Newsletter

amphibian ark

AArk Newsletter No. 13, December 2010

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The Amphibian Ark team is pleased to send you the latest edition of our e-newsletter. We hope you enjoy reading it.

The Amphibian Ark

Just shoot me! *An Amphibian Ark photography contest*

We're running a really cool photographic competition so you can contribute your best amphibian photos for the 2012 AArk calendar!

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Read this before you do any holiday shopping!

Please make every gift count this holiday season. You can find coupons at more than 1,600 top stores (including Amazon, Macy's, J.Crew, Toys R Us, Best Buy, Gap and more) and a percentage of virtually every sale will be donated to the Amphibian Ark.

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Argentinean Amphibian Conservation Needs Assessment

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A well-attended and very successful Amphibian Conservation Needs Assessment workshop for Argentinean species was held in October at the Buenos Aires Zoo in Argentina.

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Amphibian Biobanking Workshop: an overview

Rhiannon Lloyd, Biobanking Officer, Amphibian Ark

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News

Orana Wildlife Park Maud Island Frog Habitat

Nathan Hawke, Marketing, Public Relations & Visitor Services Manager, Orana Wildlife Trust, New Zealand

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Oregon Spotted Frog project at Cedar Creek Corrections Center

Kelli Bush, Sustainable Prisons Project Manager, The Evergreen State College

Since 2009, The Sustainable Prisons Project has been working with Washington Department of Fish and Wildlife and Cedar Creek Correction Center to raise endangered Oregon Spotted Frogs. Captive-bred offspring are now being reintroduced into the wild.

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A new method for semen collection from live Urodeles and standardization of *in vitro* fertilization

Nabil Mansour, Department of Organismic biology, University of Salzburg, Austria

Recently, motile and fertile semen from live Urodeles has been collected by gentle abdominal massage after hormonal stimulation. This protocol is very promising as it may be transferable to endangered Urodeles.

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Sperm cocktail celebrates conservation breakthrough

Dr Robert Browne, AArk Research Officer

Mix an amphibian sperm cocktail, freeze it, and thaw when needed; in other words a sperm bank for amphibians. Conservation breeding programs, when supported by sperm banking could save many amphibian species.

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Preparations for the battle against chytrid: Building capacity for chytrid detection in Madagascar

Gerardo García PhD, Head of Herpetology Department, Durrell Wildlife Conservation Trust

To date Madagascar appears to be chytrid-free. However, it may only be a matter of time given its proximity to mainland Africa and the trade routes that exist with countries where the disease is present.

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Geocrinia programs at Perth Zoo

Debbie Read, Media and Communications Manager and Daniel Scarparolo, Communications and Interpretation Officer, Perth Zoo, Western Australia

Perth Zoo staff are currently involved with a number of projects focussing on the genus *Geocrinia*. This article reports on two of these projects.

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Mantella breeding success

the text-only version of this newsletter

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Wayne Woods, Zoo Attendant I: Primary Amphibian Keeper, Edmonton Valley Zoo

Prior to the Year of the Frog campaign, the Edmonton Valley Zoo in Alberta Canada only had four amphibians, today we have over two hundred. In 2008 we decided that we would focus our Year of the Frog display to the displaying and breeding of frogs from the genus *Mantella*.

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An update from the Association of Zoos & Aquariums

Shelly Grow, Conservation Biologist, AZA

AZA's Conservation Biologist Shelly Grow, provides an update on amphibian projects in North America.

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Recent activities in Mesoamerica

Yolanda Matamoros, President, Mesoamerican Association of Zoos

Branko Hilke, PHd student from the Universidad de Puerto Rico presented two papers during the Mesoamerican Society for Biology Conservation Annual Congress held in San Jose, Costa Rica, 8-12 November, 2010, and Gilberto Alvarado, veterinary student at the Veterinary Medicine School, Universidad Nacional, Costa Rica, presented his thesis. The abstracts from both papers are included in this update.

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Just shoot me! *An Amphibian Ark photography contest*

Amphibian Ark will be producing another sensational amphibian calendar for 2012, featuring your stunning photos!

We're running a really cool photographic competition so you can contribute your best photos for the calendar. Our panel of judges will select the twelve best images to be used in the calendar, with the overall winner featuring on the front cover.

Javan gliding frog (*Rhacophorus margaritifer*) in West Java, Indonesia. Photo courtesy Mike Ready.

Calendars will be available for sale in August 2011. Proceeds will be used to assist Amphibian Ark conservation programs which are saving threatened amphibians. The production of our calendars is generously supported by Fowler Printing & Graphics, Randolph, Massachusetts.

Who can enter

The Amphibian Ark photo competition is open to all photographers, anywhere in the world, except for the members of the competition judging panel.

Competition categories

Amongst the twelve winning entries, one photo will also be selected as the best images in each of these three categories:

In the wild – photos must be of amphibians in the wild. The location where the photo was taken must be provided.
In captivity – photos of amphibians in a captive environment, e.g. zoos, aquariums, pets etc.
Youth – open to photographers who are under 18 years of age.

Subject matter

All photos must contain one or more amphibians, either in the wild or captivity. Images must not contain inappropriate or offensive material.

Prizes

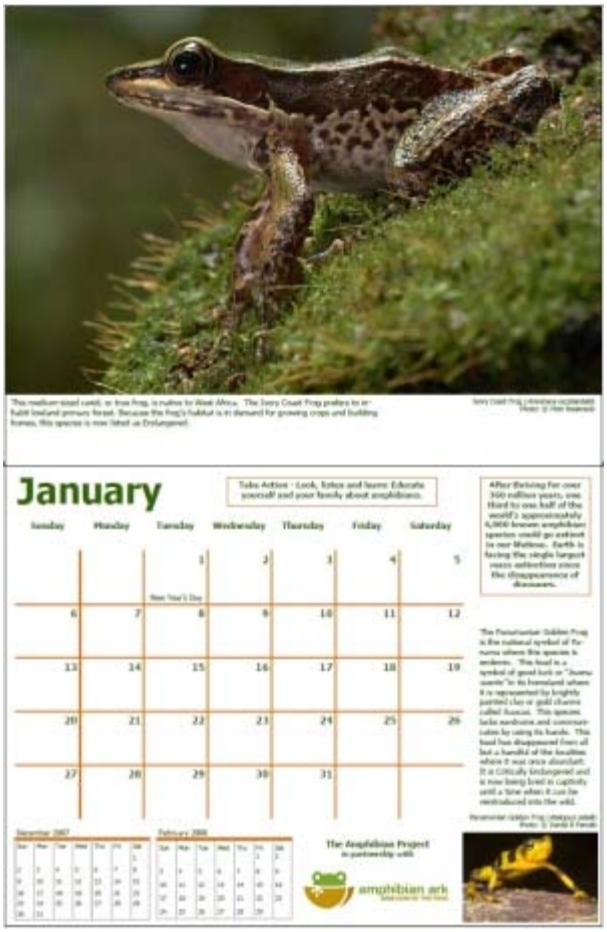
The twelve best photos will be selected for use in the 2012 Amphibian Ark calendar. Additionally, amongst the twelve winning entries, one photo will also be selected as the best images in each of the categories of Youth, In the wild, and In captivity.

