2024 Impact 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: *Oophaga vicentei* Vicente's Poison Dart Frog is an Endangered species that has a very restricted distribution and is bred in our captive assurance colony. It was presumed to have disappeared from the area around El Valle according to the last IUCN Redlist Assessment but was rediscovered at Cerro Gaital National Monument with the help of passive acoustic monitoring. This individual frog was discovered at Bajo Bonito.



Time for a refresh! Pod 7 has a viewing window and was the original pod that we began this project with in 2009, after 15 years the old racks and tanks needed replacing. Here Nate Weisenbeck is working to create a new setup that will house all PARC species and will provide a single exhibition pod that visitors will be able to access during special tours.

Goal 1: Grow and sustain captive populations of priority amphibians

TARI initiative

Thanks to a pivotal contribution from the Bezos Earth Fund, we developed a 5-year, \$2 M reintroduction plan in partnership with the Amphibian Survival Alliance and partners in Venezuela, Ecuador and Colombia. This funding will enable partners to enhance collaborations, increase captive frog populations, conduct release trials to restore wild populations, and assist conservation planning and education in some of the world's highest amphibian biodiversity landscapes.

Breeding successes

We successfully bred all harlequin frog and dart frog species as well as crowned treefrogs (*Triprion spinosus*) and lemur leaf frogs (*Agalychnis lemur*) this year. Notably, we successfully bred a Limosa harlequin frog (*Atelopus limosus*) founder collected from a surviving Bd decimated population. This could be useful for our selective breeding research if this animal has developed a resistance to Bd. Ultimately, we will need to rapidly grow this population of frogs in order to test this hypotheses. We have not been successful at breeding *Craugastor evanesco* or *Gastrotheca cornuta*. Plans are being made to attempt cryopreservation of cell line cultures next year.

Table 1: 2024 Population management report card for the priority conservationspecies maintained by the Panama Amphibian Rescue and ConservationProject.

	2024	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)	Pairs bred to F5 (Goal = 10)	Total number of frogs in collection (Goal = 300)
Andinobates geminisae		25	16	24	3	0	0	177
Oophaga vicentei		51	23	10	1	0	0	136
Triprion spinosa		19	11	8	6	2	1	72
Agalychnis lemur	1	14	5	6	3	0	0	297
Atelopus varius (lowland)		25	13	12	1	0	0	500
Atelopus certus		11	2	10	3	0	0	225
Atelopus limosus		16	3	11	0	0	0	126
Atelopus glyphus		12	6	5	0	0	0	123
Atelopus zeteki	1	3	1	5	2	0	0	169



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

Variable harlequin frog *Atelopus varius* produced from invitro fertilization

Improving genetic diversity of golden frogs in Panama

This year we held meetings with the executive leadership of the Maryland Zoo—owners of the Panamanian golden frogs in the USA—where they agreed to repatriate some frogs to increase the genetic holdings we have in Panama, which are currently descended from just three individuals.

New veterinary collaborations

We have initiated a collaboration with the veterinary departments from the Maryland Zoo, the National Zoo, Zoo New England and a medicinal chemist at the University of Michigan to conduct a veterinary research project. The goal is to improve frog health, increase the effectiveness, and reduce treatment times and doses of itraconazole by studying its pharmacokinetics and optimizing treatment protocols.

Meanwhile, we have improved harlequin frog and dart frog survivorship following pathology reports from the National Zoo Pathology Department that identified vitamin A deficiency as a likely cause of the failure to thrive issues. These issues have been partly resolved by increasing vitamin A gut loading. In collaboration with Tim Cernak at the University of Michigan, we have developed a water-miscible vitamin A solution that is now used to treat deficient frogs.



Goal 2: Restore wild populations of priority species

Plan and execute new release trials

In 2025, we will begin amphibian release trials in the Altos de Campana National Park in Panama to explore novel mechanisms that allow animal populations impacted by disease-related declines to recover in disease positive environments. With funding from the Panamanian government (SENACYT), we will conduct golden frog tadpole release trials in the park and conduct preliminary observations on *Agalychnis lemur* and *Triprion spinosus*. Additionally, we will be investigating a translocation strategy to recover a relatively common dart frog species that vanished from the park following Bd declines.

