2010 ANNUAL REPORT PANAMA AMPHIBIAN RESCUE AND CONSERVATION PROJECT

A project partnership between: Africam Safari, Cheyenne Mountain Zoo, Defenders of Wildlife, Houston Zoo, Smithsonian's National Zoological Park, Smithsonian Tropical Research Institute, Summit Municipal Park and Zoo New England.



SUMMARY

The Panama Amphibian Rescue and Conservation project was launched in 2009 to rescue amphibians in grave danger of extinction due to a deadly fungal disease spreading through Central America. The objectives are to create assurance colonies of species likely to go extinct due to the disease, and to develop a cure that will oneday allow species to be reintroduced into the wild. In 2010, we conducted several expeditions to central and eastern Panama, bringing four described and three undescribed species into the Gamboa Amphibian Rescue Center to establish adequate founding populations and we documented the continued spread of the disease, as predicted, throughout central Panama. We successfully bred three species in captivity for the first time. Meanwhile, the El Valle Amphibian Rescue Center (EVACC) continued to expand its collection and bred nine priority rescue species, and produced a second generation of captive-bred Crowned tree frogs (Anotheca spinosa). The rescue project also conducted the

first probiotics cure trials to find a cure for the deadly, invasive amphibian pathogen Batrachochytrium dendobatidis (Bd). We used captivebred Panamanian golden frogs (Atelopus zeteki). These trials were not successful, but we learned a great deal that will help us succeed in our continued search for the cure in 2011. We sent three newly outfitted amphibian rescue pods to Panama in 2010, establishing 1,200 square feet of new ex situ space. We have had some difficulties, however, connecting two containers at the Summit Municipal Park with adequate electricity and water supplies, and will be moving the rescue pods to a new site in Gamboa in 2011, where we hope to resolve these issues. We made significant progress on food rearing and husbandry protocols and are grateful for the assistance of 58 international and local volunteers throughout the year with varied skills. Our website continued to garner traffic: we had 32 independent news articles covering the project and one of our expeditions is the basis of a one-hour Smithsonian Networks documentary that will air in 2011.

GOAL 1: CREATE ASSURANCE COLONIES OF PANAMA'S MOST VULNERABLE AM-PHIBIAN SPECIES

Rescue founding populations of 20 of the 54 identified priority rescue species and establish assurance colonies by 2012.

By the end of 2010 our Summit facility housed four described species and three undescribed species. We had 15 male and 15 female founders for three species: Toad Mountain harlequin frog (*Atelopus certus*), Pirre harlequin frog (*Atelopus glyphus*) and La loma tree frog (*Hyloscirtus colymba*). EVACC had 61 species (15 only for exhibition), with at least 15 male and 15 female founders for the Crowned tree frog (*Anotheca spinosa*), Horned marsupial frog (*Gastrotheca cornuta*), Banded horned tree frog (*Hemiphractus fasciatus*) and Rusty robber frog (*Strabomantis bufoniformis*). We began some of the collection five years ago and those animals are aging.

We conducted six expeditions throughout the year to sites in central and eastern Panama. The trips were possible thanks to funding from the US Fish and Wildlife Service and the Mohamed bin Zayed Species Conservation Fund and through collaboration with other research groups, including La Sociedad Mastozoológica de Panamá (SOMASPA). We learned that most sites in central Panama, including lowland areas, are *Bd* positive, and amphibians were experiencing steep declines. The two Darien sites, Cerro Sapo and Cerro Pirre, in eastern Panama remain *Bd* free and had abundant populations of species that are regarded as very sensitive to *Bd*. Given the rate at which *Bd* spread through central Panama, however, we would expect *Bd* to appear in the Darien National Park any time in the next few years. In January of 2010, Doug Woodhams, a research associate at the Smithsonian Tropical Research Institute, detected *Bd* in amphibians in Torti, which is right on the Darien border.

The very first rescue expeditions in late 2009 brought back diseased Limosa harlequin frogs (Atelopus limosus) and La loma tree frogs (Hyloscirtus colymba) that experienced high mortality rates when treated for Bd. Veterinarians Eric Baitchman from Zoo New England and Della Garelle from Cheyenne Mountain Zoo made several trips to Panama. Working closely with staff, Garelle and Baitchman made significant progress in establishing best practices for managing recurring issues and served as telephone consultants during emergencies. Limosa harlequin frogs (Atelopus limosus) remain a grave concern and securing 15 male and 15 female founders of both the chevron-patterned color form and the brown color form is our highest priority for 2011. This may be our last opportunity to save this species.

A recent genetics study published in the *Proceedings* of the National Academy of Sciences from Biff Bermingham's lab at STRI showed that several species at El Cope in western Panama went extinct before they could even be described. The fact that three species in our collection from central Panama are undescribed really highlights the need to collaborate closely with geneticists. As such, we maintain a close working relationship with Andrew Crawford from Universidad de los Andes in Colombia, who joined us on the Cerro Sapo expedition.

