2019 Annual Report

Panama Amphibian Rescue and Conservation Project

A project partnership between: Cheyenne Mountain Zoo, Houston Zoo, Smithsonian's National Zoo, 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.

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

We continued to make progress on increasing the total available frog space in Gamboa. With help from the Cheyenne Mountain Zoo, Zoo New England and numerous volunteers, we outfitted two pods to make new space for frogs and began construction on our new insect production facility. The recently outfitted containers bring us to a total of 7 completely outfitted frog pods increasing our total frog holding space to 2,800 square feet.

In 2019, upon the expiration of an agreement with the Nispero Zoo we closed our second facility in El Valle de Anton (known as EVACC). We moved a portion of the living collection of animals that ensured continued genetic representation from most lineages of animals to our expanded facilities in Gamboa. We also transferred a portion of the living animal collection and equipment to the newly formed, independent EVACC Foundation who continue to operate independently in El Valle de Anton.

New cricket room under construction as part of the insect production facility. This 1,660 sq ft facility improvement will include dedicated space for fruitflies, crickets and storage.

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

We continued our work on maintaining priority species in 2019 (Table 1) and have more than 1,600 adult frogs in our captive collection. Management highlights for 2019 include conducting a ZIMS inventory to ensure that our records are up to date.

Table 1: 2019 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 spinosa	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

In the coming year, captive management priorities will be to breed any unrepresented founders and to start working on F2 generations of frogs. We will need to conduct population management training for our staff, now that we are getting into trickier F2 and F3 pairings.

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

Dr. Luke Linhoff soaking a frog to understand the anti-Bd properties of the skin mucus.

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

We completed a project with support from the Morris Animal Foundation investigating spindly leg syndrome and the role of calcium and phosphate in tap water. The experiment, led by Elliott Lassiter and Orlando Garces found clear evidence linking spindly leg syndrome to calcium/phosphate deficiencies in the water used to rear tadpoles. These findings have implications for solving a prevalent animal welfare concern in captive amphibians, and we have submitted the findings to a peer-reviewed journal for publication.

We began work investigating the comparative susceptibility of species in our captive collection using a non-lethal mucusome approach. The project led by Dr. Luke Linhoff is funded by the National Geographic Society and the Smithsonian scholarly studies and is collaborating with Doug Woodham's lab. It involves taking a skin mucus sample from the frog and exposing that to the chytrid fungus in a test tube. A dye that selectively stains live zoospores helps us to quantify the inhibitory activity of the frog skin mucus. In addition to ranking the Bd susceptibility of species in our existing amphibian collection, we hope to identify species that have a wide range of susceptibility, that could allow us to work on selective breeding for resistance traits in that species.

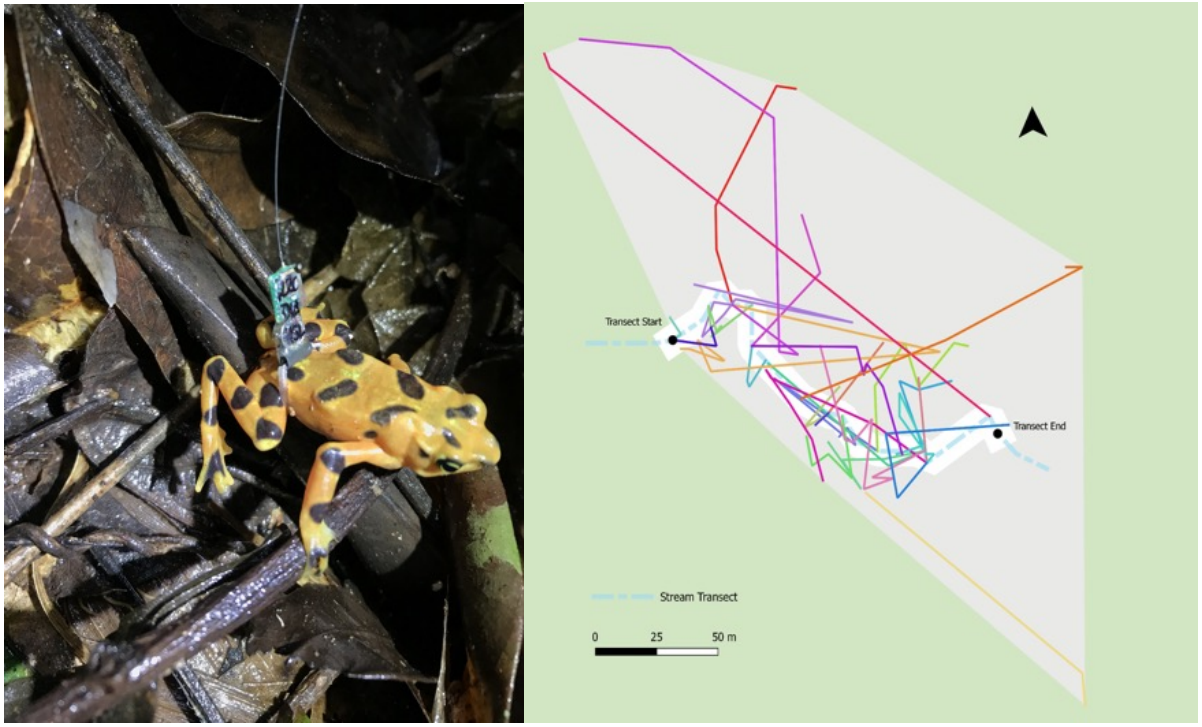
Dr. Gina DellaTogna from the InterAmerican University in Panama leads our assisted reproduction research program. Dr. DellaTogna used hormone treatments that have successfully resulted in offspring from five captive species. She has recruited two Panamanian students, Dionel Rodriguez who is focused on the differences in sperm quality between wild and captive *Atelopus limosus* and Yineska Otero who is focused on developing optimal hormone dosing methods to breed two – Rusty Robber frog (*Strabomantis bufoniformis*) and Vanishing Robber frog (*Craugastor evanesco*) that are very difficult species to breed in captivity.

