2021 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: Panama's national amphibian is the Panamanian Golden Frog, *Atelopus zeteki* this species is likely extinct in the wild and persists in captivity in the US and in Panama. It has been used as a model species that is highly susceptible to the chytrid fungus in disease and conservation research.

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

In 2021, as the human COVID pandemic impacted all of our lives, our heroic staff pulled a lot of extra weight in terms of additional challenges to get to work, balance personal safety, cover for colleagues out due to quarantine rules, and without any interns or volunteers that we normally rely on. In spite of covid restrictions, we achieved important breeding successes in our captive collection, completed construction of our insectarium and resumed some high-priority research activities. We are especially grateful to our supporting donors and foundations who recognized the extent of the COVID19 impact on our operations and kindly extended performance periods on existing grants and to the individuals who kindly maintained, or even increased their regular financial contributions.

Construction of our new insect facility was completed and fire suppression systems installed. This new 1,600 square feet insectarium has two climate-controlled rooms that can be maintained at different temperatures, allowing a diversity of food items with varying sizes and nutritional properties to meet the needs of our diverse amphibian collection. A huge thanks to the STRI Office of Facilities and Engineering who oversaw this project and to the many individual donors, the Cheyenne Mountain Zoo, Zoo New England, the Houston Zoo, the Holtzman Foundation, the Shared Earth Foundation and several individual donors for enabling us to complete it.

There are no local sources of captive-reared insects, so we are 100% reliant on our own production and have redundant capacity to accommodate any unexpected changes in the populations of the various springtails, fruit flies, crickets, pantry moths, soldier flies, cockroaches and earthworms that we produce. Under the leadership of Nancy Fairchild and Jennifer Warren, our insect production capacity is now one of the incredible success stories of our project. This team has also conducted original research to develop gut loading protocols for springtails and demonstrated improved outcomes of juvenile frogs reared on this nutritionally supplemented prey, compared to their normal yeast-based diets. On the frog-side of the house, we have now completed Spanish-language husbandry manuals for all the species in our care, that will assist greatly with protocol standardization and training.

Nancy Fairchild (left) and Jennifer Warren (right) maintain 200 colonies of crickets, 300 colonies of springtails and 200 colonies of fruit flies and other prey items to sustain our amphibian collection.

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

In 2021 our breeding focus shifted more to obtaining F2 frogs as our original founding population for many species are aging out and are mostly represented by living decedents. The breeding and rearing protocols for our *Atelopus* are well-established and we reared a good number of clutches this year, with the aim of replenishing losses from an air conditioner failure in one pod in 2020. Much breeding focus oriented to *Atelopus varius* as we are conducting research into the potential for genetic rescue of this species through crossing individuals collected from different geographic localities. We have formally requested *Atelopus zeteki* and highland *Atelopus varius* from the U.S. AZA-managed species survival program to bolster the genetic diversity of the Panama captive population, as all offspring of these two groups of animals are closely related.

Table 1: 2021 Population management report card for the 12 priority conservation species maintained by the Panama Amphibian Rescue and Conservation Project.

	Founders alive or represented (Goal = 20)	Pairs Bred to F1 (Goal = 10)	Pairs bred to F2 (Goal = 10)	Total number of frogs in collection (Goal = 300)
Andinobates geminisae	40	18	3	128
Gastrotheca cornuta	22	12	1	29
Oophaga vicentei	36	10	0	50
Atelopus varius (lowland)	51	11	0	552
Craugastor evanesco	45	3	0	61
Atelopus limosus	18	8	0	78
Atelopus certus	17	8	3	85
Atelopus glyphus	17	8	0	242
Triprion spinosus	11	6	6	65
Agalychnis lemur	8	6	0	55
Strabomantis bufoniformis	8	2	0	8
Atelopus varius (highland)	6	4	0	30
Atelopus zeteki	4	5	2	241

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

A breeding pair of *Atelopus varius* with founders collected from different localities. We are investigating a genetic rescue hypothesis to understand how outbreeding may affect susceptibility to the amphibian chytrid fungus.

We have made excellent progress understanding the husbandry of the two dart frog species *Andinobates geminisae* and *Oophaga vicentei,* and rearing success of juveniles has been significantly improved by provisioning water supplemented with calcium to tadpoles, that eliminated spindly leg conditions. We removed bromeliads all together from *Andinobates* tanks as they are not a habitat utilized by this species, and simplifies husbandry and hygiene protocols for this species. *Oophaga vicentei* require larger bromeliads that can hold significant volumes of water, but these did not last well in our small dart frog tanks, switching to a combination artificial bromeliads and 'shotglasses' that hold good amounts of water that are topped off by hand misting with calcium-supplemented water significantly improved our tadpole capacity of these breeding setups.

