UTM 10 x 10 km squares). Depending on the conservation status a minimum number of sites were included for each species (40 sites for Vulnerable species: 1 species; 20 sites for Near Threatened and European Habitat Directive Annex VI/HD—VI species: three species; Near Threatened or HD—VI 10 sites: six species). Moreover there were included 20 sites of each of the five more common or more valuable amphibian habitat types (cattle ponds, ponds with vegetation, temporary ponds, temporary streams and concrete water basins). All sites are sampled twice in the breeding period, including two detection possibilities per species per year with one weekend in winter and one in spring. Sites were grouped into areas that are sampled during both day and night. Each group consists of several participants including an experienced herpetologist, a local volunteer and others and is equipped with field materials (nets, wading—suits, lamps, field guide in color, photo-aquaria, etc.). The sampling methods are standardized and combine audio and visual survey, lifting and sampling by net. Authorization is obtained from the regional government (Consejería de Medio Ambiente de la Junta de Andalucía). Data is collected on standard forms and pictures and videos are taken to validate the determination and sampled site. After sampling, data is directly evaluated and validated: confirmed-probable-possible-improbable-confirmed incorrect (reliability 1-5). Data is stored in a database together with the images. “Confirmed” (picture or video) or “probable” (lifestage can be distinguished by expert) data is accepted for further analyses.

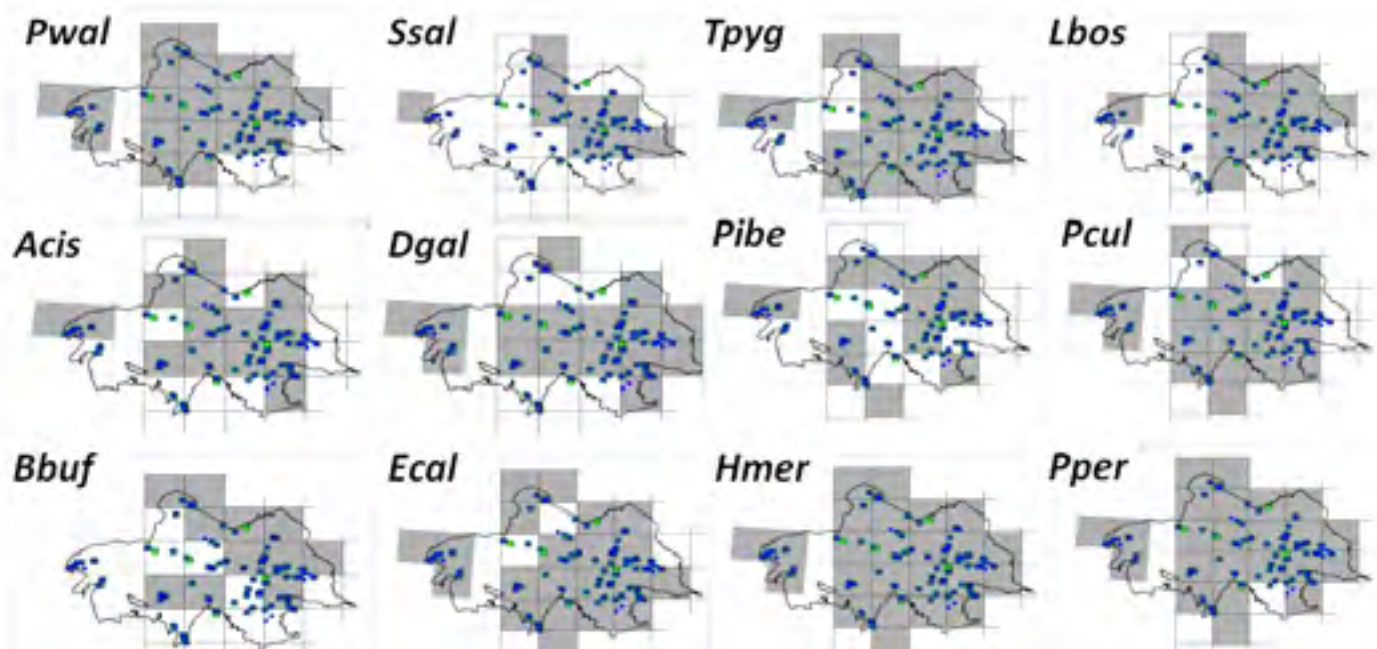
RESULTS

In 2011 and 2012 63 persons were directly involved in the monitoring, among which 6 professional herpetologists, 25 locals, 7 volunteers of the Red de Voluntarios de la Sierra Norte, 3 nature managers, 15 students, 5 landowners and 8 foreigners. Since several persons participated in both years, the efficiency off the field work and data collection increased and determination of the species in the larval stages improved. One hundred and ten preselected sites

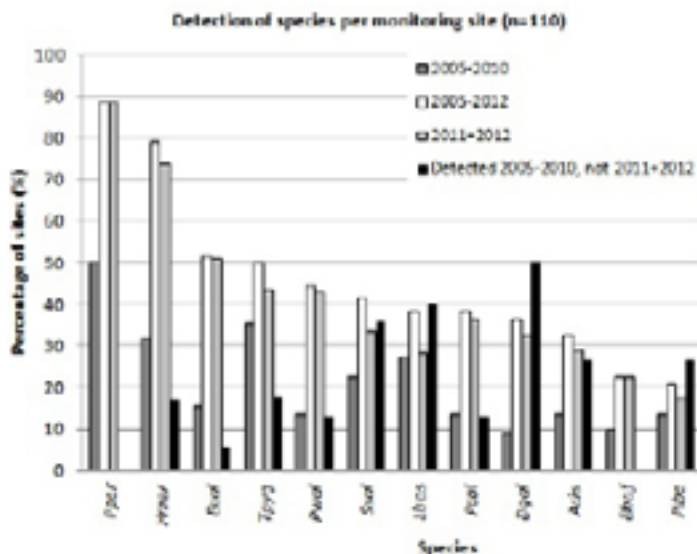


Night sampling with small field aquaria and torches. Photo: G.W. de Vries.

were sampled in both 2011 and 2012, including 20 sites of each habitat with exception of temporary ponds (n=14). Participants could observe all 12 species in the weekends in the first year for it was a wet year and the metereological conditions were optimal. In 2012, on the contrary, was an extremely dry year with low temperatures during the sampling and 11 species were recorded during each weekend. In 2011 and 2012 there were recorded respectively 11.484 and 2.858 larvae and 2.312 and 775 adult amphibians. Already in 2011, each of the target species was detected in the minimum number of required sites and the distribution of most species was mapped for the entire natural park territory within the monitoring sites (Fig. 1). Participants learned to recognize amphibians in the distinctive developmental stages and to recognize valuable amphibian habitats. The results from 2011 were available during the sampling in 2012, allowing participants to observe amphibians in the field and, on paper, the population that used the site in the previous season. The less favorable conditions in 2012 had as a result that much less amphibians were observed and none of the species was recorded in more sites than the previous year. From eight out of 10 target species on the average 12.0 % (± 10.9) additional sites



Distribution of the 110 sampled sites (blue and green dots) and species distribution on a UTM 10 x 10 km square as detected by the monitoring in 2011. Pwal: *Pleurodeles waltl*, Ssal: *Salamandra salamandra*, Tpyg: *Triturus pygmaeus*, Lbos: *Lissotriton boscai*, Acis: *Alytes cisternasii*, Dgal: *Discoglossus galganoi*, Pibe: *Pelodytes ibericus*, Pcul: *Pelobates cultripes*, Bbuf, *Bufo bufo*, E/Bcal: *Bufo calamita*, Hmer: *Hyla meridionalis*, Pper: *Pelophylax perezi*.



Detection of species previously to and during the monitoring. Percentage of sites with recordings of each species in 110 preselected sites previous to the monitoring (2005-2010) and accumulated previously+monitoring (2005-2012), during the sampling in both monitoring years (2011+2012) and percentage of sites in which the species was previously recorded (2005-2010), but not so in the 2011+2012 monitoring (abbreviations of species, see Fig. 1).

were recorded in 2012. After two years, there is a high proportion of sites in which the species were recorded in 2005-2010 period but not in 2011 or 2012 (Fig. 2). This is partly caused by conservation problems, but also a consequence of difficulties to detect or determine some of the species and strong currents in stream habitats in 2011.

The results from 2011 and 2012 stress the needs for conservation actions even without evaluating breeding success. Habitat had been destroyed in 17.3 % (14.5 % in 2011, plus 2.8 % in 2012) of the preselected and sampled sites with valuable habitat for one or several of the target species in 2005-2010. They were altered so much that the populations of target species cannot reproduce anymore: active filling in and dumping, introduction of fish, presence of American crayfish (*Procambarus clarkia*), broken concrete water basins and eutrofication. In 2011 an additional 13 % of sites had unsuitable conditions that might be temporary or belonging to the local conditions: filling due to erosion, active cleaning of basins and fish population in natural habitat. The drought of 2012 caused that 55.4 % of sites were dry or just filled with rain the days before sampling and stimulated land users to clean in winter concrete water basins that are normally washed in summer or had been without use for years. In 2012 several of the ponds that were filled up due to erosion had been cleaned by the land users and fish disappeared naturally from several stream stretches, demonstrating that temporary unavailability forms part of the dynamic in the area. Apart from obvious conservation problems, other less conspicuous problems were observed such as road kills, drowning of amphibians in constructions, leeches on amphibians at eutrophic sites or areas with intensive livestock. Analyses in the laboratory of the National Museum of Natural Sciences (CSIC) on a dead *Pelophylax perezi* confirmed the suspicion that the Chytrid fungus *Batrachochytrium dendrobatidis* is present in the Natural Park territory, though, during the monitoring, no large mortalities were recorded.

Since the need for conservation actions is demonstrated by the first results, the regional authorities have expressed their interest

to support conservation actions in the Natural Park territory and various small scale projects were designed. The positive feedback of landowners, users and local naturalists form a good basis for conservation on the long run and already various locals will remove fish from their sites or otherwise improve conditions for amphibians. Without exception the involved persons have obtained a wider understanding of the conservation needs and ecological requirements of amphibians and participants have started amphibian research and monitoring outside the Natural Park region. We hope the educative amphibian monitoring will reach amphibian populations in other regions with few conservationists and a high amphibian conservation value and needs.

SUMMARY

The educative and participative amphibian monitoring combines education and awareness rising on different levels to reach an improvement of nature conservation on a landscape scale while the amphibian community, habitats and natural dynamics are monitored by a mixed group with locals and experts. The method incorporates species distribution, relative abundance of the species, development of habitat, education of naturalists, students, nature managers, scientists, land owners and users as well as nature managers. Moreover, it gives a frame work for directing and evaluating nature conservation actions and scientific research. By combining the monitoring with education a wider audience is reached and involved than in conventional monitoring by individual volunteers or professionals, while at the same time the impact on the valuable protected area is limited. The involvement of professionals and the international character combined with inhabitants of the region allow exchange and initiatives. The large set of habitats and changing conditions over the years, make the monitoring an interesting event to participate in for many years. To lower the effect of the monitoring on the amphibian community, monitoring is done annually during two weekends and disinfected (local) materials are used. The monitoring weekends in 2011 and 2012 gave valuable information for conservation and will form the basis for a conservation plan.

Acknowledgments

The initiation of the monitoring project was coordinated by Edo Gorse from RAVON Foundation and financed by WWF Netherlands (INNO-Fond), the continuation was financed by participants, AMBOR and Natura Cerca and the Red de Voluntarios de la Sierra Norte (JA) financed the participation to several members. The educative monitoring was designed by combining key approaches on monitoring or education from Amphi Consult and Pohjakonn (exchange-survey workshops), RAVON Monitoring Network (monitoring with volunteers), AMBOR (educative studies with volunteers), IVN (training of guides) and SARE (Spanish Amphibian Monitoring Program). Permissions for the monitoring study were obtained from the regional government (Consejería de Medio Ambiente de la Junta de Andalucía) and thanks to the support from the Técnicos de Medio Ambiente from Sierra Norte Natural Park, Seville University, Doñana Biological Station (EBD-CSIC), Estonian Ministry of Environment and the Spanish Herpetological Asociación (AHE). Moreover the monitoring would not be successful without the cooperation of both public and private land owners and users and all participants. Accommodation was offered by Granja Escuela La Sierra and La Fundación.

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Amphibians in India and Their Role in Animal Science Teaching

By Suman Pratihar

When we hear the word amphibian, it instantly reminds us of a picture of the rainy season with the croaking sound of “Wak.... Waak.... Wak...” of frogs. It is common for all the amphibian species to come out of hiding as soon as the rain starts. The males announce their presence by inflating their vocal sacs (not in all species, some of the species have internal vocal sacs, which do not protrude) to produce croaking sounds at the edge of a water body. The croaking sound is an invitation for the female of the respective species. Each species has a characteristic sound and females of that particular species only can distinguish that sound. The croaking sound is also helpful in identification of the frog species. The monsoon is the best season to study amphibians or to simply observe them as most breed in this season. Unfortunately most of the protected areas are closed during the monsoon and the administrative authority of the state forest department does not permit visitors.

Identification of amphibians is tricky for many non experts as compared to other vertebrates like reptiles, birds and mammals. This is because the taxonomy of amphibians is complex (in comparison to other tetrapods) and not yet certain or stabilized (especially frogs and toads). Lack of literature on the subject has added to this complexity. Classification of the amphibians is based on external characters as well as the internal anatomy. Often, habitat preference, behavior and calls can all be used to help with amphibian identification. Each species has its own distinctive call, just like the thumbprints in humans. Amphibians are divided into three orders known as: a) Gymnophiona or Caecilians (apoda = amphibia who lack limbs; example—Ichthyophis) b) Caudata (Urodela = amphibia who possess tail; example—salamanders and newts) c) Anura = (frogs and toads). As of October 2012 there were 7,054 described amphibian species of which 6,221 are anurans, 642 caudatas and 191 caecilians (1). One third of all amphibians are currently threatened with extinction (2).

In 2010, the Zoological Survey of India documented a total of 311 amphibian species in India (3). The amphibians of India show a high level of endemism. Incredibly India has almost 50 “lost” species which is the highest number of “lost” species from any country in the world. Twenty percent of all Indian amphibians are facing extinction. A new family of caecilians, Chikilidae, has been recently discovered beneath the monsoon-soaked soils of remote northeast

India (4). This discovery was led by the University of Delhi professor Sathyabhama Das Biju. This finding explored new evidence in the study of prehistoric species migration, as well as evolutionary paths influenced by continental shift. Another surprising find is the Indian purple frog (*Nasikabatrachus sahyadrensis*) from Western Ghats. Indian purple frogs have a small range and are Endangered under the IUCN Red List (5). They spend eleven months underground and emerge for reproduction for only two weeks. Habitat loss and degradation are major cause of decline of *Nasikabatrachus sahyadrensis* from the Western Ghats, large areas of which have been deforested for agricultural purposes. *Rhacophorus lateralis* is an Endangered species of Rhacophoridae tree frog endemic to the Western Ghats in South India. The frog was described by George Albert Boulenger in 1883 based on a single specimen from

Malabar (present-day Kerala) and collected by Richard Henry Beddome. Several later surveys did not report the species until its rediscovery in the year 2000 during an expedition to the Western Ghats by a team from University of Aberdeen (6). A proposed hydroelectric project in Western Ghats region may be a threat to this species.

The Dehradun stream frog, rediscovered recently in India, was previously known from only a single specimen found in 1985. Species are disappearing a thousand times faster than historic rate. Amphibians are at forefront of this extinction wave. This is prob-

ably an alarm for a sixth extinction. The Anamalai dot-frog (*Ramanella anamalaiensis*), recently spotted by Indian scientists, was first named in 1937. The narrow-mouthed frog calls loudly from marshy areas during the monsoon season but hides the rest of the year under stones and logs on the forest floor. The colorful Chalazodes bubble-nest frog (*Raorchestes chalazodes*), missing since 1874, was found recently in India. It has an unusual combination of fluorescent green dorsum, ash blue thighs, and patchy yellow eyes. An Indian team rediscovered Silent Valley tropical frog (*Micrixalus thampii*) under leaf litter in forests within the Kunthi River watershed.

