



## In Search of the Limon harlequin frog

The stiffened corpse of a female lies sprawled in the shallow water as a male clings to her back in a desperate effort to mate. He is among the last of the surviving Limon harlequin frogs – or *Atelopus* sp. #13 – a brilliant bright yellow and black species that is known to breed in just one stream in southern Ecuador: but for how much longer? The scene is a poignant symbol of an all-too-familiar phenomenon.



*Limon harlequin frog* © Robin Moore

On the evening of June 4 I arrived in Quito to meet up with Luis Coloma and Santiago Ron from the Catolica University and Joel Sartore and Jennifer Holland from National Geographic. Armed with cameras, video and recorders, we planned our trip to Limon, a small town in the south of Ecuador, to document the species.

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## IN SEARCH OF THE LIMON HARLEQUIN FROG

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We set off early and drove all day along breathtaking and sometimes nerve-wracking roads from Quito to Limon until finally we neared the home of harlequin frog #13. As we turned the final corner to overlook the ravine, our hearts sank.



cal habitat for the frog. “We are too late” sighed a dejected Luis Coloma, eyes to the ground. He could not bear to watch as the habitat was destroyed in front of us.

Pessimistic of our chances of finding the frog, we waded solemnly upstream away from the falling rocks. Not only is the barrage of debris

down the slopes an environmental hazard; it also presents a human hazard. David Salazar, who has been studying this population for the last 4 years was hit by a falling rock last week and is reluctant to work here any longer because it is no longer safe.

Suddenly, a jubilant cry

“We may be watching a species go extinct before our very eyes.”

Bulldozers and workmen lined the road, shoveling rubble indiscriminately over the sides of the stream that provides the last criti-



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**IN SEARCH OF THE LIMON HARLEQUIN FROG**

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*Limon harlequin frog* © Robin Moore

from upstream raised our spirits like a generous shot of rum. We raced up to find a small male clinging to a leaf: a surge of excitement that the species is still alive.

On our way back down the stream, however, we found



*Jennifer find dead frogs* © Robin Moore

It is not a fight between conservation and development.

decision is made to consign a species to the annals. The road expansion we witnessed is destroying the resources that support local communities. Dynamited hillsides invite erosion and landslides, and tossing of rubble into rivers is turning their clear

another male. Rather than provide reason for celebration, this one was trying to mate with a dead female. “We may be watching a species go extinct before our very eyes” exclaimed Joel Sartore. “I knew it was bad, but I had no idea it was this bad.”

Another symbol of the destruction: glass frog eggs were found hanging over dry ground, waiting to hatch.

It is not a fight between conservation and development. Questions need to be asked before the



*Glass frog eggs* © Robin Moore

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## IN SEARCH OF THE LIMON HARLEQUIN FROG

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waters brown and suffocating the life out of them - in the name of development.

It is not the improvement of this road that is the problem per se: it is the way in which it is being done. It is a rushed job, with no proper assessment of its impacts and no measures to mitigate these impacts.

Just two weeks earlier the Minister of the Environment emphatically reiterated Ecuador's commitment to reducing biodiversity loss by 2010. Here is the perfect place to start.

We are working with local partners from the Católica University to try and protect some of the habitat upstream of the development. A captive

breeding program for the species will hopefully provide individuals for reintroduction. In the meantime, we will shine the spotlight on the government and question their commitment to protecting this and other valuable habitats in the hope that our voices will be heard.

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## AROUND THE WORLD

### Rapid survey on amphibian skin diseases in a mountain forest at the northern andes of Peru

Marco A. Enciso, Mirella Villena, Ana P. Mendoza, Germán Chávez

The skin of amphibians is highly permeable and directly involved in an important physiologic processes, including water absorption, osmoregulation, and, to different degrees, respiration. In the amphibian epidermis, the combination of a fragility, with minimal keratinization, and lack of potentially protective structures makes it very sensitive to environmental perturbations and cutaneous injury (Pessier, 2002).

Investigators have described many infectious and noninfectious diseases that occur among various species of captive and wild amphibians, and there is con-

siderable overlap in diseases of captive versus free-ranging populations (Densmore & Green, 2007). Generally, the microorganisms involved in

the infectious diseases are bacterias, fungus and viruses.