> Bob Chastain from Cheyenne Mountain Zoo and Korin Anderson from Smithsonian Networks on a rescue expedition to Cerro Sapo.



Breed at least 70 percent of founding populations to obtain F2 generations.

At Summit we bred three priority species: Limosa harlequin frog, Toad Mountain harlequin frog and La loma tree frog. EVACC bred nine priority species: Horned marsupial frogs (Gastrotheca cornuta), Lemur leaf frogs (Hylomantis lemur), Crowned tree frogs (Anotheca spinosa), Banded horned tree frog (Hemiphractus fasciatus), Pirre harlequin frogs (Atelopus glyphus), Costa Rican variable harlequin frog (Atelopus varius), Limosa harlequin frog (Atelopus limosus), Robber frog (Craugastor tabasarae), Rusty robber frog (Strabomantis bufoniformis); and three low-priority species: Green climbing toad (Incilius coniferus), Monkey frog (Phyllomedusa venusta), Cocle mushroom tongue salamander (Bolitoglossa schizodactyla). A major milestone has been the production of the first of many tadpoles from the Crowned tree frog (Anotheca spinosa), giving us confidence that we can maintain this species in captivity over multiple generations.

Project staff collaborated closely with EVACC director Edgardo Griffith and assistant director Heidi Ross and other visiting zoo professionals. We documented the lessons that we learned in the first draft of our husbandry manual that we'll use in the future to perfect our food rearing and animal husbandry practices. After many hours of painstaking care for the animals, we were rewarded by the first ever breeding of the Limosa harlequin frog (Atelopus limosus), Toad Mountain harlequin frog (Atelopus certus) and La loma tree frog (Hyloscirtus colymba) in captivity. The La loma tree frog (Hyloscirtus colymba) juveniles died shortly after metamorphosis-so this was a hollow victory-but by the end of 2010, the juvenile Toad Mountain harlequin frog (Atelopus certus) and Limosa harlequin frog (Atelopus limosus) juveniles were healthy and growing.

GOAL 2: RE-ESTABLISH HEALTHY WILD POPULATIONS OF PANAMANIAN AMPHIBI-ANS

Actively explore potential methods to re-establish wild populations of amphibians from assurance colonies (e.g. probiotics, captive-release of surplus -bred amphibians, or other novel developments in the field).

In 2010, we conducted the first probiotics trials using captive-bred Panamanian Golden Frogs (Atelopus zeteki), led by Matthew Becker of Virginia Tech. The Maryland Zoo in Baltimore provided the frogs via the Association of Zoos and Aquariums' Panamanian Golden Frog Species Survival Program. We inoculated frogs with the known probiotic bacteria Janthinobacterium lividium and found that the bacterium did not confer protection from Bd to the frogs because the bacterium did not persist on Golden Frog skin. We began prospecting for new anti-Bd bacteria in Panama and harvested more than 600 bacterial isolates from frog skin. These were challenged with Bd in vitro, and 50 isolates were found to inhibit Bd growth 100 percent. We will continue this collaborative research with James Madison, Vanderbilt and Virginia Tech Universities in 2011 and gratefully acknowledge generous support from the Anele Kolohe Foundation, Shared Earth Foundation and the USFWS.

We also attended and presented our findings from this first experiment at the first international meeting on mitigating Bd spread, hosted by Doug Woodhams of the University of Zurich. At this meeting, we systematically reviewed all existing zoonotic disease control strategies for possible ways to prevent Bd spread. Some of the meeting participants had published white paper *Mitigating Amphibian Disease*: Strategies to maintain wild populations and control *chytridiomycosis* in the open-source journal Frontiers in Zoology. Doug's research on Panamanian glass frogs also provided the first evidence for transmission of probiotics bacteria from parents to offspring. In addition, EVACC hosted Denise Kung from the University of Zurich who was investigating microbial communities on amphibian skin and the effects of augmenting them with probiotics bacteria. We provided PCR analysis of skin swabs for Bd detection while Kung was in Panama.

Roberto Ibáñez, local director of the project and a scientist at the Smithsonian Tropical Research Institute, also worked with Joyce Longcore to isolate and culture *Bd* from three different frogs. This is a major milestone for *Bd* research in Panama, as it gives us the capacity to conduct *Bd* experiments in-country, which may help us manage the disease.





GOAL 3: BUILD CAPACITY IN PANAMA TO SAFEGUARD THE NATION'S AMPHIBIAN FAUNA OVER THE LONG-TERM

Secure a combined 5,000 square feet of biosecure holding space at EVACC and Summit locations in Panama by 2012.