India is an agriculture dependent country. Farmers are more concerned with crop production rather than biodiversity loss and therefore we must provide proper education regarding pesticide and insecticide use. Agrochemical pollution of aquatic habitats is the major threat of many frog species.

Toads are very common and easily accessible species for dissecting in laboratories. In India, higher secondary education, college



Huge numbers of toads are sacrificed for educational programs in India. Photo: Suman Pratihar.

education and University education is dominated by toad dissection. Huge numbers of toads are sacrificed for educational programs in India. People for the Ethical Treatment of Animals India (PETA), urged us to eliminate dissection from zoology courses offered by our university and affiliated colleges. Now the University Grants Commission (UGC), the apex regulatory body for higher education in India, has published official recommendations calling for an end to animal dissection and experimentation for university and college zoology and life-sciences courses. Following an extensive campaign by PETA India, scientists and other concern people, the Ministry of Environment and Forests (MoEF) has issued guidelines to the Medical Council of India (MCI) and Pharmacy Council of India (PCI) to completely stop dissection and experimentation on animals for training both undergraduate and post-graduate students and to use non-animal methods of teaching. Ministry of Environment and Forests agreed with PETA that animal experiments should be stopped when alternatives are available, according to section 17(d) of the Prevention of Cruelty to Animals Act, 1960 in India.

In this background non-animal science teaching and research is a good alternative option to save our biodiversity. Several techniques

are now available to teach animal sciences without sacrificing animals. Not only would banning dissection save the lives of countless animals every year, it would also ensure that every student benefits from the very latest and most effective teaching methods. As the US affiliate office explains, dozens of studies have repeatedly shown that non-animal teaching method are equivalent—and often superior—to dissection in providing students with an understanding of anatomy and complex biological process. There are other benefits as well. Non—animal teaching methods save time and money as they can be used again and again. Students also respond well, particularly those who are uncomfortable with dissecting and experimenting on animals in the classroom.

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The Cane Toad in Experimental Physiology and Pharmacology

By Lindsay Brown

The Cane toad (*Bufo marinus*) has a natural range in Central and Southern America from southern Texas to central Brazil. The species was introduced from Hawaii to north-eastern Queensland in 1935 to control sugar cane beetles. This introduced pest is a prolific breeder that continues to spread throughout tropical Australia. Research on cane toads has spanned this almost 80-year time-frame (1). Websites with information on Cane toads include: <http://australianmuseum.net.au/Cane-Toad>; http://www.daff.qld.gov.au/4790_8270.htm; <http://animals.nationalgeographic.com.au/animals/amphibians/cane-toad/>; <http://www.canetoadsinoz.com/>; and http://www.csiro.au/proprietaryDocuments/CSE_ctfacts.pdf.

Cane toads are toxic to domesticated animals, including dogs, although one report on 90 cases in dogs reported 96% survival (2). However, the major impact of this invasive species is on native fauna resulting from the lethal toxic ingestion by frog-eating predators (3). The Cane toad has some important ecological advantages over Australian native frogs: the Cane toad is much larger with some specimens up to 1.2 kg, is poisonous throughout its life cycle, has few predators in Australia, and the female can produce up to 35,000 eggs per spawn, all without reduction of sugar cane beetle numbers. Toads survived up to seven days in aqueous solutions of up to 50% seawater, showing a tolerance to short periods of salinity (4). This study described the time-course of changes in plasma and

urine osmotic pressure, the concentrations of contributing solutes, and the hydration state of cane toads in different salinity. Control measures have been unsuccessful, and the spread of the Cane toad seems to be accelerating (1, 3).

Cane toads have been used in a wide range of physiological research projects. Oocytes from the South African clawed toad, *Xenopus laevis*, are an extremely useful and sensitive method to translate mRNAs into proteins. Markovich & Reeger (1999) showed that the oocytes from *Bufo marinus* expressed plasma membrane proteins including sulphate, dicarboxylate, and amino acid transporters, and sodium/glucose cotransporters, making this a viable heterologous system for the expression of proteins (5). The usefulness of *Bufo marinus* oocytes was extended to the expression of ion channel proteins, with electrophysiological characterization (6).

The major toxins produced by Cane toads and stored in the parotid glands are the cardioactive bufadienolides; these compounds have a six-membered α , β -unsaturated C17 lactone ring, in contrast to the five-membered α , β -unsaturated lactone ring in plant-derived cardenolides such as digoxin from *Digitalis lanata*. Cardenolides and bufadienolides increase the force of contraction of the heart at low concentrations by inhibiting $(\text{Na}^+ + \text{K}^+) \text{—ATPase}$, but cause cardiac arrhythmias and death at higher doses. Hayes and co-workers (2009) found complex changes in bufadienolide composition throughout Cane toad ontogeny (7). Total bufadienolide concentrations were about 30-fold lower during the tadpole stages, compared with concentrations in eggs and after metamorphosis, correlating with a lower toxicity of tadpoles to native frogs.

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The intestine of the Cane toad contains the substance P-like neuropeptide and potent long-lasting spasmogen, bufokinin (Lys-Pro-Arg-Pro-Asp-Gln-Phe-Tyr-Gly-Leu-Met.amide). Liu *et al.* (2000) showed that bufokinin produced a long-lasting and dose-dependent fall in blood pressure without changes in heart rate in anaesthetized Cane toads, suggesting that bufokinin has a role in haemodynamic regulation and/or sensory nerve function in the toad (8). In contrast, galanin is a neuropeptide producing vasopressor responses in anaesthetized Cane toads; the responses to galanin were inhibited in the presence of galanin antagonists (9).

Further studies have investigated electrophysiological changes to define the role of ion channels and exchangers in the functioning of the toad heart. Metabolic inhibition with cyanide slowed pacemaker activity in the heart by reducing $\text{Na}^+/\text{Ca}^{2+}$ -exchanger activity, thus slowing the inward current (I_{NCX}) during calcium efflux (10). In single pacemaker cells, ATP showed biphasic effects on intracellular calcium concentrations; these changes were mimicked by a P2Y_1 -selective agonist and inhibited by a P2Y_1 -selective antagonist, but not by compounds acting at other purinergic receptors (11). These P2Y_1 purinoceptors modulated Ca^{2+} release from the sarcoplasmic reticular Ca^{2+} store (11).

Single fibers of the rectus abdominis muscles from juvenile and adult Cane toads were examined for myosin heavy chain isoform expression and Ca^{2+} -stimulated MgATPase activity (12). The fibers showed a large proportion of hybrid isoforms expressing twitch and tonic or twitch and twitch isoforms, with differing ATPase activities.

Retrograde labeling with horseradish peroxidase was used to label the ascending projections to the major midbrain auditory center in the cane toad, showing that the major input comes from the ipsilateral superior olive (13). The central control of breathing in the cane toad was controlled by α_2 -adrenoceptors shown as increased respiratory activity with clonidine, a selective agonist, which was blocked by yohimbine, a selective antagonist at these receptors (14).

Impulse conduction in the frog heart can be demonstrated by the Stannius ligatures, first described by Stannius in 1852 (15). The first ligature is placed around the junction between the sinus venosum and the atrium while the second ligature is placed around the atrioventricular junction. After tying each ligature, the heart stops and then recommences at a slower rate, showing that each chamber possesses automaticity and that the sinus venosum is the pacemaker area. This experiment can also be carried out on the isolated Cane toad heart as a demonstration for undergraduate students.

The toad heart can also be used to demonstrate the inotropic and chronotropic responses to adrenoceptor agonists such as noradrenaline. The heart can be dissected free in a pithed toad, and then the apex joined by a hook to a simple recording device. Solutions of noradrenaline can then be administered dropwise onto the heart; the heart will beat stronger and faster within seconds. This is a quick demonstration, but it is not possible to obtain a measurement of potency of the agonists.

Relatively few undergraduate courses in physiology or pharmacology at Universities in Australia are now using Cane toads. Cane toads will survive in an animal house for several weeks with appro-

priate husbandry. Most students in physiology classes are enrolled in biomedical science, medicine, pharmacy or dentistry where human biology is the major focus. Students in veterinary science courses are an exception, but these students rarely use toads as they are perceived as not relevant to their future profession. Students in all groups will question the relevance of studies on Cane toads to humans or domesticated animals as toads are cold-blooded with a single ventricle, unlike mammalian hearts. Further, Cane toads are much more prevalent in summer (December-February in Australia), during University holidays, in areas such as south-east Queensland where the major state-based Universities are located. The next major University hub is Sydney, right at the limit of the range. The University teaching year in Australia begins in late February and ends in mid-November. During winter (June-August), toads need to be transported from north Queensland; Cairns to Brisbane is two hours' flight while Brisbane to Melbourne is a further two hours' flight.

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Frogs Boom, but Bite the Dust

By Karthikeyan Vasudevan

The monsoon is synonymous with frog chorus. The adult frogs faithfully respond to this environmental cue to breed. The breeding crescendo rapidly grows with the onset of the monsoon and several seasonal breeders call, deposit their eggs, with the expectation that their efforts would yield offspring of their own. The breeding frenzy wanes with rapidly with progressing monsoon; some laggards and first time breeders might take a shot at breeding at this stage. It is obvious that their chances at successful breeding are poor. I was intrigued by this and I punched in some numbers in my computer. If I were to start with a small population breeding frogs consisting of 10 adults weighing 10 grams each (seven males and three females) and kept replacing some males and females from time to time from the wild during my experiment; set my success at hatching at 1%; mortality at tadpole stage at 90%; recruitment into the adult population next year at 1%, I was startled to notice that on the sixth year, the biomass of frog exceeded the mass of the earth! Obviously, the population parameters I had set, imitating their survival probabilities in the wild, were hugely optimistic.

I probed further into the breeding habits of frogs and I came across a term—"explosive breeding." I referred to the most reliable source of information on amphibians—*Biology of Amphibians*, by Linda Trueb and Bill Duellman. "Explosive breeding involves a short, synchronous burst of breeding activity once or a few times each year... the selective advantage of synchronous oviposition in such species may be the satiation of predators by larvae and thus the survivorship of some offspring" (1). Then, I used Google Scholar™ to search for the use of this term. I found 37,400 hits; the first and second 100 hits had 92 and 81 articles on amphibians respectively. In the lexicon of a batrachologists this term seem to have taken an important place. Explosive breeding is so primal to frogs and it is the most common mode of breeding, and it must have been

an important adaptation responsible for their survival. Natural selection has favored a breeding prowess that could be the frogs' single most powerful armament. However, such prolific breeding is looked upon as unimportant by many. Consequently, the numerous deaths of frogs on roads, or the frogs hunted by people, or the mortality caused by polluted ponds, or the use of frogs in education, are ignored assuming that there are millions left in the wild!

In an unprecedented fashion, a world-wide phenomenon took us by surprise! Frogs have exhibited major population declines, susceptibility to disease, morphological deformities and well-documented extinctions in the last two decades. Every major scientific journal during the last decade has carried at least one article highlighting declines in the population of frogs. These texts have sunk in well amongst us. However, I believe that batrachologists-cum-conservation biologists in this part of the world (that includes me) have responded to this by "staring into the headlights." Evidently, questions pertaining to their rampant mortality have still not found central place in our research. As a coalition of ASG members, we should also actively engage in advocating solutions that reduce the mortality of frogs, on roads, from pesticides, habitat loss, hunting, epidemics and vivisections. While it might take many years of painstaking research to understand: why frogs are explosive breeders? We would have missed out an important conservation opportunity. Clearly, the intensity of threats that amphibians face today, far exceed their ability to cope. Among many species of frogs the "armaments" they have acquired during their evolution, would still fall short of their ability to survive the declines they are currently facing. Therefore, the steps we take for conservation of amphibians will matter for them.

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Thoughts on the Abolition of Dissections in Biological Science

By Ashim K. Chakravarty

More than fifteen years ago, I attended a seminar on *Necessity of Animal Dissections in Practical Classes* at G. B. Pant auditorium of National Council of Education and Research Training (NCERT), New Delhi. The focus question of that seminar was "Why one should do the act of killing and dissecting the animals when one can very well use demonstrative models alternatively?" The representatives from universities, colleges, schools and some NGOs attended and presented papers in the seminar. Inflicting unnecessary pain to animals was a star point

in the seminar. Nobody could deny that. There were some presentations to stress that we are unnecessarily disturbing the natural populations of animals we used for dissections. The all inclusive striking point of "disturbance of ecological balance" was gathering momentum but not yet vogue at that time.

Being a biologist, I made it a point in my presentation that we may not need to dissect some species like rabbits, Guinea pigs and pigeons, but could breed fish, frogs, toads, and mice for dissection purposes. Students need to have the real feeling and necessary knowledge of how to operate on an animal and trace out the branches of the blood vessels and nerves, and the way the different systems remain connected with each other. To test some drugs or cosmetics one may need not to use several rabbits, cats or even

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mice. The use of in vitro cell cultures prepared from cells of an individual animal can serve the same purpose of multiple animals. The rabbits need not to be blind when testing chemicals in cosmetics or the effects of changes to the cornea. The in vitro culture point was well accepted at that time in the domain of research. Several participants objected during discussions regarding the idea of breeding for dissections; in their view it was the most inhumane breeding to give pain.

One or two participants elaborated on the model systems and two interesting models of frogs were shown—they could be unzipped from the ventral side to see the organs which were nicely colored. These models seemed to be good demonstrative materials for the 6th standard students in schools, beyond that, these were simply colorful toys. With much asking I determined that they were a kind of benevolence of some foreign company. I was bit inquisitive about the origin of the models for many reasons.

SEMINAR AT VIDYASAGAR UNIVERSITY

I recounted this whole episode of my seminar some fifteen years ago to give my opinion on doing away with dissections of animals in another day-long Seminar, “Non Animal Science Teaching and Research” enacted at Vidyasagar University, Midnapore, in the end of February 2011. The agenda was almost the same; in addition to cruelty to the animals, this time the card of “ecological balance” was played strong by some speakers. Models were only mentioned; no specific presentation of models was given. The seminar was sponsored by the University Grants Commission, New Delhi and People for Ethical Treatment of Animals (PETA). My basic opinion on the topic did not change much during fifteen years despite my off and on thinking. Certain additional points may only be added.

POINTS TO CONSIDER

If a certain number of specimens of a species with the virtue of prodigal reproduction are removed from the nature, someone needs to study whether that would be bad for survival of species or the vacuum will be filled in quickly in absence of intra-species fight. Will it be cruel or unethical to breed some small animals like fish, frogs, toads and mice for the purpose of dissections in biology practical, when there are slaughter houses for millions of big mammals and slaughtering of goats, sheep and chickens are taking place all the time at roadside meat shops, and frog legs are exported? Are all of these seminars organized with Government funding to make the audience aware that they need to quit dissections to buy beautiful models for practical purposes? Have these models been developed and will be soon supplied by some multinational companies?

Now India is a big market for all kinds of things starting from atomic reactors, aircrafts to GM crops and underwear, all developed in distant lands. The present ministers are over the board interested in foreign investment in the country. If the Government compels the use of the biological models, then let the Indian biologists develop them in collaboration with some companies in our country for our use and export to the other countries (which will be competitive for cheap materials and labor).

Ethically we are bound not to kill animals unnecessarily and the number of animals to be killed should be as minimal as possible. A specimen may be dissected more than once keeping it in preserved in between. In our university chicken heads obtained from meat shops are provided to the students to trace the origin and distribution of cranial nerves instead of supplying a live pigeon. To me, the demonstration of different systems to the students of higher secondary or college level, with the dissected specimen in preservation is much better than the model.

Is Frog Dissection Essential for Life Science Teaching? The Dissection Controversy

By Manab Kumar Saha

The importance of frog dissection in life sciences and its associated educational value is a heated controversy. It involves policy makers, curriculum developers, administrators, teachers, educators, students, researchers, animal rights activists and the law. Policy makers have begun to reevaluate the science curriculum, to justify the role of frog dissection in life sciences. Teachers, students and parents are also questioning the importance of frog dissection in the classroom. Recently, a new wave of court cases and animal rights activity has brought animal dissection to the forefront of life science education issues, students' moral values and emotion. Many teachers think that this controversy may hamper the academic freedom in the classroom and the possibility of a less effective educational environment. Therefore, the controversy grows more heated, in that school boards feel as if control over their curriculum and their standards are dwindling, while

anti-dissection educators and researchers are gaining momentum with innovative technology supporting their cause.