Bacterial infections are one of the first causes of morbidity and mortality in captive



*Pristimantis corrugatus* at the ACP Huiquilla, Amazonas, Peru

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## RAPID SEARCH FOR AMPHIBIAN DISEASE IN NORTH PERU

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and wild amphibians. These bacteria are a part of the normal flora of these species, and only cause disease when there is a breakdown in the natural defense mechanisms. Among bacterial diseases, the most overdiagnosed and misdiagnosed disease is red leg syndrome. The most frequently implicated etiological agent is *Aeromonas hydrophila*; however, many other gram-negative bacteria can be involved, like a *Acinetobacter*, *Proteus*, etc (Crawshaw, 1992; Mauel et

directly attributed to, severe population declines, extirpation of populations and extinctions of many amphibian species around the world (Daszak et al., 1999). The chytrid fungus, *Batrachochytrium dendrobatidis*, causes a severe hyperkeratosis of the skin, affecting the cutaneous respiration and thermoregulation of amphibian host, causing death. In addition, secondary bacterial or other fungal infections can be present (Pessier, 2002; Paré, 2003). The agent is recognized as a

**Bacterial infections are one of the first causes of morbidity and mortality in captive and wild amphibians.**

It is necessary to know to what extent these infectious agents occur in susceptible groups like amphibians, principally in free-ranging individuals. In Peru, we do not have reports of bacterial diseases in wild amphibian populations. In relation to the fungus, it has been identified in the southern region of the Andes (Seimon et al., 2005; Seimon et al., 2007), but amphibian populations in the northern Andes had not previously been evaluated.

The aim of this study was to investigate the presence of *B. dendrobatidis* and bacterial agents in the skin of free-ranging amphibians in the northern andes of Peru. The study was carried out in the mountain forest and puna region of the Área de Conservación Privada Huiquilla, Amazonas, located in 06°23' S, 77°29' W and 2800 m.a.s.l., northeast of Peru. The field work was car-



***Skin swab for bacterial analysis in a *Pristimantis* individual***

al., 2002).

In the case of fungal infections, chytridiomycosis is a disease associated with, or

global threat to a broad host range of wild amphibian populations (Berger et al., 1999).

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ried out in October 2007 and February 2008. The samples were collected from amphibians of the genus *Pristimantis* (*P. corrugatus*, *P. schultei* and *P. melanogaster*) and *Gastrotheca* (*G. monticola*). Skin samples of distal phalanx (n=23) were obtained for histopathological analysis. This was performed in the Laboratory of Histopathology, Faculty of Veterinary Medicine (FVM), Universidad Nacional Mayor de San Marcos (UNMSM), Lima-Peru. Established protocols of haematoxylin/eosin stain for chytrid identification were used (Berger et al., 2000). For the bacteriological analysis, skin swabs were taken (n=19). The analysis was carried on in the Laboratory of Microbiology, FVM, UNMSM, Lima-Peru. Established protocols for *Aeromonas* and enterobacteria isolation were used.

## The most over- and misdiagnosed disease is red leg syndrome.

With respect to *B. dendrobatidis*, the results prove the absence of fungus in the samples

of the species collected, indicating that frogs in this sample area are not developing the disease. However, the presence of the pathogen cannot be ruled out in this area, because while the individuals sampled may not have developed the clinic disease, they may still have been exposed to the pathogen. More studies at the molecular level are necessary with the use of PCR technique.

In the bacteriological analysis we found *Aeromonas caviae* in the 10.51% of the samples, and to a lesser extent we found other enterobacteria of the genus *Enterobacter*, *Serratia* and *Hafnia*. The results suggest the potential threat of infection and development of disease in these species with *Aeromonas*, because if it is true that the bacteria can be a normal skin inhabitant, any event that provokes stress in the amphibian (like a high temperatures, chytridiomycosis, etc) could trigger the disease.

This rapid survey gives us an overview about the health status of amphibian populations in the sampled area. More studies on chytrid fungus and other diseases are necessary in order to understand the degree of impact and diseases distribution in Peru.

## Acknowledgements

We thank Iniciativa de Especies Amenazadas-IEA Perú, Programa de Becas Maria Koepcke, Grant No. 57-2007-APECO-C.I. for funding. Instituto Nacional de Recursos Naturales (INRENA): Authorization N° 118-2007-INRENA-IFFS-OCB. Dr. Alfonso Chavera and Siever Morales (FVM-UNMSM) and Mr. José La Torre (ACP-Huichilla).

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## African Amphibian Working Group 12-13 April 2008, Ilboro Safari Lodge, Arusha, Tanzania

**Kim Howell and Flora Stephano**

The 13th meeting of the AAWG was held from 12-13 April 2008 at Ilboro Safari Lodge, Arusha, Tanzania. The 28 registered participants came from Nigeria, Kenya, Uganda, Tanzania and South Africa as well as the UK, Europe and the USA.

The papers and posters presented are listed below, grouped according to topics, with the name of the presenter.