Understanding the host immune responses to the chytrid fungi

We conducted work to determine if pre-exposure and clearance of Bd is a viable option for improving survival of highly susceptible *Atelopus* frogs supported by Revive & Restore. The results showed that Bd exposure was ineffective at reducing Bd loads or increasing survival. In fact, prior exposure impaired immune function and worsened disease outcomes, and altered the skin microbiome of pre-exposed animals. Two follow up experiments were initiated with our partners as part of an NSF BBSRC grant to better understand the immune mechanisms associated with these observations. One study investigates *Atelopus* gene expression of host immune responses at different stages of infection, while the second study uses immune cell promotors to manipulate the host immune response to Bd with the aim to understand the role of various immune cells in disease outcomes and host microbial interface.



Surprisingly, our experimental work showed that pre-exposure of *Atelopus glyphus* worsened disease outcomes upon re-exposure. Researchers have found that in other species, pre-exposure can lead to a vaccination effect beneficial to the frog.

Acoustic monitoring for lost frogs

To detect amphibians that are rare or even presumed to be extinct, we deployed audiomoths at three sites with six stations in Panama, when many species breed and call. The sites were selected for their high biodiversity and well-known localities for several rare and possibly extinct amphibians. Audiomoths were placed in trees at breast height and set to record one min every 10 mins for a total of 144 recordings per day. About 100,000 recordings were collected at the 18 stations. We used pattern-matching to identify frog calls of species of conservation concern as well as all dart frogs present. Later the recordings were uploaded to arbimon.org for further analysis and annotation.

From the recordings, we discovered *Ecnomiohyla veraguensis*, *Triprion spinosus*, *Oophaga vicentei* and *Silverstoneia nubicola* that were thought to have disappeared from these sites. We also documented the recovery of *Colostethus pratti* at one of the sites following Bd declines, while the species disappeared from a second site, where reintroductions are planned.



Sonograms of several target monitoring species in our passive acoustic monitoring project.

Climate modelling to determine Bd refuges

Dr. Carrie Lewis at George Mason University collaborated with various groups monitoring chytrid in Panama to compile a nationwide database of more than 4,000 chytrid swabs and combined with high-resolution weather data for the country from 2005 to 2018. She has produced a seasonal chytrid prevalence map for the whole country that resembles a weather map, predicting Bd prevalence. The total number of suitable seasons reflect potential climatic refuges for frogs from the chytrid fungus.

Goal 3: Foster an appreciation of amphibians.

Highlight the contributions of scientific work and how it positively impacts communities

We held a premier for the PBS episode of Wild Hope – Frog Ark at the National Zoo on amphibian day (May 7) this year for about 300 attendees, including the director of the USFWS. It has been viewed more than a million times on YouTube, making it one of the most popular episodes in this series. Click this link to see the overwhelmingly positive public comments on the video. <u>https://www.youtube.com/watch?v=WXK6fTluM48&ab_channel=NatureonPBS</u>

Our project was also featured on <u>CNN Espanol</u> with a five minute piece about our amphibian center <u>"La carrera por recuperar especies de ranas consideradas extintas en Panamá"</u>

We have an active social media presence with 11,000 Facebook followers 7,000 Instagram followers and 1,000 followers on Threads.

We produced a 5th edition of frog posters (3,000) to give away to kids and teachers as part of our education and outreach work.



save these rare and cherished species. ...more

Scientific publications from our amphibian conservation program in 2024

Bletz, M.C., Gratwicke, B., Belasen, A.M., Catenazzi, A., Duffus A.L.J., Lampo, M., Olson, D., Toledo, F., and Vasudevan, K. 2024. Chapter 6: Infectious diseases: Advances and challenges in addressing emerging pathogens, pp. 148-176. In: Wren, S. and Angulo, A. (eds.), The IUCN Global Amphibian Conservation Action Plan. IUCN SSC Amphibian Specialist Group. Gland, Switzerland. https://portals.iucn.org/library/node/51531

Cossio, R., Ibáñez, R., Rodríguez, A., and Pröhl, H. 2024. Do sexes differ in consistent individual differences and behavioural syndrome in an aposematic poison frog? Journal of Zoology 323:139-149. https://doi.org/10.1111/jzo.13164

Gratwicke, B., Baitchman, E.J., Evans, M., Guerrel, J., Ibáñez, R., Klaphake, E., Riger, P., Ross, H., and Wilson, B. 2024. Chapter: Building an amphibian ark to avoid disease-related extinctions in Panama, 331-338 pp. In: Walls, S.C. and O'Donnell, K.M. (eds.), Strategies for Conservation Success in Herpetology. Herpetological Conservation 4, Society for the Study of Amphibians and Reptiles, USA.