Educators and researchers in support of frog dissection in the biology classroom claim that comparative anatomy and physiology can be taught more effectively through animal dissections. On the other hand, anti-dissection educators and researchers claim that the only information that students gain from dissections is how to perform dissections and this information is irrelevant to the students' future educational careers.

The specific objectives for dissection are: (a) To identify internal organ structures that are part of the digestive, circulatory, reproductive, respiratory and excretory systems of the frog. The primary objective of dissection is to allow students to observe structures that cannot readily be seen in their normal environment. (b) To learn the anatomy and morphology of the frog and be able to name each structure and describe the functions of each structure. (c) To

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learn the functions of the frog skin to protection from predation through camouflage and secretion of poison.

The problems of dissection are: some students responded primarily with anxiety and dread, virtually all of the students felt at least somewhat negative, hesitant, uncomfortable or ambivalent toward the prospect of dissecting. Even those eager to dissect often described conflicted feelings. By far, the most common problem for students was squeamishness. Nearly every student reported feelings of disgust when they thought about the impending dissection. In many cases, students openly admitted that they or others in the class thought that dissection was “gross,” “sick” or “disgusting”. Students feel uncomfortable because they are making physical contact with animals in ways they would usually define as inappropriate.

Dissection can create negative attitudes towards animal as well as creating psychological trauma in students. According to Orlans (1988), when dissection was first introduced some students lost their interest in biology due to the negative feelings and psychological trauma evoked by dissection classes. The National Association of Biology Teachers (NABT) (1990) supports the use of interactive videodiscs and computer simulation as alternatives to hands-on dissection wherever necessary. The National Anti-Vivisection Society (NAVS) (1997) claimed that the use of simulations in dissection would create compassion in student attitudes toward animal life.

A related issue is whether dissection is gender biased. Science educators are becoming increasingly concerned about gender differences with respect to expectations, types of experiences and participation in science classrooms. Some might argue that males are more likely than females to enjoy dissection. From this viewpoint, requiring dissection in biology classes contributes to a male oriented dominance in science and contributes to gender inequities in science careers. Some gender differences in attitudes toward dissection were present.

There are alternative methods of dissection, i.e., (a) models, mannequins, and mechanical simulators; (b) films and interactive videos; (c) computer simulations and virtual reality systems; (d) self-experimentation and human studies; (e) plant experiments; (f) observational and field studies, (g) waste materials from slaughterhouses and fisheries; (h) in vitro studies on cell lines; (h) and dead animals from a humane and ethical source (for example, animals which have died naturally or which have been killed humanely after scientific procedures).

Computer simulation covers all of the standards, grasp students' interests, and provide useful information that students can take with them on their journeys through college, into their careers, and ultimately on their quests to mature into well-informed contributors to society. Computer simulations provide the following: (a) provide comprehensive pre-dissection information on the anatomy and morphology of frog parts; (b) clarify unanswered questions that sometimes arise during dissections and to provide interactive, in-depth lab experience on the physiology of amphibians; (c) the program was similar to traditional curriculum that emphasizes identification of anatomical structures and functions of frog organs; (d) most importantly, the program operates on the Macintosh computer which is available in nearly all the middle school

science laboratories. When students worked on the software, they were given a worksheet for key words and definitions to complete. Computer simulated instruction gives students the opportunity to observe a “real” world experience and interact with it. Pedagogical simulations can be used in teaching students the anatomy and morphology of complex organisms or understanding complex relations of animal parts and their basic functions without actually dissecting real animals.

Advantages of alternatives depend on the learning objectives. Animal-free models have several advantages over animal experiments. In cases where students are not well prepared for work with animals, the emotions aroused by being confronted with a dead or live animal might detract from the actual learning experience. Non-animal models can be developed in such a way as to achieve the learning objectives more effectively. For example: (a) a specific animal experiment might only be offered once, whereas an alternative model can often be used repeatedly without constraints on time and place of study; (b) alternative models can offer unambiguous and complete data, and can avoid the negative learning experience of an unsuccessful experiment; (c) an alternative can have built-in self-assessment to allow students to gauge whether staged learning objectives have been achieved; and (d) alternatives which make use of modern audio-visual techniques offer the possibility of demonstrating phenomena that are normally unobservable in the equivalent animal experiment, such as animations of organ and cell functions and flythrough of organ systems.

Although in some cases the development of an alternative model can be expensive, it could be used repeatedly. Overall, the alternative model is cheaper than purchasing and caring for large groups of animals. The use of an alternative can also often save time for both the teacher and the students. Ultimately, in some situations, virtual biology learning experiences may be better than the hands on learning experience. The visual images and videos are more realistic. Videos followed in order of preference by class debates, discussions, lectures, work groups, printed matter and questions and answers.

In a survey, 80 biology teachers from different districts of West Bengal provided their opinion. The participants ranged in age from 24 to 58 years old. These teachers had some prior experience in animal dissection, but had no experience in computer-simulated interactive dissection. Eight-seven percent of teachers give there opinion in favor of anti-dissection teaching.

From the above answers, we can conclude that (animal) frog dissection is not an essential part of knowledge construction. There are now so many alternative methods of dissection, which are equally or sometimes more efficient in teaching students, that actual dissection is no longer necessary. In situations where real animal dissections are required, we should first provide a virtual lesson that covers all goals of the lesson plan. After the virtual dissection is, completed students can then dissect the physical animal if desired. In this case, the knowledge gained from the virtual lesson brings more meaning and understanding when dissecting the physical frog.

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Conservation and Ecology

Reproductive dynamics of three amphibian species in Mediterranean wetlands: The role of local precipitation and hydrological regime

By Hugo Cyuela, Aurélien Besnard, Arnaud Béchet, Vincent Devictor & Anthony Olivier

Although the influence of water availability and precipitation regimes on amphibians has been studied at large scales, whether and how inter-annual rainfall and hydrological variations affect amphibians dynamics at a local scale has rarely been addressed. In this respect, accounting for variations in species detectability in space and time has also been overlooked. We assessed the effects of rainfall and hydrological variations on the breeding dynamics of three amphibian taxa: *Pelodytes punctatus*, *Hyla meridionalis* and *Pelophylax* spp. in 20 ponds of the Camargue region (southern France) over a seven-year study period. We used multiple season occupancy models to test the effect of winter-spring rainfall and inter-annual variations in hydroperiod, mean water depth and drought events on tadpole presence in spring (March to June), a proxy for breeding dynamics. We used an independent survey with spatial replicates (dipnet sweeps) to disentangle the relative contributions of phenology and detectability to the absence of records in a given month. For the three taxa considered, the probability of missing a species when that species was actually present in a pond was most often negligible. Hence, we could consider that multi-season models properly tracked changes in species phenology. *Pelodytes punctatus* was first detected in March while the two other taxa appeared later in April. *Hyla meridionalis* appeared as a mid-season species with much more synchronous pond occupancy than *Pelodytes punctatus*. The detection peak of *Pelophylax* spp. was short and unexpectedly early for this taxon. Seasonal



Pelodytes punctatus tadpole from the Camargue, Rhone delta, southern France. Photo: Simon Baudouin.

winter-spring rainfall was associated with a decrease in extinction rates and even more strongly with an increase in colonization rates at individual ponds. Colonization rate increased following an annual drought and was best modelled as a negative quadratic effect of the variance of pond hydroperiod. Extinction probability was best modelled by a negative quadratic effect of mean water level. Hence, breeding was more stochastic i) in unpredictable and shallow ponds because of yearly drying up and ii) in highly predictable and deep ponds, possibly due to the presence of predators such as fish and crayfish. Overall, we show that ponds with intermediate rather than extreme variations in environmental conditions currently correspond to optimal breeding sites. Our study demonstrates that amphibian monitoring coupled with fine-scale analysis of environmental conditions is necessary to understand species dynamics in the long run and to inform conservation efforts for these species.

H. Cayuela, A. Besnard, A. Béchet, V. Devictor, A. Olivier, *Freshwater Biol.* **57**, 2629 (2012), DOI: 10.1111/fwb.12034

Global amphibian declines: A review

By Qazi A. Hussain & Ashok K. Pandit

Declines in the populations of amphibians is a complex problem at the global level and for explaining these declines a number of hypotheses have been propounded. Further, in order to curb and reverse this trend of amphibian decline a number of remedial measures have been proposed. In addition, directions for further research in this arena have been outlined by many scientists. This review is an attempt to compile some facets of the knowledge base available on the above cited aspects regarding amphibian declines so as to facilitate the researchers to get an overview of the problem and to help the public at large to understand the complex problem of global amphibian declines. Many amphibian populations in varied habitats are declining and it has been estimated that the present rate of extinction is greater than any known in the last 100,000 years. More than 32% of amphibian species are threatened and 7.4% of amphibian species are categorized as Critically Endangered. Studies have indicated that current rates of amphibian extinction are 211 times the background extinction and this rate may rise to 25,000 to 45,000 times if all the presently threatened amphibian species go extinct. The main hypothesized causes

of amphibian declines are introduction of alien species, over-exploitation, land use/land cover change, global environmental change, increased UV radiation, presence of contaminants and newly emerging infectious diseases. The remedial measures suggested to cope with the problem of amphibian declines include habitat protection, providing legal protection, captive breeding, education and awareness among masses, continued additional research and better understanding of amphibian diseases. Further, in order to better understand amphibian declines some of the important areas of research are utilization of a comprehensive monitoring program, study of metapopulation dynamics, use of molecular genetic techniques, and multifactorial studies.

Q. A. Hussain, A. K. Pandit, *Int. J. Biodivers. Conserv.* **4**, 348 (2012).

Neotropical primary productivity affects biomass of the leaf-litter herpetofaunal assemblage

By Jessica L. Deichmann, Catherine A. Toft, Peter M. Deichmann, Albertina P. Lima & G. Bruce Williamson

Soil fertility and plant productivity are known to vary across the Amazon Basin partially as a function of geomorphology and age of soils. Using data on herpetofaunal abundance collected from 5 × 5 m and 6 × 6 m plots in mature tropical forests, we tested whether variation in community biomass of litter frogs and lizards across ten Neotropical sites could be explained by cation exchange capacity, primary productivity or stem turnover rate. About half of the variation in frog biomass (48%) could be attributed to stem turnover rate, while over two-thirds of the variation in lizard biomass (69%) was explained by primary productivity. Biomass variation in frogs resulted from variation in abundance and size, and abundance was related to cation exchange capacity (45% of variation explained), but size was not. Lizard biomass across sites varied mostly with individual lizard size, but not with abundance, and size was highly dependent on primary productivity (85% of variation explained). Soil fertility and plant productivity apparently affect secondary consumers like frogs and lizards through food webs, as biomass is transferred from plants to herbivorous arthropods to secondary consumers.

J. L. Deichmann, C. A. Toft, P. M. Deichmann, A. P. Lima, G. B. Williamson, *J. Trop. Ecol.* **28**, 427 (2012). Copyright © 2012 Cambridge University Press.

The invasive Cane toad (*Bufo marinus*) in West New Britain, Papua New Guinea: Observations and potential impacts on native wildlife.

By Dylan van Winkel & John Lane

Since the introduction of Cane toads (*Bufo marinus*) to East New Britain 75 years ago, they have spread rapidly across Papua New Guinea and several of its offshore islands. Their dispersal was thought to be limited by habitat constraints such as primary rainforest and altitude. However, during a scientific expedition into the Hargy Caldera and Nakanai Mountains in 2011, we made several observations of *B. marinus* existing within undisturbed primary rainforest habitat and at altitudes greater than 800 m above sea level. Given their propensity to reach considerable population densities and threat towards naïve predators, these amphibians have the potential to have negative adverse effects on native Papua New Guinean wildlife. Future research on the potential ecological impacts of *B. marinus* in Papua New Guinea should be prioritized.

D. van Winkel, J. Lane, *Biol. Invasions*. **14**, 1985 (2012).

Advantages of long-term, multi-scale monitoring: Assessing the current status of the Yosemite toad (*Anaxyrus* [*Bufo*] *canorus*) in the Sierra Nevada, California, USA

By Cathy Brown, Katie Kiehl & Lucas Wilkinson

A comprehensive bioregional evaluation of the current status of at-risk species is critical for effective management and conservation. As part of a long-term, multi-scale amphibian monitoring program, we evaluated the current status of the Yosemite toad (*Anaxyrus* [*Bufo*] *canorus*) on national forest lands across the species'



Yosemite toad, *Anaxyrus* [*Bufo*] *canorus*, male calling during the spring breeding chorus. Photo: L. Wilkinson.

range in the Sierra Nevada, California, USA. We conducted monitoring at extensive (rangewide) and intensive (individual population) scales with small watersheds (2-4 km²), individual meadows and breeding areas within meadows as sample units. Yosemite toad breeding was found in an estimated proportion of 0.84 ± 0.03 (SE) of recently occupied watersheds (locality data from 1990-2001), and 0.13 ± 0.04 of historically occupied watersheds (locality data prior to 1990). Rangewide, breeding was found in an estimated proportion of 0.22 ± 0.01 of watersheds. We quantified demographic parameters (abundance and survival of breeding males, abundance of egg masses, successful metamorphosis) in six meadows in two watersheds. Abundances were small, with the largest populations having only 16-21 and 18-19 breeding males each year. Annual survival rates of males by meadow ranged from 0.49-0.72. Numbers of egg masses per year were low, and the proportion of breeding areas with successful metamorphosis ranged from 0.14-0.73. Using the multi-scale data, we examined Yosemite toad spatial and temporal occupancy patterns. Yosemite toads tended to breed in one or two sites (lakes, meadows, stream reaches) per watershed every year, and occasionally in other sites. This assessment of the status of the Yosemite toad will inform management decisions at both bioregional and population scales and the development of conservation priorities.

C. Brown, K. Kiehl, L. Wilkinson, *Herpet. Conser. Biol.* **7**, 115 (2012).

Population structure and landscape genetics of two endangered frog species of genus *Odorrana*: Different scenarios on two islands

By Takeshi Igawa, Shohei Oumi, Seiki Katsuren & Masayuki Sumida

Isolation by distance and landscape connectivity are fundamental factors underlying speciation and evolution. To understand how landscapes affect gene flow and shape population structures, island species provide intrinsic study objects. We investigated the effects of landscapes on the population structure of the endangered frog species, *Odorrana ishikawae* and *O. splendida*, which each inhabit an island in southwest Japan. This was done by examining population structure, gene flow and demographic history of each species by analyzing 12 microsatellite loci and exploring causal environmental factors through ecological niche modeling (ENM) and the cost-distance approach. Our results revealed that the limited gene



Ishikawa's frog in Amami Island (*Odorrana splendida*). Photo: S. Oumi.

flow and multiple-population structure in *O. splendida* and the single-population structure in *O. ishikawae* were maintained after divergence of the species through ancient vicariance between islands. We found that genetic distance correlated with geographic distance between populations of both species. Our landscape genetic analysis revealed that the connectivity of suitable habitats influences gene flow and leads to the formation of specific population structures. In particular, different degrees of topographical complexity between islands are the major determining factor for shaping contrasting population structures of two species. In conclusion, our results illustrate the diversification mechanism of organisms through the interaction with space and environment. Our results also present an ENM approach for identifying the key factors affecting demographic history and population structures of target species, especially endangered species.