The winning photo in each of the three competition categories will receive:

- A copy of ***Frogs of Panama*** by Dr. Douglas Woodhams which explores the diversity of amphibians and the impact of disease on Panamanian populations (see www.blurb.com/bookstore/detail/174126).
- A copy of ***Sapos***, a beautiful book from Ecuador by Santiago Ron, Martin Bustamante, Luis Coloma and Belén Mena, which uses the surreal patterns and colors of naturally beautiful amphibians in combination with and as inspiration for graphic art (www.puce.edu.ec/zoologia/sron/sapos/index.html).

In addition, all twelve winning photos will each receive:

- A copy of the ***2012 Amphibian Ark calendar***, featuring the winning photos from this competition.
- A copy of ***Threatened Amphibians of the World*** published by the IUCN and NatureServe and edited by



Simon Stuart et al. (www.lynxeds.com/product/threatened-amphibians-world).

- A copy of ***Treefrogs…prehistoric survivors with a global message*** - the latest book from National Geographic photographer Ted Schiffman (see www.imageartisan.com/treefrogs.html).

Judging

Photos will be judged on composition, technical expertise and artistic merit. The judges' decision is final and no correspondence will be entered into. A panel of five judges has been appointed from Australia, Colombia, Italy and the USA.

Submitting your photos

All entries must be submitted using the entry form on the Amphibian Ark web site www.amphibianark.org/photocompetition/ Photos must be submitted electronically, and must be received by Amphibian Ark before April 30, 2011. Printed versions will not be accepted.

Competition deadline

All photos must be submitted before April 30, 2011. Any photos not received by this date will not be considered by the judges.

For more information, please visit the photo competition page on the AArk web site.

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Read this before you do any holiday shopping!

Please make every gift count this holiday season. You can find coupons at more than 1,600 top stores (including Amazon, Macy's, J.Crew, Toys R Us, Best Buy, Gap and more) and ***a percentage of virtually every sale will be donated to the Amphibian Ark***.

Just go to GoodShop.com, designate Amphibian Ark as the cause you support and then click over to your favorite store and shop away!.

Or, add our toolbar to your browser at http://www.goodsearch.com/toolbar - and your purchases will earn a donation for us even if you forget to go to GoodShop first!

If you are stuck for a gift idea this holiday season, why not make a donation to Amphibian Ark to help save threatened amphibians, in honor of your friends or loved ones? You can specify who the gift is for, and we'll snd them a cool gift card from you! ***Just click the Donate button!***

DONATE - click here

There's no easier way to support us this holiday season! Please spread the word.

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Model *ex situ* amphibian conservation facilities

Kevin Johnson, Taxon Officer, Amphibian Ark

Amphibian Ark considers that there are two steps which are vital in any good *ex situ* conservation program, especially those that might eventually involve release back into the wild:

- the program should be based within the range country; and
- the population being managed should be housed in isolation from other populations occurring outside its range.

Where possible, all amphibian programs that will ultimately result in reintroduction or translocation programs should be operated within the native range of the species. Maintaining these populations within the range country generally results in lower disease risks than programs that are located outside the native range of the species. This helps to reduce the risks of introducing non-native pathogens into the environment around the facility holding the amphibians, and the possibility of introducing novel local pathogens to amphibians that are collected and housed outside of the range country.

Amphibian Ark has been compiling a list of *ex situ* programs for threatened amphibians which includes the progress of each of these programs. We commend a number of these programs as some of those which are based within the range country of the species, and maintain the populations in isolation from other amphibian populations occurring outside the range. These “model facilities” are highlighted on the Model Facilities page on our web site. The full details for each program can be seen by clicking on the species names.

The AArk has produced a set of husbandry and biosecurity standards that provide more detail on how *ex situ* conservation programs should be managed in the safest and most responsible way. This document is available on the Husbandry Standards and Biosecurity page on our web site.

We have been developing a large number of pages in the Amphibian Husbandry section of our web site to help support those institutions that may be considering implementing new amphibian conservation programs, or those that might require some additional support. Please contact our Training Officer, Ron Gagliardo (ron@amphibianark.org) for additional husbandry information, to obtain information about our *ex situ* conservation workshop topics, or to discuss husbandry workshops in your area. If you have skills to share, please let us know. We are always searching for additional instructors and participants.

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Argentinean Amphibian Conservation Needs Assessment

Richard Gibson, Amphibian Ark Taxon Officer and Curator Lower Vertebrates and Invertebrates, Chester Zoo, UK

A well-attended and very successful Amphibian Conservation Needs Assessment workshop was carried out from 23-25 October. It was co-funded by a Durrell Wildlife grant from US Fish & Wildlife Service (Capacity building for conservation of amphibians in Argentina), Chester Zoo, AArk/EAZA, and the Buenos Aires Zoo who kindly hosted, providing a venue, equipment and copious refreshments.

The workshop was organised under the auspices of the IUCN Amphibian Specialists Group-Argentina, with most of the hard work being done by Marcos Vaira and his students. It was attended by eighteen amphibian biologists, PhD students, zoo professionals and government officials representing all regions of the country, and facilitated by AARK Taxon Officer Richard Gibson (Chester Zoo) and Luis Carillo (Zoofari, Mexico).

Six species were identified for urgent *ex situ* rescue programs while eighteen were considered recoverable *in situ* if action was taken swiftly. Forty-one species need significant *in situ* research in order to determine threat levels, including impact of trade, population status and protected area coverage. The information collected during this workshop can be viewed on the AArk's data portal, www.amphibianark.org/assessmentresults.htm

Much time was spent discussing the next steps in using the data from the workshop as a basis for a generic amphibian conservation action plan for Argentina and as a guide for species-specific recovery programs.

