2022 Annual Report

Panama Amphibian Rescue and Conservation Project

A project partnership between Cheyenne Mountain Zoo, Smithsonian's National Zoo & Conservation Biology Institute, Smithsonian Tropical Research Institute, and Zoo New England.

Mission

Our mission is to rescue and establish sustainable assurance colonies of amphibian species that are in extreme danger of extinction throughout Panama. We will also focus our efforts and expertise on developing methodologies to reduce the impact of the amphibian chytrid fungus (Bd) and proceed to reintroduction trials.

Cover: *Agalychnis lemur* the lemur leaf frog is recovering in some places following precipitous disease-related declines. Understanding why some species are recovering may give us clues to help restore the species to previously vanished sites.

Goal 1: Ensure adequate physical infrastructure and staffing capacity to effectively manage and breed the living collection.

Following the ongoing COVID pandemic, some operations returned to normal in 2022. We moved our insect production into the new insectarium and achieved incredible production goals for feeder insects, now supporting substantial growth of the living collection of amphibians. Construction started on hard-wiring the internet to each rescue pod in order to install essential automated alarm systems that notify staff if temperatures deviate from a predefined threshold range. In 2023, we are planning to resume our volunteer and internship programs.

Dr. Eric Baitchman from Zoo New England and Jorge Guerrel examine an *Atelopus*

Goal 2: Manage genetically viable assurance colonies of 12 species in captivity that are at risk of extinction from chytridiomycosis.

In 2022, we continued to breed several species: the *Atelopus* species*, A. certus, A. glyphus, A. limosus,* and *A. varius;* the dart frogs *Oophaga vicentei* and *Andinobates geminisae;* lemur leaf frogs *Agalychnis lemur;* and crowned treefrogs *Triprion spinosus.* We are pleased to report our first F4 generation of the crowned treefrogs. We had poor survivorship of juvenile *Atelopus* as they transitioned from springtails to crickets. We have been unsuccessful at breeding *Gastrotheca cornuta, Strabomantis bufoniformis,* and *Craugastor evanesco.* We are prioritizing testing several alternate husbandry setups and exporting specimens for pathological examination. Dr. Oz Ossiboff at the University of Florida has agreed to help us with the pathological examination of animals from the collection.

Table 1: 2022 Population management report card for the 12 priority conservation species maintained by the Panama Amphibian Rescue and Conservation Project.

2022	Founders alive or represented (Goal = 20)	Pairs Bred to F1 (Goal = 10)	Pairs bred to F2 (Goal = 10)	Pairs bred to F3 (Goal = 10)	Pairs bred to F4 (Goal = 10)	Total number of frogs in collection (Goal = 300)
Atelopus varius (lowland)	30	12	12	0	0	524
Andinobates geminisae	38	14	21	0	0	157
Oophaga vicentei	57	23	2	0	0	122
Triprion spinosa	16	4	10	4	1	59
Craugastor evanesco	19	2	0	0	0	16
Atelopus certus	11	2	10	1	0	96
Atelopus limosus	14	3	8	0	0	43
Atelopus glyphus	12	6	5	0	0	253
Agalychnis lemur	13	6	7	0	0	81
Strabomantis bufoniformis	10	2	0	0	0	5
Gastrotheca cornuta	7	4	4	0	0	7
Atelopus varius (highland)	3	2	0	0	0	30
Atelopus zeteki	3	1	5	0	0	72

Progress towards goal
0-24%
25-49%
50-74%
Goal met

Juvenile crowned treefrog *Triprion spinosus;* this is the first species with 4 captive-bred generations.

Dr Gina Della Togna (left) and Dr. Leon Grayfer working on some tissue cultures at the STRI laboratories in Gamboa.

Goal 3: Research factors to improve long-term sustainability of the captive collections and increase success of release trials.

Dr. Luke Linhoff wrapped up his mucosome (anti-Bd properties of amphibian skin) work aimed to predict disease susceptibility from skin mucus secretions, supported by the National Geographic Society. Dr. Linhoff also started a new project in collaboration with Dr. Ralph Saportio at John Carroll University to "make poison dart frogs spicy again" by restoring their alkaloid skin defense, normally derived from alkaloids in their diet that are lost in captivity. This research will help us when it comes to reintroduction work with these aposematically colored frogs, hopefully reducing predation-related mortality.