T. Igawa, S. Oumi, S. Katsuren, M. Sumida, *Heredity* **110**, 46 (2013). DOI: <http://dx.doi.org/10.1038/hdy.2012.59>

Designing a diet for captive native frogs from the analysis of stomach contents from free-ranging *Leiopelma*

By Stephanie D. Shaw, Lee F. Skerratt, Ruud Kleinpaste, Lisa Daglish & Phillip J. Bishop

Diets for captive amphibians are often inadequate and lead to poor health. To determine the natural diet of two New Zealand frog species we analyzed the stomach contents of 16 *Leiopelma archeyi* from the Moehau Range of the Coromandel Peninsula and nine *Leiopelma hochstetteri* from the Moehau Range of the Coromandel Peninsula, the Hunua Ranges and Maungatautari. These specimens were obtained as bycatch from invertebrate pitfall traps from 2002-2008. Both species ate a wide range of invertebrates including springtails, mites, ants, parasitic wasps, amphipods and isopods. *Leiopelma archeyi* also ate snails. The mean ratio of maximum

prey size ingested to snout-vent length in *L. archeyi* was 0.31 (range 0.16–0.5), and in *L. hochstetteri* was 0.42 (range 0.21–0.75). We suggest a reformulated captive diet based on the species and size of invertebrates ingested in the wild. This diet may assist in the prevention of metabolic bone disease.

S. D. Shaw, L. F. Skerratt, R. Kleinpaste, L. Daglish, P. J. Bishop, *New Zeal. J. Zool.* **39**, 47 (2012).

Newt decline in Western Europe: Highlights from relative distribution changes within guilds

By Mathieu Denoël

The recent increase in the number of monitoring schemes has formed the basis for high quality distribution atlases. This provides the opportunity of estimating global and specific decline patterns across regional and national borders. In this framework, this study focused on four sympatric newt species—including the Great crested newt (*Triturus cristatus*), an Annex 2 European Habitats Directive species, over six geographic areas (five countries) in Western Europe. A relative comparison of distribution maps across time is used here and is based on more than twelve thousands occupied grid cells. It benefits from the definition of a guild, as these species are simultaneously detectable in wetlands. *T. cristatus* and the Alpine newt (*Mesotriton alpestris*) were the most and the least threatened newt species, respectively, whereas the Palmate (*Lissotriton helveticus*) and Smooth newt (*Lissotriton vulgaris*) had an intermediate decline level at both coarse and fine grain resolutions. However, regional variations across Europe and scale effects were also found. On one hand, these results show that *T. cristatus* is not only regionally



The Great crested newt (*Triturus cristatus*) is declining in several European countries. Photo: M. Denoël.

threatened but suffers from a global decline in Western Europe. On another hand, the results indicate that patterns of decline are not uniform within Europe and that species often considered as common and not threatened are, in fact, declining more than others. Finally, the proposed methodology, i.e., using guilds to assess relative decline, would be useful as a complement to other standardized methods in correctly advising conservation managers and policy makers, particularly for species with more subtle declines.

M. Denoël, *Biodivers. Conserv.* **21**, 2887 (2012). DOI: <http://hdl.handle.net/2268/128290>

Positive effects of nonnative invasive *Phragmites australis* on larval bullfrogs

By Mary A. Rogalski & David K. Skelly

Background

Nonnative *Phragmites australis* (Common reed) is one of the most intensively researched and managed invasive plant species in the United States, yet as with many invasive species, our ability to predict, control or understand the consequences of invasions is limited. Rapid spread of dense *Phragmites* monocultures has prompted efforts to limit its expansion and remove existing stands. Motivation for large-scale *Phragmites* eradication programs includes purported negative impacts on native wildlife, a view based primarily on observational results. We took an experimental approach to test this assumption, estimating the effects of nonnative *Phragmites australis* on a native amphibian.

Methodology/Principal Findings

Concurrent common garden and reciprocal transplant field experiments revealed consistently strong positive influences of *Phragmites* on *Rana catesbeiana* (North American bullfrog) larval performance. Decomposing *Phragmites* litter appears to contribute to the effect.

Conclusions/Significance

Positive effects of *Phragmites* merit further research, particularly in regions where both *Phragmites* and *R. catesbeiana* are invasive. More broadly, the findings of this study reinforce the importance of experimental evaluations of the effects of biological invasion to make informed conservation and restoration decisions.

M. Rogalski, D. Skelly, *PLoS ONE* **7**, e44420 (2012).

Survival of adult smooth froglets (*Geocrinia laevis* complex, anura, myrobatrachidae) in and around a hybrid zone

By Michael P. Scroggie

I obtained skeletochronological data from phalanges of adult frogs of the *Geocrinia laevis* species complex collected at breeding aggregations in and around a hybrid zone in southwestern Victoria, Australia. I estimated rates of survival among adults by fitting the geometric probability distribution to these data using maximum likelihood methods. Information-theoretic comparison of alternative models for survival rates revealed a strong effect of sex on survival rates, but little evidence of differences between populations, or between hybrid and parental populations. I found adult males to have a mean annual rate of survival of 0.29, compared with a rate of 0.41 for adult females. The absence of a gross deficit in rates of survival within hybrid populations suggests that differences between hybrid and parental populations in this component of individual fitness are relatively unimportant in determining the structure and dynamics of the hybrid zone.

M. P. Scroggie, *Herpet. Conserv. Biol.* **7**, 196 (2012).

An eDNA approach to detect Eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water

By Zachary H. Olson, Jeffrey T. Briggler & Rod N. Williams

Context. Environmental DNA, or eDNA, methods are a novel application of non-invasive genetic sampling in which DNA from organisms is detected via sampling of water or soil, typically for the purposes of determining the presence or absence of an organism. eDNA methods have the potential to revolutionize the study of rare or endangered taxa.

Aims. We evaluated the efficacy of eDNA sampling to detect populations of an amphibian of conservation concern, the Eastern hellbender (*Cryptobranchus a. alleganiensis*), indirectly from their aquatic environments.

Methods. We developed species-specific primers, validated their specificity and sensitivity, and assessed the utility of our methods in silico and in laboratory trials. In the field, we collected water samples from three sites with known densities of hellbenders, and from one site where hellbenders do not occur. We filtered



Eastern hellbenders, *Cryptobranchus a. alleganiensis*, are declining across their range in the eastern United States. The eDNA methods developed here may be useful in documenting landscape-level occupancy patterns as we move towards identifying existing populations for conservation action. Photo: Jeff Briggler.

water samples, extracted DNA from filters, and assayed the extraction products for hellbender DNA by using polymerase chain reaction (PCR) and gel electrophoresis.

Key results. Our methods detected hellbenders at densities approaching the lowest of reported natural densities. The low density site (0.16 hellbenders per 100 m²) yielded two positive amplifications, the medium-density site (0.38 hellbenders per 100 m²) yielded eight positive amplifications, and the high-density site (0.88 hellbenders per 100 m²) yielded 10 positive amplifications. The apparent relationship between density and detection was obfuscated when river discharge was considered. There was no amplification in any negative control.

Conclusion. eDNA methods may represent a cost-effective means by which to establish broad-scale patterns of occupancy for hellbenders.

Implications. eDNA can be considered a valuable tool for detecting many species that are otherwise difficult to study.

Z. H. Olson, J. T. Briggler, R. N. Williams, *Wildlife Res.* 39, 629 (2012). <http://www.publish.csiro.au/nid/144/paper/WR12114.htm>

Decline of an endangered amphibian during an extreme climatic event

By Ben C. Scheele, Don A. Driscoll, Joern Fischer & David A. Hunter

Climate change is a poorly understood, emerging threat to many amphibian species. One of the ways climate change is likely to affect amphibians is through increased recruitment failure associated with more frequent climatic extremes. To understand the risk posed by this threat,

we combined 13 years of annual monitoring and multi-scaled habitat modelling at the site ($n = 60$), pool ($n = 105$) and nest ($n = 170$) levels to investigate the decline of the endangered Northern corroboree frog (*Pseudophryne pengilleyi*), during the most severe drought on record in southern Australia. We documented the local extinction of 42% of *P. pengilleyi* breeding sites during the climatic extreme. Using logistic regression we investigated habitat variables associated with extinction sites. We found that locally extinct sites now resemble historically absent sites, with fewer pools, less water and drying-related tree invasion. Extended periods of limited water availability at extinction sites is likely to have restricted breeding, contributing to localized extinctions. Habitat variables recorded at the pool and nest level did not significantly influence *P. pengilleyi* presence/absence, indicating that site level wetness had an overriding effect. We anticipate that increasing climate variability is likely to disproportionately threaten seasonal pool-breeding amphibian species, exacerbating the global amphibian biodiversity crisis. However, our work with *P. pengilleyi* suggests there are a range of simple habitat manipulations that could help to ameliorate the impacts.



Gravid female Northern corroboree frog (*Pseudophryne pengilleyi*). Photo: D. A. Hunter.

B. C. Scheele, D. A. Driscoll, J. Fischer, D. A. Hunter, *Ecosphere* 3(11), 101 (2012). DOI: <http://dx.doi.org/10.1890/ES12-00108.1> [Open Access].

Rapid increases and time-lagged declines in amphibian occupancy after wildfire

By Blake R. Hossack, Winsor H. Lowe & Paul Stephen Corn

Climate change is expected to increase the frequency and severity of drought and wildfire. Aquatic and moisture-sensitive species, such as amphibians, may be particularly vulnerable to these modified disturbance regimes because large wildfires often occur during extended droughts and thus may compound environmental

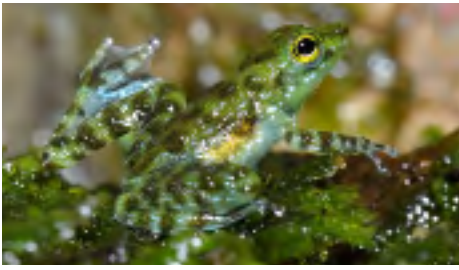
threats. However, understanding of the effects of wildfires on amphibians in forests with long fire-return intervals is limited. Numerous stand replacing wildfires have occurred since 1988 in Glacier National Park (Montana, U.S.A.), where we have conducted long-term monitoring of amphibians. We measured responses of three amphibian species to fires of different sizes, severity and age in a small geographic area with uniform management. We used data from wetlands associated with six wildfires that burned between 1988 and 2003 to evaluate whether burn extent and severity and interactions between wildfire and wetland isolation affected the distribution of breeding populations. We measured responses with models that accounted for imperfect detection to estimate occupancy during prefire (0-4 years) and different postfire recovery periods. For the Long-toed salamander (*Ambystoma macrodactylum*) and Columbia spotted frog (*Rana luteiventris*), occupancy was not affected for six years after wildfire. But 7-21 years after wildfire, occupancy for both species decreased $\geq 25\%$ in areas where $>50\%$ of the forest within 500 m of wetlands burned. In contrast, occupancy of the Boreal toad (*Anaxyrus boreas*) tripled in the three years after low elevation forests burned. This increase in occupancy was followed by a gradual decline. Our results show that accounting for magnitude of change and time lags is critical to understanding population dynamics of amphibians after large disturbances. Our results also inform understanding of the potential threat of increases in wildfire frequency or severity to amphibians in the region.

B. R. Hossack, W. H. Lowe, P. S. Corn, *Conserv. Biol.* (2012) DOI: [10.1111/j.1523-1739.2012.01921.x](https://doi.org/10.1111/j.1523-1739.2012.01921.x)

The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*

By Doris Preiningner, Anton Weissenbacher, Thomas Wampula & Walter Hödl

The Bornean frogs of the genus *Staurois* live exclusively along fast-flowing, clear water rainforest streams and are famous for displaying a variety of visual signals including foot flagging. Their extraordinary behavior and the continued loss of their natural habitat due to deforestation and subsequent pollution make them a group of target species for captive breeding as well as behavioral research. The Vienna Zoo has pioneered in the development of a research and conservation project for *S. parvus* and *S. guttatus*. We implemented two breeding



Juvenile *Staurois parvus* performing a foot-flagging behavior. Interdigital webbing is transparent grey and not yet white as observed in adults. Photo: N. Potensky.

and research arenas, offering an artificial waterfall and different options for egg deposition in a bio-secure container facility. Two month after introducing the frogs we observed amplexant pairs and the first tadpoles of *S. parvus* and *S. guttatus*. The Vienna Zoo is the first Zoo worldwide that has succeeded in breeding foot-flagging frog species and meanwhile has recorded over 900 tadpoles and at least 470 juveniles. One of the most striking observations has been the use of foot-flagging signals in recently metamorphosed *S. parvus*. This corroborates our assumption that foot flagging is employed as intraspecific spacing mechanism. The breeding success of two *Staurois* species at the Vienna Zoo can help in species conservation as it increases our knowledge on conditions necessary to breed tropical stream-dwelling anuran species found to be particularly threatened in nature. Furthermore, the captive colony provides research conditions to better understand the role of foot flagging as a visual signal component in anuran communication.

D. Preininger, A. Weissenbacher, T. Wampula, W. Hödl, *Amphib. Reptile Conserv.* **5**, 45 (2012), (e51). [Open Access; found at: <http://www.redlist-ARC.org>].

The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics and morphology

By R. K. Browne, H. Li, Z. Wang, P. M. Hime, A. McMillan, M. Wu, R. Diaz, Z. Hongxing, D. McGinnity & J. T. Briggler

The Cryptobranchidae, commonly called the Giant salamanders, are the largest surviving amphibians and comprise two extant genera, *Andrias* and *Cryptobranchus*. There are three cryptobranchid species, the Chinese giant salamander (*Andrias davidianus*; 180 cm, 59 kg), the Japanese giant salamander (*A. japonicus*; 155 cm, 55 kg) and the North American giant salamander (*Cryptobranchus alleganiensis*; 74 cm, 5.1 kg). Because of their iconic status as the world's largest amphibians

and their biopolitical significance, all cryptobranchids are subject to major and expanding initiatives for their sustainable management. Cryptobranchids are biologically similar in many ways; however, within these similarities there are differences in their habitats, diet, size, reproductive behavior and seasonality, fecundity and egg size, paternity, and growth and development. These characteristics are a consequence of their palaeontology, phylogeny, genetics and morphology. Cryptobranchid conservation genetics reveal the evolutionary significant units (ESUs) toward which conservation and research efforts must be directed to provide genetically competent individuals for rehabilitation or supplementation programs. Knowledge of these scientific fields in concert with cultural, political and economic factors all contribute to cryptobranchid conservation biology and the formulation of optimal strategies for their sustainable management. However, there has previously been no comparative review of the numerous scientific fields contributing to the knowledge of cryptobranchids, and little peer-reviewed material on *A. davidianus* and *A. japonicus* has been published in English. Here we present the first article in a series about cryptobranchid salamanders, "The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics, and morphology."

R. K. Browne *et al.*, *Amphib. Reptile Conserv.* **5**, 17 (2012), (e54). [Open Access; found at: <http://www.redlist-ARC.org>].

Conservation biology, husbandry, and captive breeding of the endemic Anatolia newt, *Neurergus strauchii* Steindachner (1887) (Amphibia: Caudata: Salamandridae).

By Bogaerts S, Janssen H, Macke J, Schultschik G, Ernst K, Maillat F, Bork C, Pasmans F & Wisniewski P

The long-term experiences of different private breeders on husbandry and breeding of the Anatolia newt, *Neurergus strauchii* are presented. This information is introduced and discussed in respect to the ecology, systematics and conservation of *N. strauchii*. Our knowledge and data of husbandry and captive breeding is collated and compared with the literature. We present our experiences to provide information and advice for the successful long-term keeping, breeding, and raising of *N. strauchii* and also an example and model that may be used for private contribution to Conservation Breeding Programs for endangered *Neurergus* species and other

semi-aquatic salamanders. *Neurergus strauchii* has proved relatively easy to keep in captivity under a range of aquatic and terrestrial housing and with adequate diet. However, although breeding is successful under a variety of conditions survival from egg to adult is low. Cold husbandry temperatures in winter increase reproduction. Eggs are laid very irregularly in time and number, and oviposition may depend on the condition of the female, particularly her nutritional condition through diet. There may be up to 285 eggs per female. The best temperature for egg laying is about 14.5 °C. Hatching success of eggs can vary enormously from 0% to 80%. Most larvae hatch from 11.5 to 14.5 mm. Larvae are easy to raise, with low mortality over a wide range of temperatures, and metamorphose in three to seven months, mostly from 55 to 63 mm and about 0.6 g. Several diseases are known to affect these newts and high temperature stress may exacerbate pathology.