A unique aspect of this AAWG

was that it was supported by the local private companies, Nomad Safaris, Ngare Sero Lodge and WEGS consultants. Their generous support meant that participants did not have to incur registration costs. A night of frogging on the grounds of Ngare Sero Lodge was preceded by a meal, and they also funded a closing cocktail. Nomad Safaris provided transport as well as driver guides for the evening, and these guides

participated actively in the night's frogging.

Dr. Michele Menegon led a discussion focussed on how Tanzania might take advantage of experience gained in southern Africa as regards Amphibian conservation, and it was noted that there was a need for Tanzania to establish an Amphibian Red Data book. In training sessions, Dr Menegon and Prof. Alan Channing

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**AFRICAN AMPHIBIAN WORKING GROUP***Continued from previous page*

Moyer gave a demonstration on techniques used in recording amphibian vocalisations. The next meeting of the AAWG will be hosted by Prof. Alan Channing in Stellenbosch, South Africa in 2010.

TOPIC	SPEAKER	TITLE
<b>Species, Systematics, Distribution</b>	Alan Channing	Just what is that species?
	Abigail Imasuen	Biodiversity of Amphibians in Okomu National Park, Nigeria
	Annika Hilliers	From leaf-litter frogs' phylogeography to West Africa's forest history
	John Visser	<i>Heleophryne</i> re-visited – How secondary sexual characters confuse the species specific.
	Michael Cunningham	The geography of speciation in Karoo Toads ( <i>Vandijkophrynus</i> spp.)
	Kirsty Bell	<i>Breviceps macrops</i> distribution
	Andrew Turner	Post-Miocene evolution in the Cape Fold Mountains: A moss frog's perspective.
	Patrick Malonza	Vertebrate species richness in the Eastern Arc Mountains: revisited using amphibians as a model
	Rafeal de Sa	Preliminary analysis of the genetic diversity of <i>Hemisus marmoratus</i> (Anura, Hemisotidae).
	John Measey	Investigating ancient and modern population fragmentation in <i>Amietophrynus pantherinus</i> , the endangered Western Leopard Toad
	Michele Menegon	1997-2007, summary of the results of ten years of fieldwork on the Amphibians of the Eastern Arc Mountains
	Mathias Behangana	On vocalisations of some Ugandan anurans
<b>Reproduction</b>	C.L. Henderson	Reproductive potential in Southern African frogs
	Flora Stephano	Stages of Development of Clawed Frog, <i>Xenopus muelleri</i> from hatching to metamorphosis
<b>Techniques etc</b>	Kim Howell	The benefits of pitfall traps when sampling amphibians

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**AFRICAN AMPHIBIAN WORKING GROUP***Continued from previous page*

	Elizabeth Harper	A Field Guide to the Amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya
<b>Parasites &amp; Disease</b>	David Moyer	Chytridiomycosis in anurans in the Udzungwa Mountains, results of a survey
	Marika Gericke	The culturing and distribution of <i>Batrachochytrium dendrobatidis</i> in South Africa
	Leon Meyer	Seasonal variation and the influence of environmental gradients on <i>Batrachochytrium dendrobatidis</i> infections in South African frogs
	Martins Aisien	Observations on the reproductive biology of <i>Polystoma erabensis</i> parasitic in <i>Amietophrynus regularis</i> from Nigeria
	Louis Du Preez	Madagascan polystomes-the African connection
<b>Feeding, Diet</b>	Beryl Bwong	Diet composition of <i>Xenopus borealis</i> in natural and disturbed habitat in the Taita Hills
<b>Conservation</b>	Abeda Dawood	Genetic variability between populations of the critically endangered frog <i>Microbatrachella capensis</i> (Anura: Ranidae: Cacosterninae).
	Krystal Tolley	Deconstructing a controversial local range expansion: conservation biogeography of the painted reed frog ( <i>Hyperolius marmoratus</i> ) in South Africa
	Patricia McCauley	Amphibians and Ecotourism
<b>Posters</b>	Michael Cunningham	Systematic revision of Ghost Frogs ( <i>Heleophryne</i> )
	A. Dawood	Antimicrobial activity of epithelia from selected frog species of the south Western Cape of South Africa
	Devolent Mtui	Observations on biology of the Torrent Frog <i>Arthroleptides yakusini</i> in Kihansi gorge, Tanzania
	Devolent Mtui	Observations on the status of amphibians in Kihansi Gorge, Tanzania
	Job de Graaf	Frogs of Flood River, Dodoma, Tanzania

Report by co organisers Kim (fsnyaki@udsm.ac.tz) of University of Dar es Salaam, Howell (khowell@udsm.ac.tz) and Flora Stephano Wildlife Conservation, the Dept. of Zoology & Dar es Salaam, Tanzania.