Gratwicke, B., and Savage, A. 2024. Mini saunas save endangered frogs from fungal disease. Nature 631:277-278.

Gray, H.M., Ibáñez, R., Barrios, H., and Potvin, C. 2024. Caught on camera: Ocelot (*Leopardus pardalis*) predation on foam nests of Savage's Thin-toed Frog, Leptodactylus savagei Heyer, 2005. Herpetology Notes 17:211-215.

Gray, H.M., Ibáñez, R., Barrios, H., Potvin, C., Olea, D., Casamá, A. and Salazar, I. 2024. Estudio de las ranas y sapos de las Tierras Colectivas del Río Balsas, Darién, Panamá. Tecnociencia 26:22-38.

Klocke, B., Garcés, O., Lassiter, E., Guerrel, J., Hertz, A., Illueca, E., Klaphake, E., Linhoff, L., Minbiole, K., Ross, H., Tasca, J.A., Woodhams, D., Gratwicke, B., and Ibáñez, R. 2023. Release trial of captive-bred variable harlequin frogs *Atelopus varius* shows that frogs disperse rapidly, are difficult to recapture and do not readily regain skin toxicity. Oryx 58:323-335. https://doi.org/10.1017/S0030605323001254

Lötters, S., et al. 2023. Ongoing harlequin toad declines suggest the amphibian extinction crisis I still an emergency. Communications Earth and Environment 4: 412. https://www.nature.com/articles/s43247-023-01069-w

Luedtke, et. al. 2023. Ongoing declines for the world's amphibians in the face of emerging threats. Nature 622:308-314. https://doi.org/10.1038/s41586-023-06578-4

Mantzana-Oikonomaki, V., Rodríguez, A., Castillo-Tamayo, G., Ibáñez, R., and Pröhl, H. Predator perception of aposematic and cryptic color morphs in two Oophaga species. Ecology and Evolution 14:70351. https://doi.org/10.1002/ece3.70351

Mulder, K.P., Savage, A.E., Gratwicke, B., Longcore, J.E., Bronikowski, E., Evans, M., Longo, A.V., Kurata, N.P., Walsh, T., Pasmans, F., McInerney, N., Murray, S., Martel, A., and Fleischer, R.C. 2024. Sequence capture identifies fastidious chytrid fungi directly from host tissue. Fungal Genetics and Biology 170:103858. https://doi.org/10.1016/j.fgb.2023.103858

Touchon, J.C., McMillan, W.O., Ibáñez, R., and Lessios, H.A. 2024. Flexible oviposition behavior enabled the evolution of terrestrial reproduction. Proceedings of the National Academy of Sciences 121:2312371121. https://doi.org/10.1073/pnas.2312371121

Expenses for Calendar Year 2024

Funding Source	Purpose	Expenses (US\$)
SCBI and donors	Salaries, supplies, fellows, travel.	259,749
Adrienne Arsht Community-Based Resilience Solutions Initiative	Salaries, operating costs.	22,769
The Woodtiger Fund	Research and program support	45,000
Zoo New England & Cheyenne Mountain Zoo	Salaries	37,466
National Science Foundation	Research	64,380
Revive & Restore	Research	54,108
SENACYT	Research	27,971
STRI and donors*	Supplies	4,140
TOTAL		720,641

*These funds include direct project costs incurred in the calendar year 2024, but do not reflect unexpended 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

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The Limosa harlequin frog *Atelopus limosus* from central Panama. We now have individuals collected pre- and post- declines that can be bred to help us test for evolved resistance.

Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke. Project Director, Panama - Dr. Roberto Ibáñez. Facility Manager Gamboa – Jorge Guerrel. Technical Staff - Igli Arcia, Amaranto Cabezón, Kenia Cabezón, Nancy Fairchild, Julio Artuz, Orlando Garcés, Estefany Illueca, Jennifer Warren, Endy Araúz, Erick Barría, Rafael Lorenzo, Sharon Mc Lean. Graduate Students - Carrie Lewis. Post Doctoral Fellows - Phil Jervis, Gonçalo M. Rosa, Joe Madison. Research Associates – Dr. Gina Della Togna.

Steering Committee

Dave Ruhl, Executive Vice President *Cheyenne Mountain Zoo*; Dr. Brandie Smith, John and Adrienne Mars Director 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*.

2024 Volunteers

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