2019 Research Publications involving collaborations with PARC

- Medina, D., **Ibáñez, R.**, Lips, K.R., and Crawford, A.J. 2019. Amphibian diversity in Serranía de Majé, an isolated mountain range in eastern Panamá. ZooKeys 859:117-130.
- Browne, R.K., Silla, A.J., Upton, R., **Della-Togna, G.**, Marcec-Greaves, R., Shishova, N.V., Uteshev, V.K., Proaño, B., Pérez, O.D., Mansour, N., and Kaurova, S.A. 2019. Sperm collection and storage for the sustainable management of amphibian biodiversity. Theriogenology 133:187-200.
- Lewis, C.H.R., Richards-Zawacki, C.L., **Ibáñez, R.**, Luedtke, J., Voyles, J., Houser, P., and **Gratwicke, B.** 2019. Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs. Biological Conservation 236:180–187.
- Scheele, B.C., Pasmans, F., Skerratt, L.F., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M.C., Flechas, S. V, Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., **Gratwicke, B.**, Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips, K.R., Longo, A.V, Maneyro, R., McDonald, C.A., Mendelson, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.-O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R., and Canessa, S. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459-1463.
- Mendoza, A.M., Bolívar-García, W., Vázquez-Domínguez, E., **Ibáñez, R.,** Parra Olea, G., 2019. The role of Central American barriers in shaping the evolutionary history of the northernmost glassfrog, *Hyalinobatrachium fleischmanni* (Anura: Centrolenidae). PeerJ 7:e6115.
- Martin H., C., **Ibáñez, R.,** Nothias, L.F., Reinert, L.K., Rollins-Smith, L.A., Dorrestein, P.C., and Gutiérrez, M. 2019. Viscosin-like lipopeptides from frog skin bacteria inhibit *Aspergillus fumigatus* and *Batrachochytrium dendrobatidis* detected by imaging mass spectrometry and molecular networking. Scientific Reports 9:3019.

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

We did not conduct any release trials of frogs in 2019, but we did learn from release trials in 2018 that predation by snakes, spiders and whip-scorpions was a considerable source of mortality of the released animals. We also learned that captive *Atelopus* do not have detectable tetrodotoxins in their skin and that the frogs did not recover any detectable skin toxicity within the first 60 days following a release. As such we began working with Phillip Jervis of the Institute of Zoology (London) to better understand the origins of skin toxins in poison dart frogs and harlequin frogs. This year, we aim to conduct dietary supplementation trials to see if we can increase the skin toxicity of captive *Oophaga vicentei* and *Atelopus limosus* that may help to reduce predation mortaility among released animals.



Analysis of radiotracking data from *Atelopus varius* released in the Donoso area showed that frogs dispersed well beyond the re-survey area (white), explaining the low re-encounter rate of other non-radio-tracked animals in mark-recapture surveys. Data are being analyzed and prepared for publication.

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

We continued our online and exhibit-based offerings at the Punta Culebra Nature Center and the PARC facility in Gamboa. We expanded the existing exhibit at Punta Culebra with additional space, that includes several new priority rescue species on exhibit there, including the Panamanian golden frog. We helped to coordinate and organize events around this year's Golden Frog Festival. We had pro-bono assistance from the public relations company Stratego. The event was covered by 213 different news, TV, radio and online spots resulting in publicity valued at $30,000 with a public relations value of $125,000. Festivities began with open houses at the Punta Culebra Nature Center and the PARC project in Gamboa attended by about 300 people.

Nine independent English and Spanish news stories covering our project appeared in 2019 Our online constituency continued to grow modestly. We now have 5,200 twitter followers, 11,600 Facebook fans, 6,700 Instagram followers. Website visitors declined about 10% compared to last year with 56,000 unique visitors (about 11,000 of these were Spanish-speaking).