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Amphibian Biobanking Workshop: an overview

Rhiannon Lloyd, Biobanking Officer, Amphibian Ark

Retaining high levels of genetic diversity in captive and *in situ* amphibian populations is important. This is because the genetic diversity of a population represents its potential to evolve and adapt to an ever-changing world. Freezing genetic materials, in the form of sperm, eggs, embryos and others, in a state of 'suspended animation' is therefore likely to be key to retaining the genetic diversity and maintaining the long-term well being of amphibian populations, particularly if they are small.

Creating a global network of freezers ('biobanks') containing amphibian genetic materials is not without its challenges and it is for this reason a three-day workshop on the topic was held at the Zoological Society of London and the European Xenopus Resource Centre (Portsmouth, UK) during September this year.

Over thirty delegates from different areas of amphibian biology, conservation and cryobiology came together for a series of presentations and discussions, over the first two days of the workshop, to meet, share expertise and develop a coherent approach to the topic of amphibian biobanking. Everything from which species and materials should be prioritised to which precautions (e.g. biosecurity) should be taken when using these materials to produce tadpoles for captive breeding and/or reintroduction was discussed. Efforts currently underway to cryopreserve amphibian cell cultures and sperm in the USA and Australia were presented, as well as new proposals to freeze cell cultures and/or sperm from critically endangered amphibians from the Caribbean, Panama and zoological institutions in Canada.

On the third day of the workshop, twenty-three of the delegates participated in a series of practical demonstrations at the European Xenopus Resource Centre, on amphibian sperm cryopreservation, *in vitro* fertilization and cell culture. Sperm nuclear injection (where a single sperm is injected into a single egg to achieve fertilization), a method often used to produce *Xenopus* (a laboratory amphibian), was also demonstrated. This technique is analogous to intracytoplasmic sperm injection, the most common assisted conception method used to overcome male infertility problems in humans. This is likely to be the best potential option available for generating offspring from endangered amphibians whose sperm is proving difficult to freeze and would otherwise be regarded as infertile.

Cryoprotocols being developed for the African Clawed Frog (*Xenopus laevis*), an amphibian species often found in laboratories, could help safe-guard the future of endangered amphibian species. Photo: European Xenopus Resource Centre © 2010.

For these amphibian biobanks to fulfill their potential as a genetic management tool, in addition to sperm, eggs are needed too. To date, amphibian eggs have been impossible to cryopreserve, due to their large size, impermeability to cryoprotectants and sensitivity to chilling. Fish eggs face similar problems. An alternative to egg freezing has been developed in fish and was tried for the first time at the workshop with *Xenopus*. The technique involves isolating germ cells from essentially dead, ('donor') embryos that have been snap-frozen in liquid nitrogen. These germ cells are then transplanted into another ('host') embryo, where they subsequently develop into gametes (e.g. eggs). The successful development of this method for amphibians would be a major breakthrough, as the eggs could be recovered and combined with the sperm from the biobanks, maximizing the available gene pool and eliminating the reliance on maintaining numerous individuals (both male and female) within the living populations.

For more information, including protocols from the practical demonstrations, on the "Towards a biobanking strategy for amphibian conservation" workshop, please visit the biobanking sub-portal or email cryobanking@amphibianark.org.

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Orana Wildlife Park Maud Island Frog Habitat

Nathan Hawke, Marketing, Public Relations & Visitor Services Manager, Orana Wildlife Trust, New Zealand

The Maud Island Frog (*Leiopelma pakeka*) Habitat project at Orana Park was partially funded by a 2010 Amphibian Ark seed grant, and although the project began on time, the devastating earthquakes around Christchurch in September caused major disruptions to our progress.

Work that has been completed on the project

- Plans have been developed and a Building Consent has been issued.
- The foundations have been excavated.
- The building foundations have been prepared for the first of two concrete pours and we are currently awaiting a pre-pour inspection by the Christchurch City Council. However, we have experienced delays in having the site inspected owing to the recent Christchurch earthquake (on 4 September).
- The sewer line has been completed and connects the frog habitat location to the septic tank.
- Tara Atkinson (Head Keeper, Native Fauna) and Alyssa Salton (Senior Native Fauna Keeper) attended the recent New Zealand Frog Workshop in Auckland. Both staff gained a significant amount of knowledge from this workshop and have discussed outcomes that are relevant to our exhibit with key Park staff.
- The same Park staff members have visited native frog exhibits at Auckland and Hamilton Zoos and have also spent time with Dr. Phil Bishop at Otago University to help prepare Orana staff members for the arrival of the frogs.
- Additional live food options (e.g. slater colonies) are being developed in preparation for the arrival of the Maud Island Frogs.

Above and below: The foundations of Orana's Maud Island Frog Habitat prior to the first pre-pour inspection of the concrete foundation. Photos: Orana Wildlife Park.

Work that remains to be completed

- The concrete foundations need to be poured in two separate stages. Pre-pour inspections must be completed by the Council before each concrete pour, but unfortunately, delays have been experienced in having the first inspection completed due to the earthquake.
- The chiller box, within which the climate control equipment and the frogs (in terrariums) will be housed, will be assembled by outside contractors and then installed on the foundation.
- Our development team will construct the building around the chiller box. Visitors will walk through the building to view the Maud Island Frogs through glass panels. Within the building, a range of introduced amphibian species (with their own service area and equipment) will also be displayed.
- Climate control equipment and all electrical work will be installed within the chiller box.
- The climate control equipment will be tested for at least one month to ensure it properly simulates the frogs' natural habitat at Maud Island. This is a requirement set out by the Department of Conservation.
- Interpretation (signage) is to be developed and installed. Education programs and public presentations will also be designed.
- Frogs will be collected from the wild and added to the habitat.

The project has been delayed for the following reasons

- Unfortunately we missed the 'window' this year to collect the animals, meaning the frogs will be collected next winter. Therefore, since the collection was missed, our work plan for the Park's Development Team was

altered to focus on creating a new exhibit at our sister attraction, Natureland Zoo (Nelson), in time for Christmas. As a charitable trust we must continue to add new attractions to our facilities to attract visitors. Our Development Team will construct the outdoor building work of the frog habitat over summer. The benefit of scheduling this work over summer is that our team will work in a fenced-off 'construction zone' meaning there will be no disruption to visitors (which can be difficult to avoid for development projects). The exhibit will be finished and running well before the frogs are collected.