We began work on an NSF-funded collaboration between Dr. Carly Muletz-Wolz, Dr. Leon Grayfer, Dr. Amy Ellison, and Dr. Barney Long titled "The amphibian skin microbiome-immune interface and its impact on disease outcomes." We began experiments to understand the role of the host immune system and the microbiome using *Xenopus* as a tolerant model disease organism and *Atelopus* as a highly susceptible animal. In addition, we screened the mucosome from all the amphibians at the Smithsonian's National Zoo to get a better understanding of how this varies across the amphibian tree of life. This basic research will improve our fundamental understanding of immune mechanisms as we continue the search for ways to mitigate Bd in wild situations.

Our collaborator Dr. Gina Della Togna and her students continued to work on assisted reproduction protocols, successfully producing the first *in vitro Atelopus varius* and *Atelopus certus* by fertilizing eggs collected from hormonally stimulated females with sperm collected fromhormonally stimulated males. Dr. Della Togna continues work on cryopreserving sperm and hasdeveloped hormonal stimulation protocols for male *Triprion spinosus*, *Gastrotheca cornuta, Craugastor evanesco,* and *Strabomantis bufoniformis* in the hopes of using assisted reproduction methods to increase reproductive success. We completed hormonal stimulation trials with male *Craugastor evanesco* and attempted hormone trials on female *Craugastor evanesco* to stimulate oviposition and breeding behavior. These trials included pairing stimulated females with males, and we obtained three egg depositions. However, none of the clutches were viable, and some females died (including treatment and control animals with no hormonal stimulation). After careful consideration, trials have been paused, and we are waiting for a pathology report on the possible causes of the deaths.

This year, Dr. Phil Jervis and Dr. Gonçalo M. Rosa from the Zoological Society of London were supported by a fellowship to conduct field work in Panama. They worked on an experiment to restore tetrodotoxin (TTX) toxicity in *Atelopus* skin through dietary supplementation. They also completed field work in Panama and Costa Rica to compare the skin chemistry and microbiome of wild surviving populations of *Agalychnis lemur* with pre-decline populations (museum-preserve specimens) to understand if there has been an evolution of resistance to the chytrid fungus allowing some small populations to recover. Rebounding populations offer a unique opportunity to characterize changes in skin mucosome following disease epizootics, and to assess how fit and equipped our captive animals are to deal with a naturalized pathogen in the wild.

A and B. breeding individuals of lemur frog (including amplexus) from healthy recovering populations in Limón Province (Costa Rica); C. sampling of skin secretions in the field using a norepinephrine bath; D. Project collaborator (José Salazar) weighing a wild-caught individual of lemur frog.

Given the advances made in acoustic monitoring, we were able to deploy audiomoth recorders in the field at several sites that are historical localities for many of Panama's "Lost Frogs." These are priority rescue species or potentially extinct species that are difficult to encounter because of extreme rarity or behavior. While we are still analyzing data, we were able to identify one population priority rescue species the crowned treefrog *Triprion spinosus,* previously thought extinct at this site, and significantly extended the known distributional range of an additional recently described treefrog species *Ecnomiohyla veraguensis* (we validated the species identification with a DNA sample from a buccal swab).

Santa Fe Fringe-limbed Treefrog
Ecnomiohyla veraguensis

2022 Research Publications involving collaborations with PARC members (bold)

Estrada, A., Medina, D; **Gratwicke, B, Ibáñez,** R, Belden, L (2022) Body condition, skin bacterial communities and disease status: Insights from the first release trial of the Limosa harlequin frog, *Atelopus limosus.* Proceedings of the Royal Society B. https://doi.org/10.1098/rspb.2022.0586

Kueneman, J., Bletz, M., Becker, M., **Gratwicke, B., Garcés, O.A.**, Hertz, A., Holden, W.M., **Ibáñez, R.**, Loudon, A., McKenzie, V. and Parfrey, L., 2022. Effects of captivity and rewilding on amphibian skin microbiomes. Biological Conservation, 271, p.109576.

Gratwicke, B. Context Matters in the fight to Save Frogs in Meshaka (W. ed) Exotic Amphibians and Reptiles of the United States, 2022.

Hughey, M.C., Rebollar, E.A., Harris, R.N., **Ibáñez, R.**, Loftus, S.C., House, L.L., Minbiole, K.P., Bletz, M.C., Medina, D., Shoemaker, W.R. and Swartwout, M.C., 2022. An experimental test of disease resistance function in the skin-associated bacterial communities of three tropical amphibian species. FEMS Microbiology Ecology, 98(3), p.fiac023.