S. Bogaerts *et al.*, *Amphib. Reptile Conserv.* **6**, 9 (2012), (e53). [Open Access; found at: <http://www.redlist-ARC.org>].

Factors influencing the timing of spring migration in Common toads (*Bufo bufo*)

By Holly Arnfield, Rachel Grant & Tobias Uller

Amphibians are typically very sensitive to environmental conditions. Many species use environmental cues, such as temperature and rainfall, to ensure that breeding coincides with suitable conditions for eggs and embryos. This also means that environmental change can drastically affect the timing of breeding, with potential consequences for recruitment and population dynamics. We analysed 12 years of data on the spring migration of the Common toad *Bufo bufo* L. to breeding ponds across 25 locations in Derbyshire, UK, to determine what factors that influence breeding activity. We also tested whether the timing of spring migration across years is predicted by annual variation in temperature or rainfall.

We found that more toads migrate in warmer temperatures and as the moon waxes, whereas precipitation did not have a significant effect on toad activity. Higher activity on warmer nights is expected, of course, but the adaptive significance of an effect of moon phase (if any) is unknown. Across years, spring migration begins earlier in warmer years, but the main migration of toads was not predicted by air temperatures before the onset of the breeding season. Contrary to the majority of studies of the timing of breeding in

amphibians, there has been a temporal shift towards later timing of breeding over the past 12 years. Overall, comparison of our results with that of previous studies of toads and other amphibians suggests that it can be difficult to generalize about the factors that influence breeding phenology, even within species. However, as more studies accumulate, it should be possible to establish the causes of such differences in environmental regulation of breeding activity among populations and the consequences thereof for populations in changing climates.

H. Arnfield, R. Grant, T. Uller, *J. Zool.*
288, 112 (2012).

Toad trackers: Amphibians as gateway species to biodiversity stewardship

By Rachel E. Rommel

The separation of people from nature may be implicated in a lack of broad based public support and understanding of biodiversity conservation. Increasing evidence shows this disconnection is even more apparent in today's youth and potentially the cause of many physical and mental ills in children. Biologists and educators can partner to provide nature based experiences in informal science learning to further develop knowledge, skills and attitudes of biodiversity research and conservation. With the support and expertise of the herpetological community, amphibians might serve as a more accessible vertebrate to offer opportunities for hands on interactions due to their relative abundance and proximity to urban and rural settings. One such program, Toad Trackers, engages youth, and adults, in science inquiry with wild amphibians, exploring biology related careers, and providing gateways to deeper investigation and personal meaning with the natural world. The program has been implemented with diverse audiences in an urban zoo setting in Texas, and in a rural setting with youth and educators at a conservation project in Zimbabwe. Through a combination of classroom workshops and hands-on, field-based research experiences,



Toad Trackers participants measure an *Incilius nebulifer*. Photo: Rachel E. Rommel.

participants learn aspects of data collection using live toads, population monitoring, spatial analysis, and demography. All of this information is taught within the context of global amphibian declines with an emphasis on why monitoring local populations of common amphibians is important for detecting declines regionally. Through further development and evaluation of the Toad Trackers program we hope to gain additional insight on the legitimacy of hands-on, early encounters with amphibians in helping engender lifelong biodiversity stewardship and conservation action. Preliminary results of the program show remarkable transformation in participant's knowledge, understanding, and appreciation of amphibians through verbal questioning, drawings and journaling. In post evaluations, the majority of respondents note that some variation of actually touching, holding or measuring a toad was their favorite part of the program. We hope this may provide motivation for both professional and recreational herpetologists to reach out to informal science learning institutions, and to formal and non-formal educators, to implement similar programming to build capacity through their knowledge and field expertise to garner much needed support for amphibian conservation.

R. Rommel, *Herpetol. Rev.* **43**, 417 (2012).

Stress hormones to assess the resistance of landscape to amphibian movements

By A. Janin, J. P. Léna, S. Deblois & P. Joly

In the framework of landscape ecology applied to biodiversity conservation, measuring the resistance of a milieu to animal movements contributes to the estimation of functional connectivity. Functional connectivity expresses the permeability of a landscape to migrating or dispersing animals. The resistance of a focal milieu is first a matter of the energetic costs of moving across it, but also a matter of perception by the animal of threats and risks when moving through unfamiliar environments. This paper explores new methods to assess such a perception of dangers linked to different land uses. We used corticosterone levels as sensitive indicators of both energetic demand of movement and risk assessment by the moving individual. We conducted experiments on substrate choice and measured levels of corticosterone before and after exposure of toads to three common agricultural substrates (ploughed soil, meadow, and forest litter) in female during breeding migrations. From previous

studies, we expected hormone levels to increase from forest litter (usual habitat of the toad) to meadows to ploughed soil. We succeeded in measuring corticosterone concentrations in the saliva, which is the first attempt in the amphibians. The expectations were supported as adult female toads had higher corticosterone levels on ploughed soil than on forest litter or meadow substrates. However, we did not detect difference in hormone levels between forest litter and meadow. On the other hand, the toads avoided moving onto ploughed soil. We also investigated stress hormones and substrate choice in juveniles during post-metamorphic migration. In these juvenile toads, corticosterone levels were not related to substrate type, but to humidity with the highest levels at the lowest humidity. Similarly, juveniles did not avoid moving over ploughed soil. We suppose that both the sensory perception and the cognitive processes differ between adult and juvenile toads. Adults probably take advantage from experience with different substrate types, whereas juveniles first emerging from the water probably do not. As a consequence, this lack of experience could make them particularly sensitive to the matrix composition and arable lands could act as ecological traps for them.

A. Janin, J. P. Léna, S. Deblois, P. Joly,
Conserv. Biol. **26**, 923 (2012).

The effect of riparian zones on species diversity of frogs in Amazonian forests

By José W. Ribeiro Jr., Albertina P. Lima & William E. Magnusson

We investigated the effects of riparian zones, and associated environmental variables, on the composition and number of species of frogs per sample unit in an Amazonian forest. Sample plots in riparian (up to 10 m from water bodies) and non-riparian areas were distributed over a 25 km² sampling grid to obtain a representative sample of habitats in each category. Each plot was sampled three times, over two rainy seasons. The riparian plots harbored more and different species than the non-riparian plots. In riparian areas near streams, the species composition changed along the gradient associated with stream width. The higher number of individuals and species in riparian plots highlights the importance of water courses and associated riparian areas for the conservation of anuran diversity in Amazon rainforests.

J. W. Ribeiro, Jr., A. P. Lima, W. E. Magnusson, *COPEIA* **2012**, 375 (2012).

Comparative pathology and ecological implications of two myxosporean parasites in native Australian frogs and the invasive Cane toad

By Ashlie Hartigan, Navneet K. Dhand, Karrie Rose, Jan Šlapeta & David N. Phalen

Myxosporean parasites *Cystodiscus axonis* and *C. australis* are pathogens of native and exotic Australian frog species. The pathology and ecological outcomes of infection with these parasites were investigated in this study. Gliosis was correlated to *Cystodiscus axonis* plasmodia in the brains of (9/60) tadpoles and (3/9) adult endangered Green and golden bell frogs using ordinal regression. Severe host reactions to *C. axonis* (haemorrhage, necrosis, and vasculitis) were observed in the brains of threatened Southern bell frogs (8/8), Critically Endangered Booroolong frogs (15/44) and Yellow spotted bell frogs (3/3). Severe brain lesions were associated with behavioural changes, neurological dysfunction, and spontaneous death. Both *C. axonis* and *C. australis* develop in the bile ducts of tadpoles, the plasmodia were significantly associated with biliary hyperplasia, inflammation and the loss of hepatocytes in (34/72) Green and golden bell frog tadpoles using ordinal regression. These lesions were so severe that in some cases 70% of the total liver was diseased. Normal liver function in tadpoles is necessary for metamorphosis, metabolism, and immune function. We postulate that this extensive liver damage would have significant host health impacts. Severe hepatic myxosporidiosis was more prevalent in tadpoles examined in autumn and winter (overwintered), suggestive of delayed metamorphosis in infected tadpoles, which would have serious flow-on effects in small populations. We compared the sensitivity of histopathology and species-specific PCR in the detection of *C. australis* and *C. axonis*. PCR was determined to be the most sensitive method (detection limit one myxospore equivalent of ribosomal DNA). Histology, however, had the advantage of assessing the impact of the parasite on the host. It was concluded that these parasites have the potential for significant ecological impacts, because of their high prevalence of infection and their ability to cause disease in some frogs.

A. Hartigan, N. K. Dhand, K. Rose, J. Šlapeta, D. N. Phalen, *PLoS ONE* 7, e43780 (2012). DOI:10.1371/journal.pone.0043780

Emerging myxosporean parasites of Australian frogs take a ride with fresh fruit transport

By Ashlie Hartigan, Lee Peacock, Alex Rosenwax, David N Phalen & Jan Šlapeta

Background

The spread of wildlife pathogens into new geographical ranges or populations is a conservation concern for endangered species. *Cystodiscus australis* and *Cystodiscus axonis* are two species of myxosporean parasites infecting Australian frogs and tadpoles that have been recently recognized as important disease agents impacting amphibian conservation. Yet despite their importance to wildlife health, the mechanism of emergence for these parasites is unknown. We hypothesize that these parasites are capable of being accidentally translocated with their amphibian hosts in fresh produce (agricultural, horticultural and industrial) shipments into naïve environments and host populations.

Methods

We surveyed 33 Australian “Banana box” frogs from Sydney fruit markets during 2011 using faecal smears and multiplex species specific PCR on DNA isolated from frog faeces or using histopathology to demonstrate the presence of both *C. australis* and *C. axonis*.

Results

One of the “Banana box” frogs, the Dainty green tree frog (*Litoria gracilentia*) was positive for *C. australis* and *C. axonis* in its faeces and continuously shed the parasites for eight months.

Conclusions

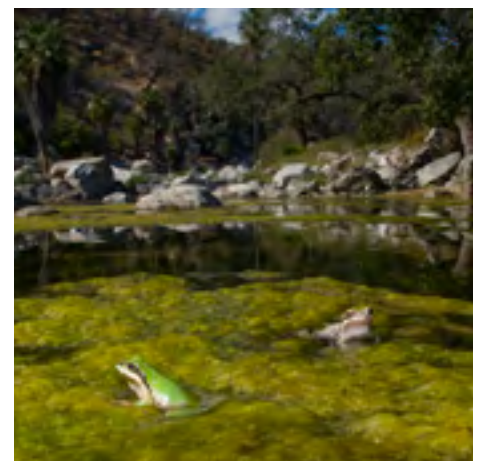
We present a possible mechanism for the emergence of *Cystodiscus* parasites and a non-invasive screening method to be used as a diagnostic test. In the future, vigilance and communication between wildlife managers/researchers and veterinarians will provide valuable information about these parasites, their host range and true distribution. This will aid risk management assessments for threatened populations within the range of *Cystodiscus* parasites and ultimately enhance conservation efforts.

A. Hartigan, L. Peacock, A. Rosenwax, D. N Phalen, J. Šlapeta, *Parasit. Vect.* 5, 208 (2012). DOI: 10.1186/1756-3305-5-208

The chytrid fungus *Batrachochytrium dendrobatidis* in isolated populations of the Baja California treefrog *Pseudacris hypochondriaca* curta in Baja California Sur, Mexico

By Victor H. Luja, Ricardo Rodríguez-Estrella, Kris Ratzlaff, Gabriela Parra-Olea & Aurelio Ramírez-Bautista

The chytrid fungus *Batrachochytrium dendrobatidis* was detected in five populations of *Pseudacris hypochondriaca curta*, an endemic treefrog fully dependent on desert oases in Baja California Sur, Mexico. Prevalence of the fungus varied among oases with values of 8, 31, 42, 60, and 100% for populations sampled. Oases with higher prevalence were inhabited by exotic species such as American bullfrogs (*Lithobates catesbeianus*) and Crayfish (*Procambarus clarkii*). These observations are the first of *B. dendrobatidis* in amphibians from lowland (<100 m) arid environments in Mexico.



A couple of Baja California treefrogs *Pseudacris hypochondriaca curta* rest on algae floating in a creek in the Biosphere Reserve Sierra La Laguna, Baja California Sur, Mexico. Photo: V. H. Luja.

V. H. Luja, R. Rodríguez-Estrella, K. Ratzlaff, G. Parra-Olea, A. Ramírez-Bautista, *The Southwestern Naturalist*, 57, 323 (2012).

Surviving chytridiomycosis: Differential anti-*Bd* activity in bacterial isolates from three lowland species of *Atelopus*

By Sandra V. Flechas, Carolina Sarmiento, Martha E. Cárdenas, Edgar M. Medina, Silvia Restrepo & Adolfo Amézquita

In the Neotropics, almost every species of the stream-dwelling harlequin toads (genus *Atelopus*) have experienced catastrophic declines. The persistence of lowland species of *Atelopus* could be

explained by the lower growth rate of *Batrachochytrium dendrobatidis* (Bd) at temperatures above 25 °C. We tested the complementary hypothesis that the toads' skin bacterial microbiota acts as a protective barrier against the pathogen, perhaps delaying or impeding the symptomatic phase of chytridiomycosis. We isolated 148 cultivable bacterial strains from three lowland *Atelopus* species and quantified the anti-Bd activity through antagonism assays. Twenty-six percent (38 strains representing 12 species) of the bacteria inhibited Bd growth and just two of them were shared among the toad species sampled in different localities. Interestingly, the strongest anti-Bd activity was measured in bacteria isolated from *A. elegans*, the only species that tested positive for the pathogen. The cutaneous bacterial microbiota is thus likely a fitness-enhancing trait that may (adaptation) or not (exaptation) have appeared because of natural selection mediated by chytridiomycosis. Our findings reveal bacterial strains for development of local probiotic treatments against chytridiomycosis and also shed light on the mechanisms behind the frog-bacteria-pathogen interaction.

S. V. Flechas *et al.*, *PLoS ONE* 7, e44832 (2012). DOI:10.1371/journal.pone.0044832

Prevalence of *Batrachochytrium dendrobatidis* infection is extremely low in direct-developing Australian microhylids

By Kim F. Hauselberger & Ross A. Alford

The emerging infectious disease chytridiomycosis has been implicated in declines and disappearances of amphibian populations around the world. However, susceptibility to infection and the extent of pathological effects of infection vary among hosts, and species with life histories that include parental care of direct-developing terrestrial eggs may tend to be less susceptible. We examined samples from a total of 595 individuals of nine species of direct-developing Australian frogs in the family Microhylidae for the presence of infection by *Batrachochytrium dendrobatidis* (Bd). Between 1995 and 2004, 336 samples were collected; 102 of these were analysed histologically and 234 were tissues stored in alcohol, which were examined using diagnostic quantitative PCR (qPCR). Swab samples were collected from 259 frogs from 2005 to 2008 and were examined using qPCR. None of the 595 samples showed evidence of infection by Bd. If these data are regarded as a single sample representative of Australian microhylids, the upper 95% binomial confidence limit for

the prevalence of infection in frogs of this family is 0.0062 (<1%). Even if only the data from the more powerful diagnostic qPCR tests are used, the upper 95% confidence limit for prevalence is 0.0075 (<1%). Our data suggest that Australian microhylids have a very low prevalence of infection by Bd in nature, and thus are either not susceptible, or are only slightly susceptible, to chytridiomycosis. This could be due solely to, or in combination with, low rates of transmission and to factors that promote resistance to infection, including ecological or behavioural characteristics, innate immune functions such as antimicrobial skin peptides, or antimicrobial symbionts in skin flora.