In 2010 we obtained two new 400-square-foot containers from Maersk, making a total of three amphibian rescue pods and providing a total of 1,200 square feet of *ex situ* space. Unfortunately we have not been able to secure electricity to the two new pods at Summit Municipal Park, and therefore have not been able to place frogs in them. As a result, we have decided to move the three pods to a new location in Gamboa in 2011. EVACC received one new container donated by APL, adding 400 square feet to its 2,400square-foot facility, giving us a combined total of 4,000 square feet of *ex situ* space towards the 5,000square-foot goal.

Secure a combined 1,200 square feet for food production facilities and additional wild collecting methods for a variety of prey types with at least one full-time keeper specializing in insect care and collection at each location by 2012.

Collaboration with Roger Williams Park Zoo and Toronto Zoo has helped us to make considerable progress on food rearing facilities. We have added one new 200-square-foot building at Summit for rearing crickets, roaches and super worms. We also completed one new 300-square-foot outdoor grasshopper-rearing cage, adding to the existing 100square-foot house for fruit-flies and springtails, making a total of 600 square feet of space at Summit. We have one full-time employee focused on food rearing, although this is a time-consuming activity and keeps many volunteers occupied. EVACC maintains about 250 square feet of space for rearing crickets and fruit flies in its main facility and a local family gathers larger katydids from the wild. A new grant through Roger Williams Park Zoo should enable EVACC to hire a full-time insect keeper in 2011.

Train and provide employment for dedicated Panamanian amphibian conservation staff and retain them by providing adequate long-term prospects for career development and growth.

We now have five full-time staff positions at each facility, representing a significant amount of capacity for conservation. EVACC had two vacancies throughout most of 2010 due to the challenges of recruiting staff in El Valle, while at the Summit location volunteer coordinator Danny Medina left for Spain to pursue a master's degree and was replaced by Angie Estrada. Mahudy Díaz started as part-time technician in qPCR methods to analyze Bd swabs, through funds provided by the Panamanian Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT). The year 2010 involved a steep learning curve for our staff and they drew heavily on the experience of EVACC and mentorship provided by project collaborators. Several staff members participated in the development of the National Conservation Action Plan meeting hosted by STRI and ANAM. By the end of 2010, the National Amphibian Conservation Action Plan had been finalized, but not yet published. This is a significant step forward for developing capacity nationally, and in engaging the Panamanian government.

Cultivate and foster an appreciation for amphibians in the public mindset.

We developed a working communications plan and a communications team led by Lindsay Renick Mayer, a public affairs specialist at the Smithsonian's National Zoo. The primary goals of this plan were to 1) build a constituency, 2) raise local, national and international awareness of the crisis, 3) raise funding and 4) recruit volunteers to assist with the program.

Online: We received more than 20,000 unique visitors to http://amphibianrescue.org in 2010, with about 37,000 page views from 129 countries around the world. New features included a regularly scheduled weekly blog post, plus a cute frog of the week photo feature that drove the most traffic to our website. Our social media strategy focused on recruiting and establishing an online relationship with constituents. In 2010 we gained 1,600 new Facebook fans for a total of 3,500 followers. Of these, 1,500 are from Panama City. Content posted to Facebook was viewed 75,000 times with 1,700 feedback actions. We also started a Twitter account in 2010 that mostly reposted Facebook updates and generated about 600 followers.

Traditional media: Thirty-two independent <u>news</u> <u>articles</u> about the project were published in 2010, including features on NPR, National Geographic, New Scientist and Panama's main newspaper, La Prensa. The prime media event last year was the making of a one-hour documentary on the project, produced for Smithsonian Networks. The team focused on the rescue of the Toad Mountain harlequin frog from the Darien region, and the development of a probiotics cure. It is scheduled to air in September of 2011.

Volunteer program: With help from the Smithsonian Women's Committee, we hired a volunteer coordinator and established a formal volunteer program. Products included a recruitment video, volunteer webpage and volunteer travel guide to support the project. In 2010 we recruited 24 international volunteers and 34 local Panamanian volunteers with a total of 58 "graduates" of the Panama Amphibian Recue and Conservation Project. This far exceeded the stated project goals of 15 international volunteers. On average we had two volunteers at the center at any one time who devoted around two weeks of labor to the project, adding up to about two extra full-time employees over the course of the year.

Ensure the financial sustainability of the project beyond 2012.

In 2010 we tried an innovative "Text Frog" campaign, and solicited \$5,000 in donations in partnership with NimbleBit, a company that makes the popular iPhone app called Pocket Frogs. Overall, the communications campaign has been reasonably successful in building a constituency, but we will need to raise this by at least an order of magnitude in order to grow public contributions to a level that could help sustain the project in the longer term. While we have had some small successes, development of a business and conservation financing plan is a top priority in 2011.