- The Christchurch earthquake on 4 September caused major disruptions to the project, particularly in getting an inspection completed by the Council. It is likely that we may experience further delays in completing the project owing to availability of contractors and Council inspections due to the cleanup/redevelopment in Christchurch after the earthquake.

Revised timeline for the project

- October/November 2010 – concrete foundations poured ad inspected by the City Council.
- November – outside contractors to assemble the chiller box and (when complete) install it on the foundations.
- December 2010/January 2011 – Orana development team construct the outdoor building around the chiller box.
- February 2011 – climate control equipment and all electrical work completed.
- March 2011 – run the habitat to test that the climate control equipment properly simulates the frogs' natural habitat.
- April 2011 – install interpretation, complete educational programs and design a public presentation about native frog conservation and the plight of amphibians in general.
- Winter 2011 – collection of the frogs, transfer to Orana and then once they have settled in, the exhibit will be officially opened.

Note that as we are relying on outside contractors for parts of the project, the timeline above may be subject to change owing to availability of such contractors in the cleanup/redevelopment following the earthquake.

A link to the complete proposal for this facility can be viewed here.

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Oregon Spotted Frog project at Cedar Creek Corrections Center

Kelli Bush, Sustainable Prisons Project Manager, The Evergreen State College

The Mission of the Sustainable Prisons Project is to “bring science and nature into prisons. We conduct ecological research and conserve biodiversity by forging collaborations with scientists, inmates, prison staff, students, and community partners. Equally important, we help reduce the environmental, economic, and human costs of prisons by inspiring and informing sustainable practices.” This work was started by Dr. Nalini Nadkarni, a forest ecologist at The Evergreen State College, as a pilot project to conduct research on mosses at the Cedar Creek Corrections Center (CCCC) near Olympia, Washington, USA.

By 2008 the work expanded to include four Washington State prisons. With the help of collaborators, The Sustainable Prisons Project offers: a monthly science and sustainability lecture series at each prison; conservation projects raising Oregon Spotted Frogs (*Rana pretiosa*), Taylor’s Checkerspot Butterflies (*Euphydryas editha taylori*), and over 200,000 rare native prairie plants for habitat recovery; and green collar job training on subjects such as beekeeping.

Since 2009, The Sustainable Prisons Project has been working with Washington Department of Fish and Wildlife and Cedar Creek Correction Center to raise endangered Oregon Spotted Frogs. There are four institutions rearing the frogs, including Oregon Zoo, Woodland Park Zoo, NW Trek, and Cedar Creek Corrections Center. All of these groups recently came together to release this season’s frogs into the wild in a collaborative effort to stabilize the native populations. A total of 1,346 frogs were released into a wetland site on military property, Joint-Base Lewis-McCord. The prison boasts having the largest frogs of any participating rearing institution, with 100% of this year’s frogs large enough for release.

Above: An Oregon Spotted Frog (*Rana pretiosa*), part of a conservation project at Cedar Creek Correction Center, Washington. Below: Measuring Oregon Spotted Frogs. Photos: Kelli Bush.

Cedar Creek Correctional Center’s rearing success can be attributed to the amount of time and attention the inmates are able to dedicate. They form genuine bonds with the frogs and some are given names, like “Lefty” or “NASCAR.” The few deceased frogs have been placed in an offender-created “frog cemetery,” with hand-made gravestones. Inmates learn about amphibian biology, husbandry, animal behavior, data collection, and how to conduct scientific research in the process of raising the frogs. In addition, they are given the opportunity to use their time in prison in a positive and meaningful way.

In an effort to reduce the carbon footprint of the frog project, inmates began raising crickets. The goal of the cricket rearing project is to create a more sustainable and stable food supply to meet the demand of a growing population of Oregon Spotted Frogs. Crickets are one of the largest expenses for the project and are shipped from farms located in southern states. Long-distance shipping complications can impact frog feeding schedules, and greatly increase the project’s carbon footprint.

Amphibian Ark Training Officer, Ron Gagliardo recently made a visit to CCCC to advise inmates and staff on the cricket rearing operation. Ron’s extensive experience with frog and cricket rearing proved a tremendous resource. The inmates were able to ask him many questions and his input will undoubtedly improve upon the initial success of the cricket operation.

Prisons and prisoners are often overlooked as partners in sustainability and conservation efforts. The Sustainable Prisons Project has successfully demonstrated that collaboration among conservation groups, higher education, and

prisons benefits all partners, and the planet. If you would like to know more about the project or would like to make a contribution please visit our website www.sustainableprisons.org

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A new method for semen collection from live Urodeles and standardization of *in vitro* fertilization

Nabil Mansour, Department of Organismic biology, University of Salzburg, Austria

In most Urodeles, with the exception of the Cryptobranchidae, fertilization is internal. The male releases spermatophores into the water and the female picks them up into her cloaca and stores in the spermatheca at the exit of the oviduct until fertilization. Until now, the artificial reproduction of endangered Urodeles was not appropriate as semen collection was only possible through sacrificing males.

Recently, motile and fertile semen from live Urodeles such as: Axolotl (*Ambystoma mexicanum*), Common Newts (*Lissotriton vulgaris*), Crested Newts (*Triturus cristatus*) and Fire Salamanders (*Salamandra salamandra*) has been collected by gentle abdominal massage after hormonal stimulation. This is not all; fertile eggs have been collected after hormonal stimulation of females exhibiting typical spawning behaviour. Best fertilization rates were achieved by mixing the semen with eggs before the addition of a simple saline solution of low osmolality and of alkaline pH. This protocol is very promising as it may be transferable to endangered Urodeles. Additionally, we are trying to establish a successful protocol for the cryopreservation of Axolotl semen, as this may also be transferable to endangered Urodeles in the future.

For full details, please consult the following publication: Mansour N, Lahnsteiner F, Patzner RA. *Collection of gametes from live axolotl,* Ambystoma mexicanum*, and standardization of* in vitro *fertilization*. Theriogenology. 2010 Oct 19.