We collaborated with Global Wildlife Conservation to host a meeting of the '*Atelopus* Survival Initiative'. About 40 representatives from academia, zoos, non-profit and governments of *Atelopus* range countries met in Medellin Colombia. The goal was to discuss how to coordinate our amphibian conservation efforts across the range of *Atelopus* and develop a conservation plan for this imperiled genus which is in preparation.

"Fabulous Frogs!" Exhibit at the Punta Culebra nature center has been expanded.

Goal 6: Ensure the financial sustainability of the project.

We continued to raise funds to support the operational expenditures of this project. About 47% of funds was from project partners, 42% from corporate contributions, including a renewal of our agreement with First Quantum's Cobre Panama. 7% of our funding came from foundations and restricted grants.

Funding Source	Purpose	2019 Expenses (US$)
First Quantum Cobre Panama	Salaries, supplies, services	297,276
SCBI and donors	Salaries, travel, supplies, services, research, stipends, insectarium construction	246,817
Houston Zoo	Salaries, supplies, services	50,307
Morris Animal Foundation	Research, supplies, stipends, equipment	27,907
SENACYT	Research, supplies	19,367
Cheyenne Mountain Zoo	Salaries	19,292
WoodTiger Fund	Research, stipends, travel, supplies	18,306
Zoo New England	Salaries	16,275
BBVA	Services, utilities	4,630
National Geographic Society	Research	2,906
TOTAL		**703,083**

* These funds include direct project costs incurred in the calendar year 2019, 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 who have made additional contributions to the project directly: Susan and Frank Mars, Linda Mars, Anne Keiser and Doug Lapp, Holtzman Wildlife Foundation, Sey and Pearl Moskowitz, Stratego, The Shared Earth Foundation, The Anele Kolohe Foundation, Mohammed Bin Zayed Species Conservation Fund, Cervecería La Rana Dorada, Baton Rouge Zoo, and the EVIM Foundation.

2019 Online Contributions

Our sincere thanks to the following individuals who contributed $25 or more online: Beth Achenbach, Mike Baker, Thomas Bressler, Marilyn Finberg, Lawrence Hartman, Julie Krzykowski, Nicholas Lindner, Alexandra Naimoli, Martijn Oei, Eric Stubbs, Nicole Teo, Elizabeth Wade, Gregory and Alvera Wilson.

Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke
Project Director, Panama - Dr. Roberto Ibáñez
Facility Manager Gamboa – Jorge Guerrel.
Technical Staff - Lanki Cheucarama, Nancy Fairchild, Estefany Illueca, Jennifer Warren.
Interns – Amaranto Cabezón, Valeria Franco, Orlando Garcés, Kathleen Higgins, Elliott Lassiter, Alirio Membeche, Karina E. Zurique Mendoza, and Guadalupe Ureña, Nicole Vargas.
Graduate Students Blake Klocke, Alyssa Wetterau. *Post Doctoral Fellows* – Luke Linhoff.

Steering Committee

Bob Chastain, President and CEO *Cheyenne Mountain Zoo*;
Lee Ehmke, CEO, *Houston Zoo;*
Dr. Steve Monfort, Director *SCBI*
Dr. Matthew Larsen, Director *Smithsonian Tropical Research Institute*;
John Linehan, President and CEO *Zoo New England*.

Implementation Committee

Dr. Eric Klaphake & Dr. Liza Dadone *Cheyenne Mountain Zoo*; Peter Riger *Houston Zoo*; Dr. Brian Gratwicke *Smithsonian Conservation Biology Institute*, Matthew Evans *Smithsonian's National Zoological Park*; Dr. Roberto Ibáñez, Jorge Guerrel *Smithsonian Tropical Research Institute*; Dr. Eric Baitchman & Bryan Windmiller *Zoo New England*; Dr. Brad Wilson *Atlanta Botanical Gardens.*

2019 Volunteers

Ruth Aguirrebengoa, Viviane Ali, Tomas Arauz, Nicolette Ávila, Carlos Benedetti, Marek Bering, John Berkholtz, Eleodoro Bonilla, Torrey Brownell, Agustin Camano, Constanza Chaparro, Dennis Charlton, Caroline Feischl, Yimayri Figueroa, Pedro Garcia, Saúl González, Evelyn Gonzalez, Jane Harding, Sophie O'Hehir, Frederick Mckendrick, Patrick Miles, Carlos Morán, Suita Narváez, Jason Platt.

2019 Golden Frog Festival

Organizer: Jimena Pitty. Organizing Committee: Linette Dutari, Sonia Tejada, Beth King, Roberto Ibáñez, Jorge Alemán, Álvaro González, Ana Endara, Paulette Guardia, Lina González, Rebecca Rissanen, Leila Nilipour, Jenniffer Saucedo.

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