## DAPTF Seed Grants

Recipients of DAPTF Seed Grants are generally expected to publish the results of their projects in refereed journals, or as articles in *Froglog*. The following papers report work supported by DAPTF Seed Grants awarded to Johanna Delgado-Acevedo (2002), Eli Greenbaum (2002) Tibor Hartel (2004), Nancy Karraker & James Gibbs (2002), Justin Garwood (2005) and Pamela Widder & Joseph Bidwell (2004):

Delgado-Acevedo, J. & Restrepo, C. (in press) The contribution of habitat loss to changes in body size, allometry, and bilateral asym-

metry in two Eleutherodactylus frogs from Puerto Rico. *Conservation Biol.*: (johannadelgado@yahoo.com)

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Hartel, T. (in press) Weather conditions, breeding date and population fluctuation in *Rana dalmanina* from central Romania. *Herpetol. J.*: (asobeka@yahoo.com)

Karraker, N. E., Gibbs,

J. P. & Vonesh, J. R. (2008) Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. *Ecol. Applications*: 18; 724-734. (karraker@hkucc.hku.hk)

Pope, K. L., Garwood, J. M., Welsh, H. H. & Lawler, S. P. (in press) Evidence of indirect impacts of introduced trout on native amphibians via facilitation of a shared predator. *Biol. Conservation*: (kpope@fs.fed.us)

Widder, P. D. & Bidwell, J. R. (2008) Tadpole size, cholinesterase activity, and swim speed in four frog species after exposure to sub-lethal concentrations of chlorpyrifos. *Aquatic Toxicology*: 88; 9-18. (widder@vt.edu)

## ANNOUNCEMENTS

### Cure for the chytrid?

Reid Harris and colleagues at James Madison University have identified bacteria that live naturally on amphibians and help them to survive fungal infection.

Testing the mountain yellow-legged frog (*Rana muscosa*), Harris and colleagues found that the presence of bacteria helped reduce weight

loss and increase lifespan during a fungal attack.

The amphibian chytrid has been implicated in population crashes and species extinctions around the world, yet a silver bullet to cure wild individuals has continued to elude scientists. This finding is significant and provides hope that we may have a

method for combatting the fungus before many more species are lost to the disease.

Harris and colleagues plan to begin field trials within the next few years if positive results continue.

## Seeking enthusiastic volunteer to identify calls of Costa Rican amphibians

We are looking for an enthusiastic, patient and knowledgeable volunteer with expertise on Costa Rican frog calls. We are testing a new state-of-the-art acoustic monitoring protocol for frogs and defining several protocol parameters such as number of samples per night, duration of each

sample, etc. The person will have to listen to several hundreds of hours of recordings and be able to identify all the frog species calling. All recordings come from La Selva Biological station and adjacent mid elevation Braulio Carillo National Park, Costa Rica. The results from

this effort will likely result in a paper in an international journal and coauthorship will be offered. For more information please contact Jorge Ahumada, TEAM Network, Conservation International (j.ahumada@conservation.org).

## Matching Funds for Site-level Conservation

The Amphibian Specialist Group is accepting proposals to achieve the protection of critical amphibian habitat anywhere in the world. Proposals from Africa and Asia are highly encouraged. The site must have demonstrable importance for amphibians and contain a population of at least one IUCN Threat-

ened amphibian species. Sites containing species identified by the Alliance for Zero Extinction (AZE: [www.zeroextinction.org](http://www.zeroextinction.org)) as occurring in just one site worldwide will be favored. We can match up to 50% of project costs: remaining funds must be secured by the applicant. Organizations and Institutions are eligible

to apply for funding. Please submit a one-page summary of the site, the species, the opportunity and a detailed budget, up to a maximum request of USD \$30,000 to Robin Moore at [rdmoore@conservation.org](mailto:rdmoore@conservation.org). Larger requests may be considered for exceptional projects. Proposals will be reviewed on a rolling basis as they are received.

## Instructions to Authors

FROGLOG publishes a range of articles on any research, discoveries or conservation news relating to the amphibian decline phenomenon. We encourage authors describing original research to first make submissions to a refereed journal and then, if appropriate, to publish a synopsis in Froglog. Submissions should be in English, normally no more than 1000 words

and follow the style of FROGLOG Vol 83 (as should references). You may also submit images, maps, figures or tables. We encourage the submission of photographs to accompany text. Short news items and press releases are also acceptable. Please submit potential contributions to Robin Moore at the address in the box to the right.

FROGLOG is the bi-monthly newsletter of the Amphibian Specialist Group (ASG). Articles on any subject relevant to the understanding of amphibian conservation, research and / or assessments should be sent to: Robin Moore, Editor, Conservation International, 2011 Crystal Drive, Suite 500, Arlington, VA 22202, USA.

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