Agalychnis lemur are housed in groups in semi-aquatic tanks, and in breeding season, egg masses are removed for rearing separately. We produced several clutches of *Triprion spinosus* but switching males out from successful mating pairs to increase genetic representation often halts reproduction. We were unable to successfully breed *Gastrotheca cornuta, Craugastor evanesco* and *Strabomantis bufoniformis* and have begun researching hormone stimulation protocols for these species in the hopes of using assisted reproduction methods to help with reproduction.

Simplified *Andinobates geminisae* breeding setups (left) have pvc pipes for egg deposition and petri dishes for individual tadpole rearing after transportation by the male, while false bottoms help maintain tank hygiene. On the right *Oophaga vicentei* breeding has improved by provisioning artificial bromeliads and supplemental shotglasses for tadpole rearing.

Dr Gina Della Togna with some of her students from left to right: Dionel Rodriguez, Dr. Della Togna, Igli Arcia, Karina Rodríguez and Diego Samaniego. All working on developing ARTs in our amphibian species.

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

COVID-related travel restrictions eased towards the end of last year permitting resumption of research projects led by Luke Linhoff aiming to predict the disease susceptibility from skin mucus secretions, a project supported by the National Geographic Society. We were also able to successfully conduct research into the innate skin defenses in salamanders native to the USA, led by Randall Jimenez and Carly Muletz-Wolz. We found that skin secretions alone were not sufficient to explain differences in salamander susceptibility to infection, but that anti-fungal skin bacteria played a key role in helping the salamander resist chytrid infections.

Our collaborator Dr. Gina Della Togna Smithsonian Research Associate and collaborator from the InterAmerican University in Panama and her team also resumed research on hormone dose response curves to stimulate sperm production and egg laying in several key species. Gina's team focused primarily on those species that we have been unsuccessful at reproducing in captive conditions.

Several publications led by us or by our collaborators that came out this year (see list below). Of note, our experiment to genetically manipulate a core skin microbe of golden frogs to protect the frogs from disease was published in ISME communications, while we were unable to prevent disease in golden frogs we did learn much about the ecology of skin bacteria and two different probiotic approaches. Kathleen Higgens a former PARC project intern published her paper on spindly leg syndrome and husbandry in *Andinobates* tadpoles, this research has significantly helped to improve captive-breeding success in this species. Some of our UK collaborators discovered that *Perkinsia* organisms that are regarded an an emerging infections disease associated with amphibian declines elsewhere in the world are widespread in Panamanian tadpoles.

2021 Research Publications involving collaborations with PARC

Becker, M.H., Brophy, J.A.N., Barrett, K, Bronikowski, E., **Evans, M**., Glassey, E., **Klocke, B. Lassiter, E.,** Meyer, A.J., Kaganer, A.W., Muletz-Wolz, C.R., Fleischer, R.C., Voigt, C.A. and **Gratwicke, B.** 2021. Genetically modifying skin microbe to produce violacein and augmenting microbiome did not defend Panamanian golden frogs from disease. ISME Communications, 1, p.57.

Higgins, K., Guerrel, J., Lassiter, E., Mooers, A., Palen, W.J. and **Ibáñez, R.** 2021. Observations on spindly leg syndrome in a captive population of *Andinobates geminisae*. Zoo Biology, 40(4), pp.330-341.

Smilansky, V., Jirků, M., Milner, D.S., **Ibáñez, R., Gratwicke, B.,** Nicholls, A., Lukeš, J., Chambouvet, A. and Richards, T.A. 2021. Expanded host and geographic range of tadpole associations with the Severe Perkinsea Infection group. Biology Letters, 17(6), p.20210166.

Byrne, A.Q., Richards-Zawacki, C.L., Voyles, J., Bi, K., **Ibáñez, R**. and Rosenblum, E.B. 2021. Whole exome sequencing identifies the potential for genetic rescue in iconic and critically endangered Panamanian harlequin frogs. Global Change Biology, 27(1), pp.50-70.

Gratwicke B. 2021. Context matters in the fight to save frogs. In Meshaka, W. (ed.) *Exotic herpetofauna of United States*. The John Hopkins University Press.

Rodriguez, C., **Ibáñez, R.,** Mojica, L., Ng, M., Spadafora, C., Durant-Archibold, A.A. and Gutiérrez, M. 2021. Bufadienolides from the skin secretions of the Neotropical toad *Rhinella alata* (Anura: Bufonidae): antiprotozoal activity against *Trypanosoma cruzi.* Molecules, 26(14), p.4217.