FINANCIAL REPORT

EXPENDITURES	FUNDING SOURCE	
EVACC	Houston Zoo and EVACC donors	\$139,000
Outfitting 2 rescue pods*		\$87,716
	Defenders of Wildlife	\$38,400
	Cheyenne Mountain Zoo	\$25,169
	Zoo New England	\$24,147
Staff salaries		\$101,043
	STRI	\$31,000
	SI Women's Committee	\$20,000
	Cheyenne Mountain Zoo	\$19,043
	Defenders of Wildlife	\$31,000
Supplies & expeditions		\$79,072
	USFWS	\$62,541
	MBZ Species Conservation Fund	\$10,000
	SI Women's Committee	\$4,500
	Africam Safari	\$2,031
Cure research		\$44,616
	USFWS	\$25,000
	SCBI and donors	\$19,616
SCBI staff salary and travel SCBI and donors		\$131,564
		\$583,011

*Includes \$20K in kind from Maersk Line.





Atelopus limosus, the Limosa harlequin frog.

Donors

In addition to the contributions above, the Smithsonian National Zoological Park and the Houston Zoo are grateful to the following generous donors who have made additional contributions to the project: Anele Kolohe Foundation, APL, Buffalo Zoo, CBSG, Cleveland Zoo, Elmwood Park, Dickerson, Little Rock, Oklahoma City, Shared Earth Foundation, Susan and Frank Mars, Maersk, the US Fish and Wildlife Service, Mohammed Bin Zayed Species Conservation Fund, Project Golden Frog, Ronna Erickson and the Smithsonian Women's Committee.

Online Contributions

Our sincere thanks to the following people who contributed \$25 or more online:

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Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke *Project Director, Panama* - Dr. Roberto Ibáñez *Technical Staff* - Rousmery Bethancourt*, Lanki Cheucarama, Aracelys De Gracia, Angie Estrada, Nancy Fairchild, Jorge Guerrel, Mahudy Díaz, Danny Medina* (* =alumni).

El Valle Amphibian Conservation Center Director – Edgardo Griffth El Valle Amphibian Conservation Center Assistant Director – Heidi Ross Technical Staff – Santana Arcia, Matilde Peréz

Steering Committee

Amy Camacho, Managing Director Africam Safari; Bob Chastain, President and CEO Cheyenne Mountain Zoo; Jamie Clarke, Executive Vice President Defenders of Wildlife; Rick Barongi, Executive Director Houston Zoo; Steve Monfort, Director Smithsonian Conservation Biology Institute; Biff Bermingham, Director Smithsonian Tropical Research Institute; Nestor Correa, Director Summit Municipal Park; John Linehan, President and CEO Zoo New England.

Implementation Committee

Luis Carrillo Africam Safari; Dr. Della Garelle Cheyenne Mountain Zoo; Cindy Hoffman Defenders of Wildlife; Paul Crump; Peter Riger & Edgardo Griffth Houston Zoo; Dr. Brian Gratwicke Smithsonian's National Zoological Park; Dr. Roberto Ibáñez Smithsonian Tropical Research Institute; Nestor Correa, Summit Municipal Park; Dr. Eric Baitchman Zoo New England.

Communications Committee

Katie Borremans Cheyenne Mountain Zoo; Cindy Hoffman Defenders of Wildlife; Brian Hill & Edgardo Griffth Houston Zoo Inc.; Lindsay Renick Mayer Smithsonian's National Zoological Park; Beth King Smithsonian Tropical Research Institute; Brooke Wardrop Zoo New England.

2010 Volunteers

Summit volunteers: Indira Arrocha, Lauren Augustine, Idis Batista, Jeff Baughman, Megan Baumer, Rousmery Bethancourt, Michelle Brown, Nair Cabezón, Jose Canate, Melanie Carter, Katherine Castillero, Susana Castillo, Eduardo Chong, Paola Cordoba, Ricardo Cossio, Jeff Coulter, Karina De León, José Escobar, Angie Estrada, Sangie Estrada, Matthew Evans, Nancy Fairchild, Elizabeth García, Lorena García, Mandy Gaudreau, Dalia González, Jeremy Hayward, Laura Hernández, Iveth Herrera, Lizzi Herrera, Melanie Herrera, Sean Higgins, Robert Hill, Jessica Hite, Eduardo Leiva, Luke Linhoff, Tom Mason, Nadya Morales, Sarah Oogden, Iván Rodrigo Ortíz, Louis Perrotti, Heather Pong Yen Tings, Richard Quintero, Brooke Reeve, Lindsay Renick Mayer, Juan Pablo Ríos, Manuel Rivera, Mercedes Rodríguez, Alberto Rudas, Enrique Sánchez, Edwin Smith, Angélica Suira, Tania Sunsine, Marta Torres, Milagros Vargas, Collin Clark.

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