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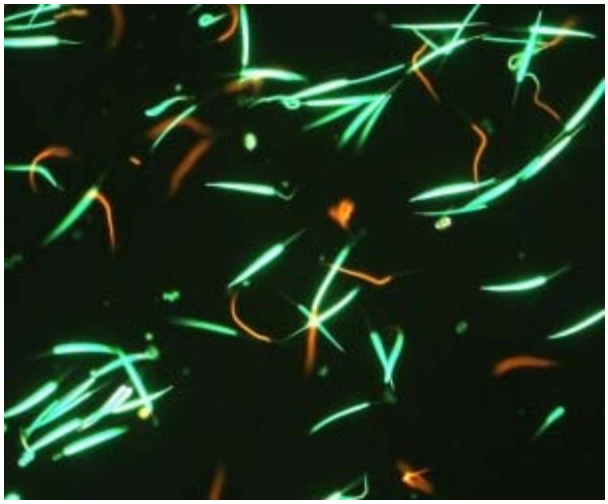
Sperm cocktail celebrates conservation breakthrough

Dr Robert Browne, AArk Research Officer

Mix an amphibian sperm cocktail, freeze it, and thaw when needed; in other words a sperm bank for amphibians. Sperm banks are established for people, for threatened fish, agricultural animals including mammals and birds, so why not for the conservation of amphibians. After all we have already lost up to 200 amphibian species. Conservation breeding programs (CBPs) when supported by sperm banking could have saved many of them.

Cryopreserved sperm not only perpetuates an amphibian species' genetic variation, but also reduces the numbers of any species needed in captivity from hundreds to tens. Therefore, for the same cost we get more species saved, more reliability, and with more efficiency. Less amphibians in CBPs also means less amphibians taken from the wild and more attention for those that are.



Research at the The Laboratory for Cryopreservation of Genetic Resource, Institute of Cell Biophysics, Moscow Region, Russia, first produced tadpoles from cryopreserved amphibian sperm in testicular tissue in 1996. Further research has established reliable techniques for the cryopreservation of testicular sperm that have reached commercial application. Rather than relying on testes for sperm, as their sampling requires killing the donors, the use of hormonally induced sperm only requires a hormone injection. The frogs can be released within twenty-four hours if in the field.

Techniques for the harmless sampling of sperm from living amphibians by induction with hormones are well established. However, the successful cryopreservation of hormonally induced sperm has eluded researchers until now. Collaboration between the Institute of Cell Biophysics, and the Centre for Research and Conservation, Royal Zoological Society of Antwerp, was formed to produce a protocol for the cryopreservation of hormonally induced sperm.

Above: After cryopreservation and staining the live sperm of *Rana temporaria* are green and dead sperm are orange. Photo: Edith Gakhova.
Below: A juvenile European Common Frog (*Rana catesbieana*) produced from cryopreserved hormonally induced sperm. Photo: Edith Gakhova.

During six weeks in the spring of 2010, Prof. Edith Gakhova, Dr. Natalia Shishova, and Dr. Victor Utesheva, and I, developed and tested new cryopreservation protocols. Hormonally induced sperm from the European Common Frog (*Rana temporaria*) was recovered in large amounts. A new cocktail of cryoprotectants to protect the sperm, with increased sophistication of freezing and thawing, resulted in a very high recovery of living sperm, and high rates of fertility and larval development. An interesting aspect of the new protocol is that foam boxes are used to cryopreserve the sperm, avoiding the use of expensive freezing equipment. The path is now open for the sampling and cryopreservation of sperm from many threatened amphibians in captivity and in the field. This can be done economically and with simple facilities. We are investigating novel technologies to enable the transport of sperm from the field without cooling, and to sample and cryopreserve salamander sperm.

We have had the ability to cryopreserve amphibian sperm since 1996. However, there have been no sperm banks representing the entire natural genetic variation of any amphibian. This is unfortunate considering the loss of so many amphibian species since then. However, an initiative by Dale McGinnity and Dr Sally Nofs, Nashville Zoo at Grassmere, Tennessee, USA, and Michigan State University, is creating a sperm bank for the North American Hellbender (*Cryptobranchus alleganiensis*). The North American Hellbender is one of three giant salamander species. These species are in an ancient family, and being the largest amphibian in North America the Hellbender is of particular conservation significance. However, the Hellbender has not reproduced over a large range and only older

salamanders remain.

These conservation concerns, and the ease of sampling sperm as semen from males, led to the development of a project to build a gene bank for the Hellbender. The project was supported by a web-based global consortium of researchers working on amphibian sperm cryopreservation. In 2009 the sperm was sampled from a hormonally induced male at Nashville Zoo and recovered motility after cryopreservation. In 2010 the field sampling of salamanders was undertaken, with sperm then transported and cryopreserved.

Sampling sperm from a Hellbender at Nashville Zoo. Photo: Robert Browne.

I was invited to participate in the field sampling and cryobiology work in 2010. We sampled a site where the Hellbenders are still recruiting juveniles, and one with only older salamanders. Hellbenders are found under large rocks and a catch rate is one that could be one male per hour. We waded upstream in water that was up to our necks in the pools, and turned rocks in the rock banks and riffles. When a male was caught we sampled the semen and immediately released the Hellbender under its rock. Then we refrigerated the sperm and cryopreserved it the next day. We are preparing articles on the sampling, cryopreservation, and electron microscopy.

Article reference: N.R. Shishovaa, V.K. Utesheva, S.A. Kaurovaa, R.K. Browne, and E.N. Gakhova. 2010. *Cryopreservation of hormonally induced sperm for the conservation of threatened amphibians with* Rana temporaria *as a model research species*. Theriogenology 2010 Oct 30. [Epub ahead of print].

http://news.nationalgeographic.com/news/2010/08/100820-hellbenders-snot-otters-sperm-amphibians-science-environment/

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Preparations for the battle against chytrid: Building capacity for chytrid detection in Madagascar

Gerardo García PhD, Head of Herpetology Department, Durrell Wildlife Conservation Trust

To date Madagascar appears to be chytrid-free. However, it may only be a matter of time given its proximity to mainland Africa and the trade routes that exist with countries where the disease is present. To ensure that the disease is detected as soon as possible if it should it reach Madagascar, the first step that has been taken was to carry out a training workshop at Parc Ivoloina on the east coast of Madagascar in October this year. The workshop was funded through the European Association of Zoos and Aquaria with additional support coming from the Regional Museum of Natural History, Turin and Conservation International.