Higgins, K. and **Ibanez, R.,** 2022. Description of two dendrobatid tadpoles (Anura: Dendrobatidae: *Andinobates* and *Oophaga*) with comments on egg clutches. Zootaxa, 5175(3), pp.395-400.

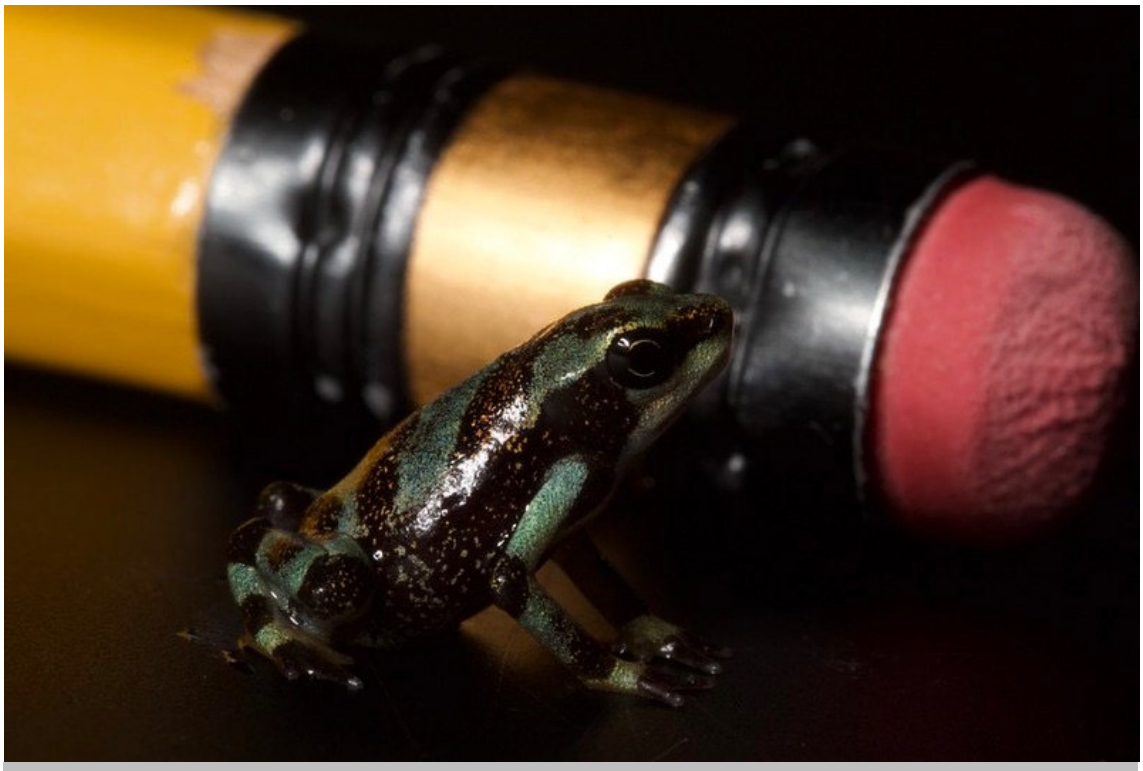
Springborn, M.R., Weill, J.A., Lips, K.R., **Ibáñez, R.** and Ghosh, A., 2022. Amphibian collapses increased malaria incidence in Central America. *Environmental Research Letters*, *17*(10), p.104012.

Geminis' dart frog *Andinobates geminisae* and tadpole

Vicente's dartfrog *Oophaga vicentei* and tadpole

Goal 4: Begin experimental frog reintroduction trials with surplus offspring.

We continued our collaboration with Dr. Carly Muletz-Wolz and Dr. Allison Byrne, funded by Revive & Restore, to cross *Atelopus* collected from different localities in order to test the idea that increasing genetic diversity may result in hybrid vigor, making offspring more resilient to disease. While we have had some success breeding these pairs and obtaining tadpoles, we have had trouble getting juvenile metamorphs to survive beyond three months, even from animals bred from the same locality. We started an intense examination of our juvenile husbandry protocols and are working to solve the problem. As a result, we have not yet been able to test the genetic rescue hypothesis. We also conducted some pilot studies with different mesocosm designs that will ultimately be used for future release trials. We learned from earlier studies that 30-day housing in mesocosms before release increased the survival and reduced dispersal of animals compared to hard-release treatments. In addition, we are looking for genes under selection following Bd-driven declines in *Atelopus* frogs using museum specimens and contemporary population surveys; lab work and DNA sequencing were completed in 2022, and analyses are in progress.