K. H. Hauselberger R. A. Alford, *Dis. Aquat. Organ.* **100**, 191 (2012).

Trenbolone causes mortality and altered sexual differentiation in *Xenopus tropicalis* during larval development

By Allen W. Olmstead, Patricia A. Kosian, Rodney Johnson, Pamela E. Blackshear, Jonathan Haselman, Chad Blanksma, Joseph J. Korte, Gary W. Holcombe, Emily Burgess, Annelie Lindberg—Livingston, Blake A. Bennett, Kacie K. Woodis & Sigmund J. Degitz

Trenbolone is an androgen agonist used in cattle production and has been measured in aquatic systems associated with concentrated animal feeding operations. We characterized the effects of aqueous exposure to 17 β -trenbolone during larval *Xenopus tropicalis* development. Trenbolone exposure resulted in increased mortality of post-Nieuwkoop-Faber stage 58 tadpoles at concentrations ≥ 100 ng/L. Morphological observations and the timing of this mortality are consistent with hypertrophy of the larynx. Development of nuptial pads, a male secondary sex characteristic, was induced in tadpoles of both sexes at 100 ng/L. Effects on time to complete metamorphosis or body sizes were not observed; however, grow-outs placed in clean media for six weeks were significantly smaller in body size at 78 ng/L. Effects on sex ratios were equivocal,



Tropical clawed frogs (*Xenopus tropicalis*) Photo: Allen Olmstead.



Necropsy of a Tropical clawed frog (*Xenopus tropicalis*) exposed to the synthetic androgen, trenbolone, revealed that the gastrointestinal tract was completely filled with air (as pictured) and the lungs were devoid of air. Observations of these types of mortality during larval exposure suggest that trenbolone causes the larynx muscles to hypertrophy, cutting off the passage of air to the lungs. Photo: Allen Olmstead.

with the first experiment showing a significant shift in sex ratio towards males at 78 ng/L and in the second experiment, no significant effects were observed up to 100 ng/L, although overall sex ratios were similar. Histological assessment of gonads at metamorphosis showed half with normal male phenotypes and half that possessed a mixed sex phenotype at 100 ng/L. Hypertrophy of the Wolffian ducts was also observed at this concentration. These results indicate that larval 17 β -trenbolone exposure results in effects down to 78 ng/L, illustrating potential effects from exposure to androgenic compounds in anurans.

A. Olmstead *et al.*, *Environ. Toxicol. Chem.* **31**, 2391 (2012).

Evidence of chytrid-mediated population declines in Common midwife toad in Serra da Estrela, Portugal

By Gonalo M. Rosa, Ibone Anza, Pedro L. Moreira, Jos  Conde, Filipe Martins, Matthew C. Fisher & Jaime Bosch

The emergence of a novel infectious disease, chytridiomycosis, is now widely recognized as a major cause of amphibian declines and biodiversity loss across local and global scales. Amphibian mortalities caused by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) were first recorded in Iberia, Europe over a decade ago. In August 2009, hundreds of post-metamorphic Common midwife toads (*Alytes obstetricans*) were found dead in the water and margins of a pond in the Serra da Estrela Natural Park, north-central Portugal. Histological and genetic analyses confirmed their infection with Bd. Given the likelihood of a new outbreak of chytridiomycosis, we evaluated the possible impacts of this



Recently metamorphosed *Alytes obstetricans* from Serra da Estrela Natural Park (Portugal). Photo: Gonalo M. Rosa.

disease on populations of *A. obstetricans* within the Park by conducting field surveys during 2010 and 2011. We compared the present distribution and abundance of *A. obstetricans* with historical records, and quantified the present prevalence and intensity of infection by *Bd*. Results showed that (1) *A. obstetricans* disappeared from 67% of the 1×1km squares where it was recorded in the past, (2) breeding is currently limited to 16% of the confirmed breeding sites in the past, and that (3) larvae are now less abundant, as well as are highly infected by *Bd* in the remaining sites. These effects were most pronounced at altitudes above 1,200 m. Our findings suggest that an outbreak of chytridiomycosis is responsible for the rapid decline of *A. obstetricans* in Serra da Estrela, and we believe that urgent conservation measures are needed to prevent local extinction of the species.

G. M. Rosa *et al.*, *Anim. Conserv.* In press

Potential influence of plant chemicals on infectivity of *Batrachochytrium dendrobatidis*

By Elizabeth W. Davidson, Andrew Larsen & Crystal Meins Palmer

We explored whether extracts of trees frequently found associated with amphibian habitats in Australia and Arizona, USA, may be inhibitory to the fungus disease, *Batrachochytrium dendrobatidis* (*Bd*), which has been associated with amphibian declines. We used salamanders, *Ambystoma tigrinum*, as the model system. Salamanders acquired significantly lower loads of *Bd* when exposed on leaves and extracts from River red gum, *Eucalyptus camaldulensis*, and loads were also low in some animals exposed on extracts of two oak species, *Quercus emoryi* and *Q. turbinella*. Some previously infected salamanders had their pathogen loads reduced, and some were fully cured, by placing them in leaf extracts, though some animals also self cured when housed in water alone. A significant number

of animals cured of *Bd* infections six months earlier were found to be resistant to reinfection. These results suggest that plants associated with amphibian habitats should be taken into consideration when explaining the prevalence of *Bd* in these habitats, and that some amphibians may acquire resistance to the fungus if previously cured.

E. W. Davidson, A. Larsen, C.M. Palmer, *Dis. Aquat. Org.* **101**, 87 (2012). DOI: <http://www.int-res.com/abstracts/dao/v101/n2/p87-93/>

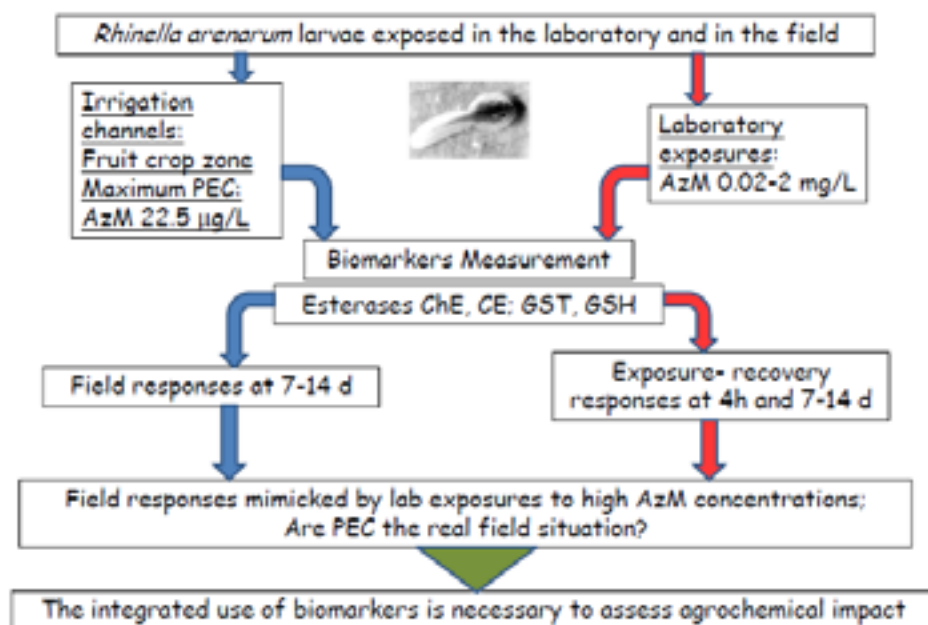
Response of biomarkers in amphibian larvae to in situ exposures in a fruit-producing region in north Patagonia, Argentina

By Enrique A. Rosenbaum, Leonardo Duboscq, J  mina Sole  o, Cristina M. Montagna, Ana Ferrari & Andr  s Venturino

The authors evaluated biomarker responses in caged larvae of the amphibian *Rhinella arenarum* in water channels during fruit production season and compared them with those elicited by a transient exposure to azinphos methyl (AzM) (0.02-2 mg/L; 4 h), the main pesticide applied in the Alto Valle region, Patagonia, Argentina, taking into account the maximum environmental concentration detected in superficial water (22.5 µg/L). The

traditional biomarkers of organophosphate exposure, acetylcholinesterase (AChE) and carboxylesterase, were inhibited in tadpoles after one week of exposure in channels potentially receiving pesticide drift, whereas the antioxidant glutathione (GSH) and the detoxifying activity of GSH S-transferase (GST) were induced. In a two-week monitoring study, AChE activity was induced in larvae exposed at the agricultural site, and carboxylesterase showed an inhibition followed by return to control values, suggesting an exposure-recovery episode. Antioxidant glutathione levels were first depleted and then surpassed control levels, whereas GST activity was continuously induced. These responses were mimicked in the laboratory by 2 mg/L AzM-pulse exposure, which notably exceeds the expected environmental concentrations. The results draw attention to the complexity of responses after pesticide exposure, strongly depending on exposure time-concentration and recovery periods, among other possible factors, and support the necessity of the integrated use of biomarkers to assess exposure episodes in agricultural areas.

E.A. Rosenbaum *et al.*, *Environ. Toxicol. Chem.* **31**, 2311 (2012).



Amphibian larvae were exposed in the laboratory to the insecticide azinphos methyl (AzM), mainly applied during fruit producing season in the region, at concentrations equal to or two orders of magnitude higher than the maximum predicted environmental concentrations (PEC) in water. The biochemical biomarkers acetylcholinesterase (AChE), carboxylesterase (CE), glutathione-S-transferase (GST) and reduced glutathione (GSH) suggest a field scenario much worse than the expected from PECs, and highlight the relevance of using an integrated approach to environmental impact evaluation.

Host invasion by *Batrachochytrium dendrobatidis*: Fungal and epidermal ultrastructure in model anurans

By Sasha E. Greenspan, Joyce E. Longcore & Aram J. K. Calhoun

The chytridiomycete fungus *Batrachochytrium dendrobatidis* (*Bd*) colonizes mouthparts of amphibian larvae and superficial epidermis of post-metamorphic amphibians, causing the disease chytridiomycosis. Fungal growth within host cells has been documented by light and transmission electron microscopy; however, entry of the fungus into host cells has not. Our objective was to document how *Bd* enters host cells in the Wood frog *Lithobates sylvaticus*, a species at high mortality risk for chytridiomycosis, and the Bullfrog *L. catesbeianus*, a species at low mortality risk for chytridiomycosis. We inoculated frogs and documented infection with transmission electron microscopy. Zoospores encysted on the skin surface and produced morphologically similar germination tubes in both host species that penetrated host cell membranes and enabled transfer of zoospore contents into host cells. Documenting fungal and epidermal ultrastructure during host invasion furthers our understanding of *Bd* development and the pathogenesis of chytridiomycosis.

S. E. Greenspan, J. E. Longcore, A. J. K. Calhoun, *Dis. Aquat. Org.* **100**, 201 (2012).

Preliminary analyses suggest absence of the amphibian chytrid fungus in native and exotic amphibians of the United Arab Emirates

By Pritpal S. Soorae, Thabit Al Abdessalaam, Christophe Tourenq, Maral Khaled Shuriqi & Mohammed Al Mehairbi

There is a lack of data on sampling and *Bd* detection within the Arabian Peninsula as can be seen on <http://www.spatalepidemiology.net/datasets/>, which shows which countries have been sampled and the number of *Bd* positive samples per country. Therefore this preliminary analysis aims to provide information from this region where there is a lack of data. The UAE has two species of native amphibians, namely the Arabian toad, *Duttaphrynus arabicus* and Dhofar toad, *Duttaphrynus dhufarensis*, which are both regional endemics. A *Bd* survey was done both on native (wild and captive populations in zoological institutions) and non-native amphibians (in zoological institutions and



Dhofar toad (*Duttaphrynus dhufarensis*) one of the UAE's regionally endemic toad species. Photo: P. S. Soorae.

pet trade) in the UAE. This was done to ensure that both native species and exotic species were covered simultaneously in this surveillance survey. In the UAE large assemblages of toads in the wild are usually only encountered during breeding periods, when surface water is available during the short rains, otherwise toads occur in very low densities in the wild and mainly in the mountainous north of the country where water flows along seasonal rivers in the mountains known as "wadis." The non-native amphibians sampled were African clawed frog, *Xenopus* spp., Floating frog, *Occidozyga lima*, Green toad, *Bufo viridis*, *Hoplobatrachus rugulosus*, Skittering frog, *Euphylyctis ehrenbergii*. This initial baseline survey of *Bd* in the UAE has fortunately not detected any presence of *Bd* both in wild and in captive collections. The possible route of *Bd* into the UAE could be via the pet trade in imported exotic amphibians that come from various countries mainly from South East Asia. In the wild the *Bd* fungus would also be challenged for survival as summer temperatures can reach 50 °C and with reduced surface water this would pose a challenge to the *Bd* fungus to survive under such extreme conditions. The possible route of transmission to the wild populations could be through the release of infected aquatic species into "wadi" systems.

P. S. Soorae, T. Al Abdessalaam, C. Tourenq, M. K. Shuriqi, M. Al Mehairbi, *SALAMANDRA*, **48**, 173 (2012). http://www.salamandra-journal.com/index.php?option=com_docman&Itemid=74

Nematode and ciliate nasal infection in captive Archey's frogs (*Leiopelma archeyi*)

By Stephanie D. Shaw, Rick Speare, Denis H. Lynn, Gregor W. Yeates, Zeng Zhao, Lee Berger & Richard Jakob-Hoff

Archey's frog (*Leiopelma archeyi*) is first on the list of evolutionarily distinct and globally endangered (EDGE) amphibians. Captive breeding is an important strategy for protection of the

species, but programs are hampered by a lack of information on diseases present in wild and captive populations. Two novel nematodes (*Koerneria* sp. and *Rhabditis* sp.) were found separately in four captive Archey's frog showing clinical signs of hemorrhagic purulent nasal discharge and weight loss. One of these frogs also had a novel protozoal infection (*Tetrahymena*) in the nasal cavity. *Koerneria*, *Rhabditis* and *Tetrahymena* have not previously been reported in amphibians in New Zealand. One frog was treated successfully with oral moxidectin at 0.4 mg/kg for the nematode infection and topical metronidazole at 10 mg/kg for the protozoal infection. The clinical signs abated only after both infections were cleared. The second frog died before treatment could be established. The third and fourth frogs were found dead.

S. D. Shaw *et al.*, *J. Zoo. Wildl. Med.* **42**, 473 (2011).

Fluorosis as a probable factor in metabolic bone disease in captive New Zealand native frogs (*Leiopelma* species).

By Stephanie D. Shaw, Phillip. J. Bishop, Cathy Harvey, Lee Berger, Lee. F. Skerratt, Karen Callon, Maureen Watson, John Potter, Richard Jakob-Hoff, Mike Goold, Nicole Kunzmann, Peter West & Rick Speare

This report describes the investigations into the cause and treatment of metabolic bone disease (MBD) in captive native New Zealand frogs (*Leiopelma* spp.) and the role of fluoride in the disease. MBD was diagnosed in *Leiopelma archeyi* and *Leiopelma hochstetteri* in 2008 at three institutions: Auckland Zoo, Hamilton Zoo, and the University of Otago. Most of these frogs had originally been held at the University of Canterbury for several years (2000-2004) but some were collected directly from the wild. Radiographs on archived and live frogs showed that MBD had been present at Canterbury, but at a lower rate (3%) than in the current institutions (38-67%). Micro-computed tomography showed that the femoral diaphyses of the captive frogs at Auckland Zoo had greater bone volume, bone surface, crosssectional thickness and mean total crosssectional bone perimeter which is consistent with osteofluorosis. On histology of the same femurs there was hyperplasia, periosteal growth, and thickening of trabeculae which are also consistent with skeletal fluorosis. An increase in fluoride levels in the water supply preceded the rise in the incidence of the above pathology further supporting the diagnosis of osteofluorosis. Analysis of long-standing husbandry practices showed that ultraviolet B exposure and the dietary

calcium: phosphorus ratio were deficient when compared with wild conditions—likely causing chronic underlying MBD. To prevent multi-factorial MBD in captive *Leiopelma* the authors recommend 1) increasing dietary calcium by incorporating into the captive diet inherently calcium-rich invertebrates, 2) increasing exposure to natural or artificial ultraviolet B light, and 3) using defluoridated water. Addressing these three factors at Auckland Zoo reduced morbidity, bone fractures and mortality rates.