The aims of the workshop were to initiate plans for a nationwide early warning detection plan for chytrid, build capacity within Madagascar to carry out this plan, and to begin raising awareness about the implications of captive management as an option should chytrid arrive on the island. Thirty Malagasy participants from a combination of government, non-government and private organisations attended the six-day training workshop. Lectures on the biology of chytrid fungus, diagnostic sampling techniques and exercises in designing disease detection field studies were run by Dr. Ché Weldon of South Africa's North-West University, and Dr Angelica Crottini, ZPL Università di Milano-Bicocca, Italy.
A draft nationwide Chytrid Fungus Early Detection Plan was developed during the workshop under the guidance of Dr. Weldon with input from course participants in the selection of the key sites to survey, following certain criteria such as sites with a high diversity and abundance (i.e. the Protected Areas such as Andasibe, Ranamafana and Betampona in the east and Ankarafantsika, Isalo and Bemaraha in the west). This plan is now with the Amphibian Specialist Group in Madagascar to determine how to turn the plan into action.

Sambava Tomato Frog (*Dyscophus guineti*) being swabbed for chytrid testing. Photo: Gerardo Garcia.

Discussions are ongoing with government and non-government organisations as to how best develop the capacity for the establishment of captive populations of threatened amphibians in Madagascar following on from the seeds sown during the course.

Basic principles of captive management and quarantine protocols for amphibians were presented during the course, in conjunction with practical interactive activities leading to the design and construction of basic units to keep frogs in Madagascar. These sessions were all conducted by Dr. Gerardo Garcia and Jamie Copsey of Durrell, Jersey. This was an initial approach into the complex world of replicating natural environmental conditions in captivity by using mostly the equipment available in-country. That was the initial challenge for this new generation of researchers, most of whom only have a background in fieldwork. A basic induction into water analysis and filtering, temperature, breeding live insects and standards of quarantine were the introductory modules relating to captive management within this workshop. Participants designed facilities to maintain specific threatened species of Malagasy frogs taking the new skills they had acquired during the course into consideration.

Malagasy students being shown chytrid testing techniques by Jamie Copsey. Photo: Gerardo Garcia.

The selection of these species came from the past prioritization exercise lead by Dr. Franco Andreone in 2006 as part of a committee of experts from different countries. During that exercise (one of the first undertaken by Amphibian Ark in the prioritization of species to be kept *ex situ*) we prioritised which taxa are most in need of *ex situ* assistance. But due to the lack of knowledge in keeping Malagasy amphibians in captivity (despite their enormous diversity), a second exercise was undertaken to select key species with different

breeding strategies (i.e. stream-breeding tree frogs, *Boophis microtympanum*). For each of the fifteen breeding strategies several species were selected to ensure that at least one of these could be found.

In 2011 the next step will be to work more closely with particular organisations in Madagascar to help in the creation of captive facilities on the island where local amphibian biologists can learn how to manage their own species *ex situ*. The plan will be to set up these facilities simultaneously with an intensive captive management course for amphibians.

This stage for amphibian conservation in Madagascar will present zoos and aquariums outside of Madagascar with a fantastic opportunity to help support the conservation of the island's unique amphibian diversity. If we can build these skills before chytrid arrives then we will be ahead of the game. Any institutions or individuals interested in being part of this process in 2011 are encouraged to contact Gerardo García at the Herpetological Department of the Durrell Wildlife Conservation Trust at gerardo.garcia@durrell.org.

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Geocrinia programs at Perth Zoo

Debbie Read, Media and Communications Manager and Daniel Scarparolo, Communications and Interpretation Officer, Perth Zoo, Western Australia

White-bellied Frog translocation a first

On 13 September, seventy White-bellied Frogs (*Geocrinia alba*) reared at Perth Zoo (two adults, six sub-adults, and sixty-two juveniles) were released into the wild near Margaret River in the south-west of Western Australia, as part of a joint agency effort to re-establish this critically endangered amphibian in an area where they have become extinct.

The translocation is part of an ongoing Threatened Fauna ARK project by the Department of Environment and Conservation (DEC) to aid the recovery of the White-bellied Frog and the Orange-bellied Frog (*Geocrinia vitellina*).

Despite the challenges of working with such a small frog species, Perth Zoo staff successfully reared seventy-five metamorphs from wild collected egg nests under the Zoo's new rear-for-release program for this species. The newly-emerged metamorphs or froglets are the size of the writing tip of a pencil and weigh just 0.03 grams.

White-bellied Frogs after release in September 2010. Photo: Perth Zoo

The wild-collected eggs and tadpoles were transferred to the Zoo for rearing to boost their chances of survival. With only 200 White-bellied adults estimated to be left in the wild and research showing heavy predation of egg nests in situ, the captive rearing of eggs and tadpoles is an important step in increasing their chances of reaching adulthood.

The White-bellied Frog is confined to a limited and isolated ecological niche and is very susceptible to changes in its environment. The destruction or disturbance of its habitat can, and has, resulted in local extinctions. Only found in higher-rainfall jarrah and karri forests in the south-west, this species has previously been recorded at 115 locations but is now locally extinct at twenty-two of these sites. The frogs tend to move only five metres during a breeding season and less than twenty metres between years, making them vulnerable to even very small-scale disturbances.

The DEC has programs to address threats that may affect the release site. Threats to the frogs' habitat include feral pigs, illegal firewood harvesting, off-road vehicles and fire.

The September release was the first White-bellied Frog translocation and the first time frogs rather than eggs have been translocated in Western Australia. Orange-bellied frog eggs have been translocated previously.

The frogs were released at the start of the breeding season in groups of ten consisting of both males and females. As these frogs don't travel large distances, they were released close to each other so that when the males call to attract a mate, the females won't have far to travel. The release went well with the frogs quickly moving into the burrows that were dug for them. The released frogs have been marked (using Visible Implant Elastomer tagging) for monitoring purposes.

After the release, Perth Zoo staff searched for new egg nests to bring back to the Zoo to continue the rear-for-release program.

The translocation was funded by State Natural Resource Management funds and grants from Perth Zoo's Wildlife Conservation Action program and the Zoological Association of Australia.

Frog hormone testing at Perth Zoo

How much urine can a centimetre-long frog make? Not much but that didn't stop Post-doctoral Research Fellow Lindsay Hogan, from The University of Queensland, in her work to determine the sex of young White-bellied (*Geocrinia alba*) and Orange-bellied

A White-bellied Frog (*Geocrinia alba*). Photo: Perth Zoo.

(*Geocrinia vitellina*) Frogs at Perth Zoo. Dr Hogan has been working with the White-bellied Frogs in Perth Zoo's Native Species Breeding Program since June, collecting urine and faecal pellets to determine their sex.

One difficulty that needed to be overcome prior to the frogs' release in September (as reported above) was determining the sex of the sub-adult and very small metamorph frogs. These frogs are a sedentary species, barely travelling more than a few metres in their entire life, so making sure that both female and male frogs were released together in the same area, was a critical factor in the release program.