This is one of the first *Atelopus varius* metamorphs produced by Dr. Della Togna using in vitro fertilization tools. This assisted reproduction tool can help us to rapidly produce crosses from a single female using sperm collected by hormonally stimulating and collecting from multiple males.

Goal 5: Cultivate and foster an appreciation for amphibians in the public mindset and work on community engagement at the field level.

In addition to our regular community engagement in Panama, we collaborated with the Atelopus Survival Initiative to develop a global action plan to save harlequin frogs in all 11 countries where they are found in nature. We participated in an expedition funded by the National Geographic Society that brought multiple National Geographic Explorers together for an interdisciplinary collaboration to share research methods, findings, and develop new outreach capabilities. We worked with the musician and educator Janni Benavides of Jacana Jacana who developed original songs about harlequin frogs for different countries and *Atelopus*-themed activity books for children.

Our primary exhibition area at the Punta Culebra Nature Center has now re-opened and features a newly renovated exhibition area showcasing Panamanian golden frogs. This year, we participated in golden frog celebrations at the Summit Zoo, featuring the popular 'frog restaurant' where people could see how we grow food for our captive collection and other talks and activities. We continue our online presence with 11,600 followers on Facebook, 6,800 followers on Instagram, and 5,400 followers on Twitter, and our website received 48,000 unique users.

Jacana Jacana at the premier performance of the golden frog song at La Minca school, Sierra Nevada de Santa Marta (top). Our hosts from Fundacion *Atelopus* (bottom left). Local children screen-printing paper and t-shirts with *Atelopus* designs (bottom right).

Goal 6: Ensure the financial sustainability of the project.

We are very grateful to our long term partners for their continued support of this important work. We have obtained new restricted research funds from the National Science Foundation that focus on understanding mechanisms of immunity to disease in amphibians. In August 2022, our most recent 3-year agreement with First Quantum Minerals expired and it has not been renewed.

Funding Source	Purpose	2022 Expenses (US$)
SCBI and donors	Coordinator salary, supplies, fellows, travel, insectarium construction.	246,495
First Quantum Minerals (Cobre Panama)	Salaries, operating costs, swab analysis	155,272
The Woodtiger Fund	Research and program support	45,000
Zoo New England	Salaries	23,856
Cheyenne Mountain Zoo	Salaries	18,960
National Science Foundation	Research	75,808
Revive & Restore	Research	30,197
National Geographic Society	Research	13,327
SENACYT	Research	17,957
STRI and donors*	Supplies	51,974
TOTAL		**678,846**

*These funds include direct project costs incurred in the calendar year 2022, but do not reflect un-expensed funds or in-kind institutional administrative support, utilities, fundraising, public affairs, and programmatic support costs generously provided by the Smithsonian Tropical Research Institute who hosts this project. The National Geographic Meridian Grant "The last refuge of Harlequin Toads: working together to save the jewels of the forest" was awarded to Re:Wild, and those expenditures are not reported here, but we gratefully acknowledge the value and impact of the activities that this grant funded.

Donors

In addition to the contributions from project partners and listed funders, we are grateful to the following donors: Susan and Frank Mars, Sey and Pearl Moskowitz, The Woodtiger Fund, The Shared Earth Foundation, The Anela Kolohe Foundation, Revive & Restore, National Geographic Society, Friends of the Baton Rouge Zoo, Gregory and Alvera Wilson, Amy Ziffer, and Society of the Transfiguration.

Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke.
Project Director, Panama - Dr. Roberto Ibáñez.
Facility Manager Gamboa – Jorge Guerrel.
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Graduate Students - Blake Klocke, Carrie Lewis.
Post Doctoral Fellows - Luke Linhoff, Phil Jervis, Gonçalo M. Rosa, Gina Della Togna.

Steering Committee

Bob Chastain, President and CEO *Cheyenne Mountain Zoo*;
Dr. William C. Pitt, *Director of Conservation and Science Smithsonian's National Zoo and Conservation Biology Institute;*
Dr. Oris Sanjur, Deputy Director *Smithsonian Tropical Research Institute*;
John Linehan, President and CEO *Zoo New England*.
Jamie Kratt, *at-large member.*

Implementation Committee

Nicole Chaney and Jeff Baughman *Cheyenne Mountain Zoo*; Dr. Brian Gratwicke& Matthew Evans *Smithsonian's National Zoological Park and Conservation*
Biology Institute; Dr. Roberto Ibáñez & Jorge Guerrel *Smithsonian Tropical Research Institute*; Dr. Eric Baitchman *Zoo New England.*

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