Gray, H.M., Green, D.M. and **Ibáñez, R.** 2021. Diurnal calling in a nocturnal frog: exceptional calling activity of túngara frogs (*Engystomops pustulosus*) on the Panamanian Island of Taboga. Herpetologica, 77(3), pp.227-231.

Gastrotheca cornuta, the horned marsupial frog

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

We collaborated with Dr. Daniel Medina to evaluate different mesocosm designs for future release trials, we have found mesocosms to be an effective tool for successfully recapturing frogs, and limiting rapid dispersal from the release site with soft releases. We are also working with a research fellow Allie Byrne and SCBI geneticist Carly Muletz-Wolz on an idea to genetically rescue *Atelopus varius.* This project is primarily funded by the Revive and Restore Foundation to answer the question can we genetically rescue *Atelopus varius*? The observations that this hypothesis is based on were made by Allie Byrne et al. on post-epidemic surviving *Atelopus varius* populations. This project involves 2 steps, the first is comparing the genes of frogs before they were impacted by the amphibian chytrid fungus with those that survived the epidemic to understand the potential role of genes in predicting resistance. We collected samples from museum specimens to compare with modern frog DNA and we are now analyzing that data. Secondly, we are crossing *Atelopus varius* collected from different localities to see if the hybrid crosses have better Bd resistance traits or "hybrid vigor" we will compare survivorship of offspring and evaluate Bd susceptibility of the different groups of offspring.

From our previous release trials we learned that mesocosms are useful tools to recapture frogs, exclude predators, limit post-release dispersal and improve post-release survivorship. We have begun studies of different mesocosm designs to optimize future release trials.

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

Covid-19 has forced our primary exhibition area at the Punta Culebra Nature center to close, and to scale back golden frog day celebrations to a series of online talks. We used the closure opportunity to remodel a former gift shop area and expand the amphibian exhibition area at the Punta Culebra Nature center which reopened in 2022. At our site in Gamboa we repaired the main mural which had degraded due to weather. We continue our online presence with 11,300 followers on facebook, 6,800 followers on Instagram and 5,400 followers on twitter and our website www.amphibianrescue.org received 40,000 unique users. In addition to our regular community engagement in Panama, we are collaborating with a new regional initiative called the Atelopus Survival Initiative and helped develop a global action plan to save harlequin frogs in all 11 countries where they are found in nature https://www.atelopus.org

Our primary exhibition space in Panama is at the Punta Culebra Nature Center, (top) while passers by can peek inside a working amphibian rescue pod at our facility in Gamboa (bottom). Both exhibitions were closed during COVID 19, but have now re-opened.

Goal 6: Ensure the financial sustainability of the project.

We gratefully acknowledge the financial support from the following primary partners, corporations, foundations and societies who have supported this work. Note these figures reflect expenditures in calendar year 2021 by funding source, not contributions.

Funding Source	Purpose	2021 Expenses (US$)
SCBI and donors	Coordinator salary, supplies, fellows, travel, insectarium construction.	292,149
First Quantum Minerals (Cobre Panama)	Salaries, operating costs, swab analysis	315,205
The Woodtiger Fund	Research and program support	45,000
Zoo New England	Salaries	13,288
Holtzman Foundation	Insectarium construction	26,633
Cheyenne Mountain Zoo	Salaries	23,480
Revive and Restore	Research	27,143
National Geographic Society	Research	450
SENACYT	Research	29,016
STRI and donors*	Supplies	21,229
TOTAL		**793,593**

*These funds include direct project costs incurred in the calendar year 2021, but do not reflect unexpensed funds or in-kind institutional administrative support, utilities, fundraising, public affairs and programmatic support costs generously provided by the Smithsonian Tropical Research Institute who host this project.

Donors

In addition to the contributions from project partners, 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 and Restore, National Geographic Society, Baton Rouge Zoo, Society of the Transfiguration, Miles Mallory, John McCulley.

Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke
Project Director, Panama - Dr. Roberto Ibáñez
Facility Manager Gamboa – Jorge Guerrel.
Technical Staff - Amaranto Cabezón, Kenia Cabezón, Lanki Cheucarama, Nancy Fairchild, Orlando Garcés, Estefany Illueca, Tina Mejía, Jennifer Warren
Graduate Students - Blake Klocke, Alyssa Wetterau, Phil Jervis.
Post Doctoral Fellows - Luke Linhoff, Allie Byrne.

Steering Committee

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

Implementation Committee

Nicole Chaney *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 & Bryan Windmiller *Zoo New England.*

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