With no visual way to accurately identify the gender of the sexually immature metamorphs, Dr Hogan measured the concentrations of two sex hormones (estrogen and testosterone in both sexes) excreted in the frogs' urine and faeces in the hope of identifying their sex.

It wasn't an easy task as the frogs don't produce a lot of urine or faeces. To overcome this, multiple samples were collected from each frog and specimens pooled together to gather a large enough sample for testing. Urine was collected from every sub-adult once a week for ten weeks before there was enough urine to run a valid hormone test.

"We also collected faeces from every sub-adult and metamorph every three days for a period of ten weeks before we had enough material to test," Dr Hogan said. "There were over eighty frogs to collect from so it was time consuming and very fiddly work but rewarding once we saw the results.

"Just in time for the release, we knew there were six male and six female sub-adults plus twenty-two male and twenty-three female metamorphs. Twenty-nine metamorphs weren't sexed as they were either housed in groups (making it difficult to identify which faeces belonged to which animal) or not enough material was collected for analysis.

"This information enabled us to release the frogs in groups comprising known males and females, together with some unknowns so they would have a greater chance of breeding. The research is also exciting as it is the first time these techniques have been used on Australian amphibians.

"I have done a lot of work with the reproductive biology of Australian mammals including wombats, and dunnarts. Applying these techniques to amphibians has opened up a lot more opportunities."

The second phase of Dr Hogan's research will be to monitor how the frogs' hormones affect their reproductive behaviours.

"I will be looking at the females to see what their hormones are like during egg development and what happens to them after the eggs are deposited," Dr Hogan said.

"I will also be investigating how testosterone in the males relates to burrowing and calling.

"Correlating the hormones with behavioural observations is well studied in mammals but again is something not done before with frogs.

"This research is really at the forefront of amphibian research and will play an important part in the conservation of amphibian species."

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Mantella breeding success

Wayne Woods, Zoo Attendant I: Primary Amphibian Keeper, Edmonton Valley Zoo

Prior to the Year of the Frog campaign, the Edmonton Valley Zoo only had four amphibians, today we have over two hundred. In 2008 we decided that we would focus our Year of the Frog display to the displaying and breeding of frogs from the genus *Mantella*. Mantellas are small colourful frogs endemic to the island of Madagascar. There are thirty species of Mantellas, many of which are endangered due to habitat destruction and the exotic pet trade. We currently have four species of Mantella: Golden Mantella (*Mantella aurantiaca*), Splendid Mantella (*M. pulchra*), Baron's Painted Mantella (*M. baroni*), and Climbing Mantella (*M. laevigata*). We have successfully bred three of the four species.

Mantellas become sexually mature at one year of age and are seasonal breeders, so in order to breed them they must go through a dry/wet cycle. In June of 2009 we put our *M. baroni*, *M. pulchra*, and *M. laevigata* through a dry cycle that lasted three months. In 2009 we only had one *M. aurantiaca* so we did not cycle him. During the dry season we reduced the temperature to below twenty degrees Celsius, increased the daylight length to twelve hours, reduced humidity by limiting misting, and decreased the feeding to every other day. During the dry cycle the Mantellas called less.

Golden Mantella (*Mantella aurantiaca*). Photo courtesy of Henk Wallays.

In September of 2009 we changed substrates, reorganized the Mantellas' enclosures and entered them into the wet season. In the wet season we increased humidity by misting four times a day as well as having a cool mist humidifier on their enclosures, we decreased the day light cycle to ten hours, and we increased feeding to every day.

In October 2009 both the *M. baroni* and *M. pulchra* produced clutches of eggs. We experimented to see how best to raise the eggs to tadpoles so we removed one clutch from the *M. pulchra* and kept it in a petri dish on a layer of moss surrounded by water. We misted it every day. The other clutches were left in with the parents. Twelve days after the discovery of the clutches the eggs turned into mass of squirming tadpoles. The eggs left in with the parents developed better than those which had been removed. The tadpoles were placed into plastic containers which were kept bare of any substrates and the only other thing in the container was a piece of pothos and an air stone.

The tadpoles were fed daily and fifty percent of the water was changed.

Within two months the tadpoles grew front legs and became froglets. The froglets were fed small fruit flies and springtails and kept in an aquarium with paper towel substrate which was changed daily. The survival rate of these first Mantella clutches was approximately sixty percent.

In 2010 we received three more *M. aurantiaca* to become mates to our solitary male. We did not cycle the new frogs through a dry cycle, just a wet season and In October of 2010 these frogs produced with two large clutches, with 211 tadpoles hatching. As of the time of writing, fifty percent of the tadpoles have become froglets, and currently our survival rate is seventy-five percent but not all the tadpoles have become froglets yet. We are really excited about our *M. aurantiaca* offspring and hope to be sending them off to other institutions to display this Critically Endangered amphibian.

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Mantella tadpoles hatched at Edmonton Valley Zoo. Photo: Wayne Woods.

An update from the Association of Zoos & Aquariums

Shelly Grow, Conservation Biologist, AZA

U.S. Fish and Wildlife Service's Notice of Inquiry

AZA, in collaboration with its members and the Amphibian Taxon Advisory Group, will submit comments in response to the U.S. Fish and Wildlife Service's Notice of Inquiry concerning a petition to list all live amphibians in trade as injurious wildlife under the Lacey Act--unless they are certified to be free of *Batrachochytrium dendrobatidis* (Bd). Comments are being accepted until December 16, 2010. Learn more at: www.fws.gov/policy/library/2010/2010-23039.html

National Science Foundation award benefits FrogWatch USA

The National Science Foundation (NSF) has awarded a $2.7 million, five-year grant to the National Geographic Society to launch a major educational initiative that will engage young people in scientific research about their environment. In this educational research and development project, National Geographic will develop and evaluate a web-based software platform called FieldScope to support citizen science projects involving geographic data.

FrogWatch USA will be one of two test bed partners for this platform, which will offer exceptional opportunities for data entry, visualization, and analysis. The project focuses on a particular type of citizen science project that National Geographic calls "community geography," where participants collect observations or measurements in different locations and analyze them for geographic patterns.

AZA is both honored and eager to work with NSF, the National Geographic Society, and Project BudBurst on this multi-year effort. Learn more at: www.aza.org/PressRoom/detail.aspx?id=16170

Become a FrogWatch USA Chapter Coordinator through new web-based training program

AZA is welcoming a new era in professional development by offering the first in a series of web-based training opportunities. The first module available allows U.S.-based organizations interested in FrogWatch USA to learn how to become a Chapter Coordinator and open a local FrogWatch USA Chapter at their facility.