S. D. Shaw *et al.*, *J. Zoo. Wildl. Med.* **43**, 549 (2012). DOI: <http://dx.doi.org/10.1638/2011-0276R1.1>

Acute toxicity of herbicide formulations and chronic toxicity of technical-grade trifluralin to larval Green frogs (*Lithobates clamitans*)

By Scott M. Weir, Shuangying Yu & Christopher J. Salice

There is considerable conservation concern regarding the impacts of agrochemicals on amphibian populations. Fewer herbicides have been investigated for amphibian toxicity than insecticides, despite higher application rates for herbicides. For example, in 2007 more than 2.5 times the mass of herbicides was applied to Texas upland cotton than insecticides. In addition, herbicides were applied to 96% of all cotton acreage compared to only 43% for insecticides. Of the few herbicides investigated, atrazine and glyphosate (active ingredient in Roundup) have received the greatest research interest and both are toxic to amphibians. We performed acute toxicity tests (96 hr) with two dinitroaniline herbicides that have received relatively little attention in amphibian ecotoxicology: trifluralin (sold as Treflan 4D) and pendimethalin (sold as Prowl 400 EC) and compared acute toxicity to an insecticide (malathion, formulated) and technical grade trifluralin. We performed experiments with tadpoles from a common species, the Green frog (*Lithobates clamitans*). We also performed chronic toxicity tests with technical grade trifluralin on green frog larvae. Chronic toxicity treatments consisted of controls, an environmentally relevant concentration of 20 µg/L and a higher (but still environmentally relevant) concentration of 200 µg/L for 62 days. Both Treflan 4D (LC₅₀ = 2.8 mg/L) and Prowl 400EC (LC₅₀ = 2.5 mg/L) were significantly more toxic to Green frog tadpoles than either malathion (LC₅₀ = 6.5 mg/L) or technical grade trifluralin (LC₅₀ = 9.8 mg/L). Chronic exposure to pure trifluralin resulted in significantly smaller Green frog

tadpoles at 20 µg/L compared to controls and 200 µg/L treatments. Acute toxicity results suggest that many herbicides may have formulations that are more toxic to amphibians than the active ingredient or even some insecticides. The LC₅₀s reported here are likely environmentally unrealistic at label rate specifications. Chronic toxicity results suggest a “non-monotonic” response of amphibians to trifluralin, but the mechanism is not known. Herbicides are applied at greater rates and are often detected more frequently in surface waters than insecticides, but very few herbicides (or their formulations) have been tested for toxicity to amphibians. Considering the toxicity found in this study and previous research into glyphosate formulations, more amphibian herbicide toxicity research is warranted.

S. M. Weir, S. Yu, C. J. Salice, *Environ. Tox. Chem.* **31**, 2029 (2012).

Developmental and polyamine metabolism alternations in *Rhinella arenarum* embryos exposed to the organophosphate chlorpyrifos

By Verónica Sotomayor, Cecilia Lascano, Ana María Pechen de D'angelo & Andrés Venturino

Organophosphorus pesticides (OPs) are widely applied in the Alto Valle of Río Negro and Neuquén, Argentina, due to intensive fruit growing. Amphibians are particularly sensitive to environmental pollution, and OPs may transiently accumulate in ponds and channels of the region during their reproductive season. Organophosphorus pesticide exposure may alter amphibian embryonic development and the reproductive success of autochthonous species. In the present study, embryos of the common toad *Rhinella arenarum* were employed to assess developmental alterations and to study polyamine metabolism,

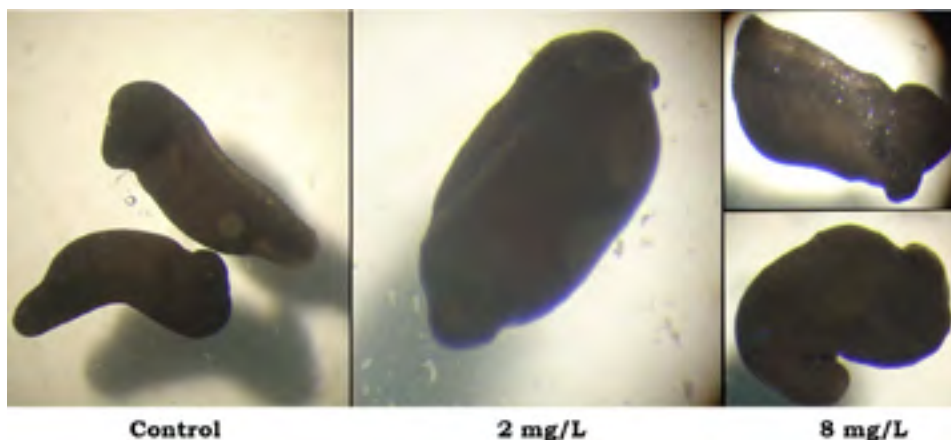
which is essential to normal growth, as a possible target underlying the effects of the OP chlorpyrifos. As the duration of chlorpyrifos exposure increased and embryonic development progressed, the median lethal concentration (LC₅₀) values decreased, and the percentage of malformed embryos increased. Developmental arrest was also observed and several morphological alterations were recorded, such as incomplete and abnormal closure of the neural tube, dorsal curvature of the caudal fin, reduction of body size and caudal fin length, atrophy, and edema. An early decrease in ornithine decarboxylase (ODC) activity and polyamine levels was also observed in embryos exposed to chlorpyrifos. The decrease in polyamine contents in tail bud embryos might be a consequence of the reduction in ODC activity. The alteration of polyamine metabolism occurred before embryonic growth was interrupted and embryonic malformations were observed and may be useful as a biomarker in environmental studies.

V. Sotomayor, C. Lascano, A. M. Pechen de D'angelo, A. Venturino, *Environ. Toxicol. Chem.* **31**, 2052 (2012).

Batrachochytrium dendrobatidis and Ranavirus in anurans inhabiting decorative koi ponds near Minneapolis, Minnesota, USA

By Brian G. Wolff, Sarah M. Conway & Clemon J. Dabney

Amphibian declines have been linked to fungal and viral pathogens and information concerning their geographic distribution, dissemination, and virulence is of substantial interest to conservation biologists. We report the presence of ranavirus and *Batrachochytrium dendrobatidis* (Bd) in anurans inhabiting



Some developmental alterations observed in *R. arenarum* embryos at “muscular response” stage caused by exposure to the organophosphate chlorpyrifos. Chlorpyrifos caused a concentration-dependent increase in the number of malformations; these included: dorsal curvature, axial shortening (reduced growth), and edema.

four decorative koi ponds in a suburb of Minneapolis, Minnesota, USA. These pathogens have been previously reported in Minnesota anurans, but we believe this is the first confirmed report of *Bd* and ranavirus in a Minneapolis/St. Paul suburban area and the first report of *Bd* and ranavirus in decorative koi ponds. Decorative koi ponds and water gardens are extremely simplified ecosystems and may be especially conducive to *Bd* propagation and viral transmission. Koi enthusiasts and water gardeners may additionally be fostering the spread of *Bd* and ranavirus by importing infected pond organisms. Bullfrogs (*Lithobates catesbeianus*), for example, are marketed to koi enthusiasts and water gardeners to control algae and have been linked to *Bd* and ranavirus dispersal. Water hyacinth from a company that advertises that its plants are grown organically and that tadpoles are used to control algae was introduced to the ponds surveyed in this study. The possibility that koi ponds and water gardens serve as loci for disease transmission and dispersal in the U.S. would seem to warrant greater attention.

B. G. Wolff, S. M. Conway, C. J. Dabney,
Herpetol. Rev. **43**, 427 (2012).

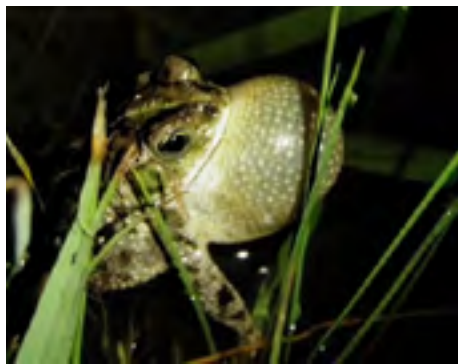
Influence of existing site contamination on sensitivity of *Rhinella fernandezae* (Anura, Bufonidae) tadpoles to Lorsban_48E formulation of chlorpyrifos

By Carolina Salgado Costa

On August 2012, we published a paper in *Ecotoxicology Journal*. The principal objective was to study the effects of the insecticide Lorsban_48E formulation of chlorpyrifos on *Rhinella fernandezae* (Anura: Bufonidae) tadpoles, a native species of Argentina, Brazil, Paraguay and Uruguay.

Amphibians are valuable indicators of local environmental changes. Pollution from agrochemical has been one of the proposed factors for global amphibian declines. Having this in mind, we choose a widely used multi-purpose organophosphorus insecticide able to bioconcentrate in different groups of aquatic organisms but with limited information of its toxic effects on amphibians, to compare the effects on different populations of *R. fernandezae* tadpoles.

We selected two sites with different degree of anthropogenic disturbance; one was an unpolluted area and the other an area with



An adult male of *Rhinella fernandezae* in reproductive activity in a temporary pond of Buenos Aires Province (Argentina). Photo: Carolina Salgado Costa.

high degree of antropogenic disturbance as a source of organisms. We assessed acute and chronic lethal and sublethal effects (behavior, growth, and abnormalities) and found that chlorpyrifos showed high toxicity on the tadpoles, inducing lethal and sublethal effects at 96 h exposure. The results allowed comparing the studied species with the existing toxicological data and ranked *R. fernandezae* tadpoles below the 30th percentile in the species sensitivity distribution. Moreover, we found that the effects of chlorpyrifos showed no significant differences ($p > 0.05$) between the studied populations considering all the acute endpoints evaluated. The insecticide exhibited severer effects at higher concentrations than at higher times of exposure.

Making a comparison of both studied sites, we conclude that contaminants in the polluted site do not seem to have induced local adaptation. Taking into account that the measured levels of chlorpyrifos in the environment are within the range of those inducing sublethal effects in the laboratory on *R. fernandezae* tadpoles, we consider that environmental concentrations of the insecticide are indicating a potential risk for populations inhabiting agroecosystems.

C. Ruiz de Arcaute, C. Salgado Costa, P. M. Demetrio, G. S. Natale, A. E. Ronco,
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Clinical trials with itraconazole as a treatment for chytrid fungal infections in amphibians

By Laura A. Brannelly, Corinne L. Richards-Zawacki & Allan P. Pessier

Due in large part to recent global declines and extinctions, amphibians are the most threatened vertebrate group. Captive assurance colonies may be the only lifeline for some rapidly disappearing species. Maintaining these colonies free of disease represents a challenge to effective amphibian conservation. The fungal disease chytridiomycosis, caused by the

fungus *Batrachochytrium dendrobatidis* (*Bd*), is one of the major contributors to global amphibian declines and also poses a serious threat to captive assurance colonies. Many treatment options for *Bd* infection have not been experimentally tested and the commonly administered dosages of some drugs are known to have negative side effects, highlighting a need for clinical trials. The objective of this study was to clinically test the drug itraconazole as a method for curing *Bd* infection. We bathed *Bd*-positive juveniles of two anuran amphibian species, *Litoria caerulea* and *Incilius nebulifer*, in aqueous itraconazole, varying the concentration and duration of treatment, to find the combination that caused the fewest side effects while also reliably ridding animals of *Bd*. Our results suggest that a bath in 0.0025% itraconazole for 5 min daily for 6 d reliably cures *Bd* infection and causes fewer side effects than the longer treatment times and higher concentrations of this drug that are commonly administered.

L. A. Brannelly, C. L. Richard-Zawacki, A. P. Pessier. *Dis. Aquat. Org.* **101**, 95 (2012).

Gonadal abnormalities in frogs (*Lithobates* spp.) collected from managed wetlands in an agricultural region of Nebraska, USA

By Diana M. Papoulias, Matt S. Schwarz & Lourdes Mena

Nebbraska's Rainwater Basin (RWB) provides important wetland habitat for North American migratory birds. Concern exists that pesticide and nutrient runoff from surrounding row-crops enters wetlands degrading water quality and adversely affecting birds and wildlife. Frogs may be especially vulnerable. Plains leopard (*Lithobates blairi*) metamorphs from RWB wetlands with varying concentrations of pesticides were evaluated for a suite of biomarkers of exposure to endocrine active chemicals. Froglets had ovarian dysgenesis, high rates of testicular oocytes, and female-biased sex ratios however, there was no clear statistical association between pesticide concentrations and biomarkers. Data interpretation was hindered because timing and duration of exposures were unknown and due to an incomplete understanding of *L. blairi* sexual development. Emphasis is on describing the complex developmental biology of closely-related leopard frogs, how this understanding can explain RWB *L. blairi* anomalies, and the need for sampling at the appropriate life stage.

D. M. Papoulias, M. S. Schwarz, L. Mena,
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AmphibiaWeb Recent Publication List

This reference list is compiled by Professor Tim Halliday (formerly DAPTF International Director; tim.r.halliday@gmail.com). It lists papers on amphibian declines and their causes and amphibian conservation, with an emphasis on those that describe methods for monitoring and conserving amphibian populations. Tim is always delighted to receive details of forthcoming papers from their authors.

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Call for recent publication abstracts

If you would like to include an abstract from a recent publication in this section of *FrogLog* please 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.

Perch substrate use by the threatened wallum sedge frog (*Litoria ongburensis*) in wetland habitats of mainland eastern Australia. *Australian J. Zool.* (j.shuker@griffith.edu.au)

Smith, G. R. and Burgett, A. A. (2012) Effects of nutrient enrichment and changes in the background tadpole community on American bullfrog tadpoles. *Herpetol. J.* 22; 173-178. (smithg@denison.edu)

Szuroczki, D. and Richardson, J. M. L. (2012) The behavioral response of larval amphibians (Ranidae) to threats from predators and parasites. *PLoS One*: 7(11); e49592. (dorina.szuroczki@utoronto.ca)

Takase, M. *et al.* (2012) Accumulation and pharmacokinetics of estrogenic chemicals in the pre- and post-hatch embryos of the frog *Rana rugosa*. *In Vivo*: 26; 913-920. (minoru@hiroshima-u.ac.jp)

Toledo, L. F. and Batista, R. F. (2012) Integrative study of Brazilian anurans: geographic distribution, size, environment, taxonomy, and conservation. *Biotropica*: 44; 785-792. (toledolf2@yahoo.com)

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van Buggenum, H. J. M. and Vergoossen, W. G. (2012) Habitat management and global warming positively affect long-term (1987-2011) chorus counts in a population of the European tree frog (*Hyla arborea*). *Herpetol. J.* 22; 163-171. (hvanbuggenum@gmail.com)

Whitfield, S. M. *et al.* (2012) Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica*: 44; 779-784. (steven.whitfield@usd.edu)

Yahnke, A. E. *et al.* (in press) Effects of the herbicide imazapyr on juvenile Oregon spotted frogs. *Envtl. Toxicol. and Chem.* (aey@uw.edu)

Zeisset, I. and Beebee, T. J. C. (in press) Donor population size rather than local adaptation can be a key determinant of amphibian translocation success. *Animal Conservation*: (t.j.c.beebee@sussex.ac.uk)

General Announcements

Upcoming Meetings & Workshops

February

21-24, Southeast PARC Annual Meeting-
Hickory Knob State Park, McCormick, SC

March

22-23, 4th Box Turtle Conservation
Workshop-North Carolina Zoological Park,
Asheboro, NC

April

19-21, Joint meeting of North Carolina
PARC and the North Carolina Herpetological
Society-NC Zoo, Asheboro, NC

May

13-24, Graduate and Professional Course-
Species Monitoring and Conservation:
Reptiles-Smithsonian Conservation Biology
Institute, Front Royal, VA, USA

Internships & Employment

Two graduate student (M.S.) positions are available, including two years of stipend and tuition remission, in the Department of Biology at San Francisco State University. These students will be co-advised by Andy Zink and Vance Vredenburg and work directly on a project investigating the relationships between communal nesting in *Batrachoseps* salamanders and the spread of fungal pathogens. Students will be expected to combine laboratory work (sampling museum specimens, quantitative PCR) with field work on salamander behavior and ecology throughout California. Interested students should email their CV, unofficial transcripts, and GRE scores to Andy Zink at (zink@sfsu.edu) with SALAMANDER POSITION (MS) in the subject line of the email.