FrogWatch USA is a citizen science program that encourages community stewardship by training volunteers to listen and report the breeding calls of frogs and toads in their communities. Chapters engage people in both science and conservation in such a way that each volunteer is empowered to do more. Opening a FrogWatch USA Chapter not only contributes to amphibian conservation, links *ex situ* programs to *in situ* conservation, and promotes AZA-accredited zoos and aquariums within their local community, but also supports a national network of FrogWatch USA Chapters and volunteers that together, like other citizen science programs, provide large scale, long-term data. Learn more about FrogWatch USA at www.aza.org/frogwatch

Register for the FrogWatch USA Chapter Coordinator training through AZA's web site (www.aza.org/professional-development/). AZA members receive a discounted registration of just $50; course registration for non-members is $75.

Captive breeding program for a Critically Endangered frog leaps ahead

AZA extends its congratulations to the Memphis Zoo and Omaha's Henry Doorly Zoo for the recent hatching of over 1,4000 tadpoles produced after researchers carried out an in vitro fertilization (IVF) for the Critically Endangered Dusky Gopher Frog (*Lithobates sevosus*). Nearly 100 of the tadpoles were produced by female gopher frogs' eggs fertilized with sperm that had been collected non-invasively from Dusky Gopher Frogs at the Memphis Zoo and shipped to Omaha overnight to be used for the IVF. This transfer of non-invasively collected sperm for IVF was a world-first for amphibians. With U.S. Fish and Wildlife Service estimates suggesting that there are only 100 Dusky Gopher Frogs left in the wild, with almost all of these residing in a single pond in Mississippi, IVF may prove to be a critical tool in the efforts to conserve this species. Read more at: www.aza.org/PressRoom/detail.aspx?id=16126

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Recent activities in Mesoamerica

Yolanda Matamoros, President, Mesoamerican Association of Zoos

Branko Hilke, PHd student from the Universidad de Puerto Rico presented two papers during the Mesoamerican Society for Biology Conservation Annual Congress held in San Jose, Costa Rica, 8-12 November, 2010, and Gilberto Alvarado, veterinary student at the Veterinary Medicine School, Universidad Nacional, Costa Rica, presented his thesis. The abstracts from both papers are included in this update.

Conservation priorities for leaf-litter frogs (Terrarana: Craugastoridae & Eleutherodactylidae) based on evolutionary distinctiveness and extinction risk (EDGE)

Branko Hilje, Department of Biology, University of Puerto Rico - Rio Piedras and Ingi Agnarsson, Asociación para la Conservación y el Estudio de la Biodiversidad

Abstract

Ongoing global change poses a major threat to organisms across the earth. Even though this crisis affects taxa from all major branches of the tree of life, amphibians seem to be particularly strongly affected.

The 'global amphibian decline' has been documented since the 1980's, demonstrated by accelerated population declines and extinction rates. Amphibians, therefore, emerge as an exemplar group to study the causes of extinction, and to design and implement effective conservation efforts. Establishing conservation priorities for threatened taxa, however, is no small task. Species imperilment often is the main criterion used to establish conservation priorities. However, phylogenies are increasingly playing an important role providing a measure of the evolutionary distinctiveness of taxa. Species are far from all being equal in terms of how much unique evolutionary history they contain. Many have closely related species that preserve the majority of their genetic and morphological diversity, while others have no close relatives and thus represent much unique evolutionary history.

Here, we use recently developed methods (EDGE/HEDGE) that combine phylogenetic information with data on species imperilment (IUCN's red list) to propose conservation priorities for the amphibian families Craugastoridae and Eleutherodactylidae. Our results rank species based on EDGE/HEDGE scores and these ranks can be used to help guide conservation decisions. In addition, taxa that emerge as high priority tend to have very limited ranges, and many are island endemics. An important percentage of high priority taxa occur only in non-protected areas. Therefore, our findings indicate that new strategies are necessary in terms of land-use planning and preserving of habitats, and suggest that conserving suitable habitat on islands is a particularly important factor in amphibian conservation. Analyzing such patterns for other taxa is an urgent priority to establish if our findings here apply more broadly to other amphibians, and other organisms.

Presence and geographical distribution of Batrachochytrium dendrobatidis in anurans of medium and highland from Cordillera de Talamanca

Gilbert J. Alvarado, Centro de Investigación en Estructuras Microscópicas (CIEMIC), Universidad de Costa Rica

Abstract

Amphibian population decline and extinction has been reported in protected areas and pristine sites like mountain areas of tropics worldwide. These declines and extinction in some of these decimated populations have been associated with a newly emerging infectious disease (chytridiomycosis) in amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). In Costa Rica many amphibian populations at locations over 500 m elevation are most affected. We determined the prevalence of infection of *Bd* in 244 anurans of forty-one species representing ten families. They come from six different areas from high and medium elevations (500 – 3000 m elev.) in Cordillera de Talamanca. They were collected between 2007 and 2008.

The biopsies were taken from the pelvic patch and processed by histopathology methods. Positive individuals were assessed by a

quantitative and morphometric analyses. We compared positive and non-positive individuals belonging to the same species in order to see statistic significances.

Positive animals were detected in every sampled location and the general prevalences were determined in 0.15 (36 specimens). The more detailed prevalence description is: 0.44 (18) of the species and 0.60 (6) of the families presented at least one positive individual. Anurans with the highest prevalence were observed within the families Strabomantidae (0.20) and Craugastoridae (0.17). Both families were composed of terrestrial anurans that inhabit in the leaf-litter (0.16), of direct development (0.17), implicating an absent tadpole and an egg not associated with a water body. Only seven of eighteen positive species presented any histopathological significative change.

Our results indicated that the abiotic variables are the stronger factors that play a relevant role over host-pathogen dynamic. That was more evident if we associated them with biotic factors (reproductive mode, individual size, immune response). All of them together could generate the ideal conditions to negatively affect an exposed population.

Our results suggest that we should focus on understanding terrestrial habitats and their abiotic variable, especially where *Bd* seems to be endemic. Also, it is important to be aware that not every positive anuran living in its natural habitat will show histopathological significant changes to generate physiopathological changes that could affect individual health status. Therefore, some species could be host reservoirs not showing disease as a result of not having a negative impact over their own populations.

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