The following information can be found at: <http://www.parcplace.org/resources/job-listings.html>. Herp jobs are posted as a service to the herpetological community. If you would like to list a job opening for your organization, please send the announcement to: herpjob@parcplace.org

Field Technicians-Anuran and Turtle
Research in Western Iowa, Western Iowa
(Posted 1/2/13; Closing 2/4/13)

Biologist I Position-CSS-Dynamic/USGS Fort
Collins Science Center, Guam (Posted 1/2/13;
Closing 1/31/13)

Biological Science Positions (2 openings),
Yosemite National Park, Yosemite, CA
(Posted 12/22/12; Closing Jan. 10, 2013)

Graduate Opportunities to Study
Salamanders, Department of Biology at San
Francisco State University, San Francisco, CA
(Posted 12/19/12; No Closing Date Provided)

Full-Time Laboratory Technician in Reptile
Facility, Stowers Institute for Medical
Research, Kansas City, MO (Posted 12/19/12;
No Closing Date Provided)

Full-Time Herpetology/Eco Evo Biology
Position, Cornell University, Ithaca, NY
(Posted 12/19/12; Closing January 30, 2013)

Biological Science Technician, Ocean Springs,
MS (Posted 12/16/12; Closing January 02,
2013)

Nongame Wildlife Biologist, Bangor, ME
(Posted 12/14/12; Closing January 11, 2013)

Biologist Technician-Herpetologist (up to
3 positions), Casper, WY (Posted 12/11/12;
Open Until Filled)

Gopher Tortoise Internships (Spring-
Summer 2013) Archbold Biological Station,
Lake Placid, FL (Application Deadline
December 20, 2012)

Herpetological Researcher/Educator
Internship-Research 4 Reptiles LLC,
Wilmington, IL (Application Deadline April
1, 2013)

Current Student/Recent Graduate Amphibian
Research Technicians, Patuxent Wildlife
Research Center, Maryland (Application
Deadline December 20, 2012)

Snake-bird predator-prey interaction
Field Technicians, Aiken, South Carolina
(Application Deadline December 18, 2012)

Amphibian and Reptile Monitoring Biological
Technician Vacancies, Cape Cod National

Seashore, Wellfleet, MA (Application
Deadline December 10, 2012)

Indigo Snake Telemetry Technician-The
Orianne Society, Archbold Biological Station,
Highlands County, FL (Application Deadline
Dec. 2, 2012)

Ph.D. Position: Giant tortoises as drivers
of the seed dispersal network of Aldabra,
Institute of Evolutionary Biology and
Environmental BozeStudies, University of
Zurich, Switzerland (Closing November 1,
2012 or until filled)

Ph.D. Position: Genetic Assessment
of Amphibian Source-Sink Dynamics
(University of Missouri), Columbia, MO
(Closing 12/15/2012)

Ph.D. Assistantship, Wildlife Ecology -
Clemson University, Clemson, SC (Fall 2012-
Open Until Filled)

Assistant Professor-Wildlife Ecology and
Management-Purdue University, West
Lafayette, IN (Posted 8/21/12-Open Until
Filled)

OPS Wildlife Field Biologist, Florida Fish and
Wildlife Conservation Commission, Panama
City, FL (Posted 7/30/12-Open Until Filled)

Curator of Herpetology and Assistant
Professor of Biology, Sam Noble Museum
and Department of Biology, the University
of Oklahoma, Norman, Norman, OK (Posted
7/30/12-Open Until Filled)

Threatened & Endangered Species Field
Technician, Florida Fish and Wildlife
Conservation Commission, Holt, FL (Posted
7/27/12-Open Until Filled)

Keep In Touch

If you would like to be added to the ASG mailing list, please send an email to:
froglog@amphibians.org with the subject heading "add me to mailing list."
Also, follow us on Facebook for regular updates on the herpetological conservation
community and the latest news from the ASG.
<http://www.facebook.com/AmphibiansDotOrg>

Amphibian Conservation Husbandry

3rd - 7th June 2013

*The ACH course immensely helped to improve my knowledge and skills of amphibian husbandry.
ACH course participant, 2009*

The Amphibian Conservation Husbandry (ACH) course is a five day intensive course held at Durrell's headquarters in Jersey, designed to expose participants to the latest theory and practice of amphibian husbandry. Participants will be equipped with the skills and knowledge to establish and manage captive populations and breeding programmes for some of the world's most threatened amphibians.

WHO IS THE COURSE FOR?

The ACH course is designed specifically for curators, zoo keepers, private breeders and others interested in the captive management of amphibians.



WHAT IS THE COURSE CONTENT?

The course involves lectures, guided tours and plenty of practical sessions to try out newly learnt skills. The course is co-directed by leading amphibian experts from Durrell Wildlife Conservation Trust and additional external experts from around the world.

This course is officially endorsed by Amphibian Ark and Amphibian Survival Alliance.

THE COURSE WILL INCLUDE THE FOLLOWING TOPICS:

- Understanding the natural history of your species
- Water quality, testing and filtration
- Temperature, lighting and UV
- Enclosure design and decoration
- Nutrition and breeding live foods
- Healthcare, disease and biosecurity
- Breeding difficult species
- Supporting *in-situ* conservation



WHAT IS THE COST?

The course fee is £750 (discounted to £600 if paid at least 8 weeks in advance). Optional full board on-site accommodation is available for £210 for six nights.

For further information please contact:
+44 (0)1534 860037 or academy@durrell.org



Durrell Conservation Academy
Durrell Wildlife Conservation Trust
Les Augrès Manor, La Profonde Rue
Trinity, Jersey, JE3 5BP

durrell wildlife conservation trust durrell.org
an international charity saving species from extinction

Funding Opportunities

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

January 2013

Animal Behavior Society—Grants and Awards 2012-2013. The ABS offers several categories of research grants for graduate students, ranging in amount from US\$500 to US\$2,000. Additionally, ABS invites applications from students and from more established researchers in developing countries for the Developing Nations Research Grant. ABS posts contact information and eligibility criteria. The application deadline is 11 January 2013.

Australian Government—AAA Small Grants. The Australia Awards in Africa (AAA) supports alumni and alumni associations in their home countries through a Small Grants Scheme. The grants are a form of seed funding to advance the aims of the Millennium Development Goals, which include actions to promote agriculture and environment (among other areas). Grants are up to AUD\$10 thousand for alumni networks and organizations, and up to AUD\$5 thousand for individual professional alumni. The first round of applications closes on 31 January 2013.

CRDF Global and Russian Academy of Sciences—Collaborative Research in the Russian Far East. CRDF Global and the Far Eastern Branch of the Russian Academy of Sciences announce research support for teams of U.S. and Russian scientists. The subject areas of interest include several related to climate change, ocean science, Far East biological resources, and others. This competition will fund a maximum of five awards, each up to US\$44 thousand over two years. The application deadline is 31 January 2013.

Ecosystem Services for Poverty Alleviation—ESPA 2013. Ecosystem Services for Poverty Alleviation (ESPA) is an interdisciplinary research program managed by a consortium of UK government organizations. ESPA's themes in 2013 are sustainable ecosystems as a pathway out of poverty; ecosystem services and the urban environment; and building on ESPA success. There are no restrictions by location or nationality of ESPA researchers or research organizations, but at least one developing country must be involved in each funded project. The program invites applications in the range of £200 thousand to £500 thousand for projects of 24-30 months. The deadline for outline proposals is 23 January 2013.

Gadfly Project—Support for Geo-Based Web Applications. The Gadfly Project offers

in-kind grants to organizations that can demonstrate novel uses for geo-based web applications. Gadfly provides services and training in the developing world to develop new geographical information systems (GIS), web maps, and geo-management practices. Proposals should aim for support between US\$10 thousand and US\$60 thousand, if possible. The closing date for proposals is 31 January 2013.

Japan Fund for Global Environment—Conservation Grants 2013. The Fund makes grants for field projects in environmental conservation in Japan and developing countries, especially in Asia. Eligibility extends to legally registered non-governmental organizations and non-profit organizations in Japan and the developing countries. Each non-Japanese applicant needs to have a representative in Japan to apply on behalf of the applicant. The average grant size is about 4 million yen. The application period is 04 January 2013 through 25 January 2013.

National Geographic Society—Buffet Awards for Conservation Leadership 2013. The National Geographic Society (USA) welcomes nominations for the 2013 Buffett Awards in Conservation Leadership. There is one award for Latin America, and another for Africa. The awards honor the unsung heroes of conservation by celebrating the recipient's past achievements, and by supporting ongoing work in conserving nature or culture. The awards include a grant of US\$25 thousand. The deadline for nominations is 15 January 2013. Buffet Award Latin America Buffet Award Africa

Royal Bank of Canada—Blue Water Project 2013. The RBC's Blue Water Project makes grants for watershed protection and safe drinking water in North America, the Caribbean, and UK. (i) The Community Action Grants are up to \$10 thousand for local and community organizations in Canada, USA, and the Caribbean. (ii) Leadership Grants ranging from \$10 thousand to \$100 thousand are available in North America and other countries where RBC does business. Application deadlines are 11 January 2013 for the Community Action Grants, and 08 February 2013 for the Leadership Grants.

UNESCO—Michael Batisse Award for Biosphere Reserve Management 2013. The United Nations Educational, Scientific and Cultural Organization (UNESCO) sponsors this award for outstanding achievements in biosphere reserve management. The award is US\$6 thousand and international travel and allowances in Paris to present the winning case study to the MAB-ICC session. The deadline for submitting case studies (English or French) is 31 January 2013.

Wildlife Conservation Network—Partnership Applications 2013. The WCN supports wildlife projects worldwide, with emphasis on Latin America, Africa, and Asia. WCN invites letters of inquiry from registered nonprofit organizations engaged in wildlife conservation or animal welfare to apply for partnerships. WCN collaborates with its partners for networking, fund raising, and organizational support. The deadline for letters of inquiry is 15 January 2013.

February 2013

European Commission (EC)—National Participation for Forest Governance in Vietnam. The EU Delegation in Vietnam will support Vietnamese organizations to build their capacity for active participation in FLEGT—the international program of Forest Law Enforcement, Governance, and Trade. Eligibility for grants extends to non-profit organizations in the EU member states and Vietnam. Grants will range from €200 thousand to €400 thousand, subject to cost shares. Reference EuropeAid/133707/L/ACT/VN. The deadline for concept notes is 21 February 2013.

European Commission (EC)—Rural Diversification in Zimbabwe. Under its Food Security program, the EC will fund increased production and diversification of small-farm producers in Zimbabwe. Grants will support opportunities for diversification such as community-based wildlife, ecotourism, fisheries, aquaculture, agroforestry, forestry, and non-timber forest products. Eligibility for funding extends to non-profit organizations in the EU countries and Zimbabwe, and to international organizations. Grants will range from €2.5 million to €3.5 million, subject to cost shares. Reference EuropeAid/133709/L/ACT/ZW. The deadline for concept notes is 12 February 2013.

Fondation Nature & Decouvertes—Grants for Nature Protection 2013. The foundation supports projects for nature protection in France and Francophone Africa. Applications for small grants ("coup de main") can be submitted throughout the year. The next application deadline for major projects (from €3 thousand to €10 thousand) is 15 February 2013.

Global Biodiversity Information Facility (GBIF)—Ebbe Nielsen Prize 2013. The GBIF supports research and discovery in biodiversity informatics. The Ebbe Nielsen Prize is awarded annually to a person or team that demonstrates excellence in combining biodiversity informatics and biosystematics research. The award of €30 thousand supports the recipient(s) to engage in research for a period of 3-6 months in another country. The deadline for nominations is 15 February 2013.

March 2013

International Tropical Timber Organization—Freezailah Fellowship Fund, First Cycle 2013. 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 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. ITTO normally invites fellowship applications twice a year. The next application deadline is 05 March 2013.

John Ball Zoological Society—Wildlife Conservation Grants 2013. The JBZS (USA) 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 should be associated with a recognized institution (e.g., zoo, educational institution, conservation organization, etc.). The deadline for applications is 04 March 2013.

Zoological Society of London (ZSL)—EDGE Fellows 2013. ZSL's EDGE of Existence Program provides fellowships for two-year projects in research and conservation of EDGE (Evolutionarily Distinct and Globally Endangered) mammals, amphibians, and corals. Applicants must be early-career conservation biologists (less than 5 years experience) who are nationals or residents of the country in which the EDGE species occur. The Fellowship includes a grant of up to £5 thousand per year, plus training and technical support. Applicants are strongly advised to discuss their projects with the EDGE team before applying. The application deadline is 31 March 2013.

April 2013

African Leadership Academy—Anzisha Prize for Young Innovators 2013. The Anzisha Prize is awarded to young (ages 15–20) innovators in Africa who develop and implement innovative solutions to challenges facing their communities. Past awards include innovations in energy, agriculture, and ecological consumer products. The African Leadership Academy will select the top-rated applicants to participate in a workshop and conference near Johannesburg. The finalist Prize winners will share US\$75 thousand. The closing date for applications (English or French) is 01 April 2013.

MacArthur Foundation—African Great Lakes 2013. The John D. and Catherine T. MacArthur Foundation make grants for conservation and development in selected regional ecosystems of the developing world. In year 2013, the Foundation invites letters

of inquiry (LOIs) for grants supporting conservation of Large Lakes in the Rift Valley, East-Central Africa. The deadline for LOIs is 05 April 2013. Note: Applications in 2013 for MacArthur's other regional conservation grants is by invitation only.

Royal Society and UK Department for International Development—Research Collaboration, UK and Africa. The UK's Royal Society collaborates with the UK's DFID to strengthen the research capacity of universities and research institutions in sub-Saharan Africa. Priority research areas in the current funding cycle are water and sanitation; renewable energy; and soil-related research. Principal investigators must hold a Ph.D. and be based in the UK or one of the eligible Sub-African countries. The program makes small start-up grants (up to £25 thousand), as well as larger program grants for 5-year periods. The closing date for applications is 04 April 2013.

May 2013

American Association of Zoo Veterinarians (AAZV)—International Grants for Conference Participation. The AAZV provides financial aid to zoo and wildlife veterinarians from outside of the USA to participate in the AAZV's annual conferences. Preference will be given to applicants from developing countries with demonstrable financial needs and focused educational/conservation goals. Grants are normally up to US\$2 thousand. The application deadline is 30 May 2013.

Amphibian Ark—Seed Grants 2013. Amphibian Ark offers the AArk Seed Grant of US\$5 thousand in support of *ex situ* conservation of globally endangered amphibian species. The grant is intended for start-up rescue projects that need seed money in order to attract larger funding. Projects should work with species in their range countries, involve range-country biologists, conform to biosecurity standards, and observe other guidelines explained on the website. Applications are due 01 May 2013.

IUCN-Netherlands—Purchase of Nature 2013. 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, mainly in tropical developing countries. The grants are to local conservation organizations in those countries. Pre-proposals are due before 01 May 2013.

McKnight Foundation—Communities and Natural Resources in Southeast Asia. McKnight's grant program for Southeast Asia (Vietnam, Laos, Cambodia) includes themes on indigenous and ethnic minority communities in relation to livelihoods and natural resources. Grants are to qualified

local and international NGOs working in the countries of interest. The annual deadline for letters of inquiry is 01 May.

Turtle Conservation Fund—Grants to Protect Tortoises and Freshwater Turtles. The Turtle Conservation Fund makes grants to organizations and individuals worldwide for conservation and research of endangered and critically endangered tortoises and freshwater turtles. Most grants are US\$2 thousand to US\$5 thousand per project. Application deadlines are 01 May and 01 November of each year.

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 bimonthly 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

James P. Lewis: jplewis@amphibians.org

EDITORIAL COMMITTEE

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Phillip J. Bishop (ASG Co-Chair)
Robin D. Moore (ASG Program Officer)
Candace M. Hansen (ASG Program Officer)
Craig Hassapakis (Co-editor, Amphibian and Reptile Conservation)
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 James Lewis at: jplewis@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 English.

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.

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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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