

2016 ANNUAL REPORT

PANAMA AMPHIBIAN RESCUE AND CONSERVATION PROJECT



A project partnership between: Cheyenne Mountain Zoo, Houston Zoo, Smithsonian's National Zoological Park, 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 and proceed to reintroduction trials.

GOAL 1: Create assurance colonies of Panama’s most vulnerable amphibian species

To date we acquired 20 pairs or bred 10 pairs, whichever ratio is greater, for 8 species and bred at least 10 founding pairs of 4 species (Table 1). Collectively, we are working with 12 species of conservation concern. This year we bred *Craugastor evanescens* in captivity for the first time and more than doubled the number of adult frogs in our collection.

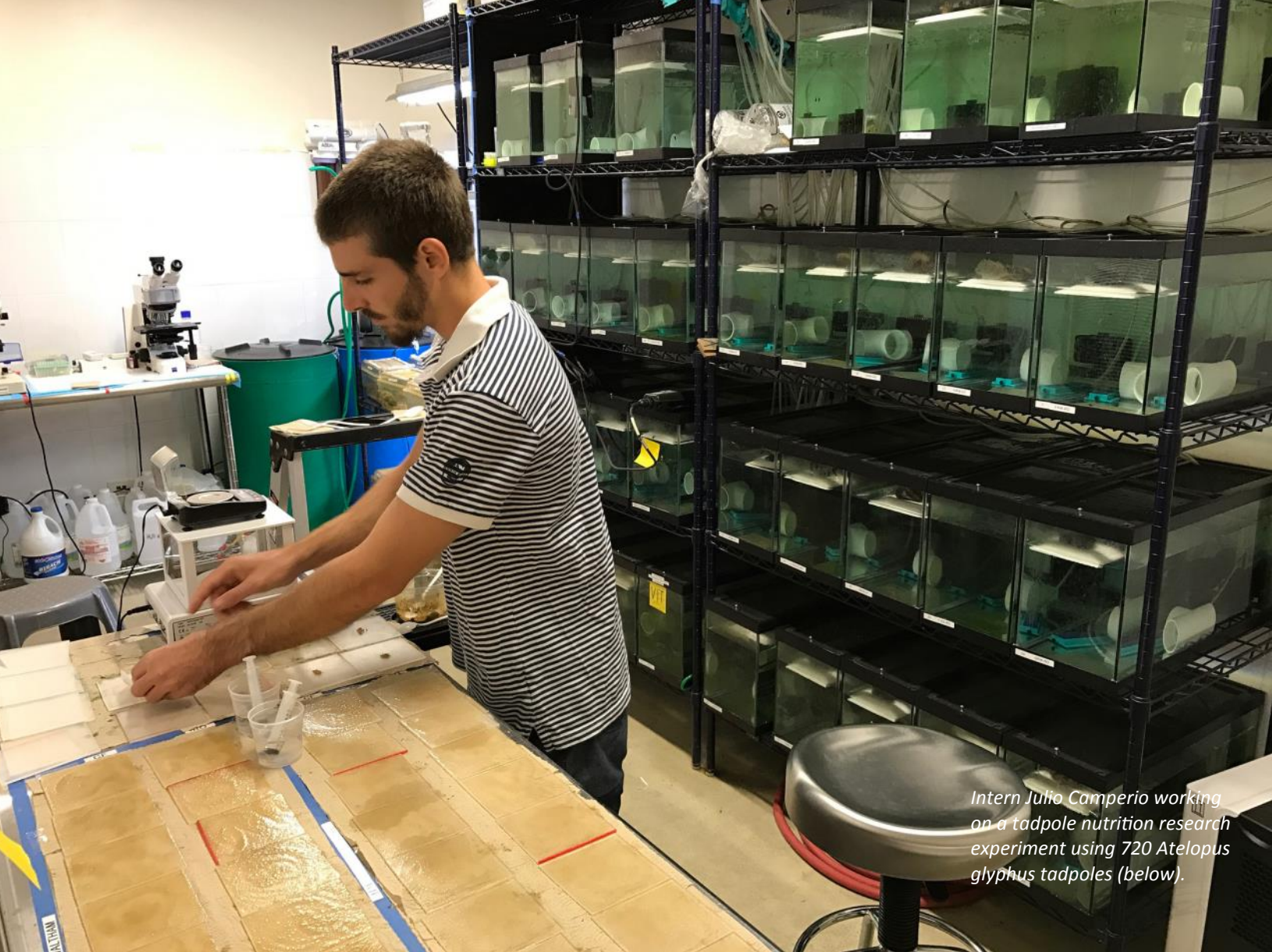
Table 1: Progress towards captive population management goals .

2016 REPORT	% min required founder population	Pairs bred to F 1 (goal 10 pairs bred)	Pairs Bred to F2	Number of frogs (goal 250-500)
<i>Atelopus certus</i>	1	12		144
<i>Gastrotheca cornuta</i>	1	13	6	158
<i>Atelopus limosus (chevron)</i>	1	13		82
<i>Atelopus glyphus</i>	1	9		52
<i>Atelopus varius (lowland)</i>	1	5		1101
<i>Andinobates geminisae</i>	1	6		27
<i>Oophaga vicentei</i>	1	1		49
<i>Craugastor evanescens</i>	1	1		44
<i>Anotheca spinosa</i>	0.5	7	8	160
<i>Atelopus varius (highland)</i>	0.4	4		426
<i>Strabomantis bufoniformis</i>	0.575	2		30
<i>Agalychnis lemur</i>	0.25	2		89
<i>Atelopus zeteki</i>	0.2	3	4	415
<i>Atelopus limosus (brown)</i>	0.2	2		30

Key
Goal attained /exceeded
75% of goal attained
50% of goal attained
25% of goal attained
Goal not met



The first ever captive-bred Craugastor evanescens (Vanishing Robber Frog) at the El Valle Amphibian Conservation Center



Intern Julio Camperio working on a tadpole nutrition research experiment using 720 *Atelopus glyphus* tadpoles (below).

Husbandry

We continued our tadpole nutrition research project to investigate factors affecting the development of tadpoles in conjunction with the Waltham Center for Pet Nutrition. We identified water composition as a potential factor affecting spindly leg development in tadpoles and will attempt to resolve the issue by switching our water supply for the Gamboa Amphibian Research and Conservation facility entirely to reverse-osmosis reconstituted water.

Expeditions

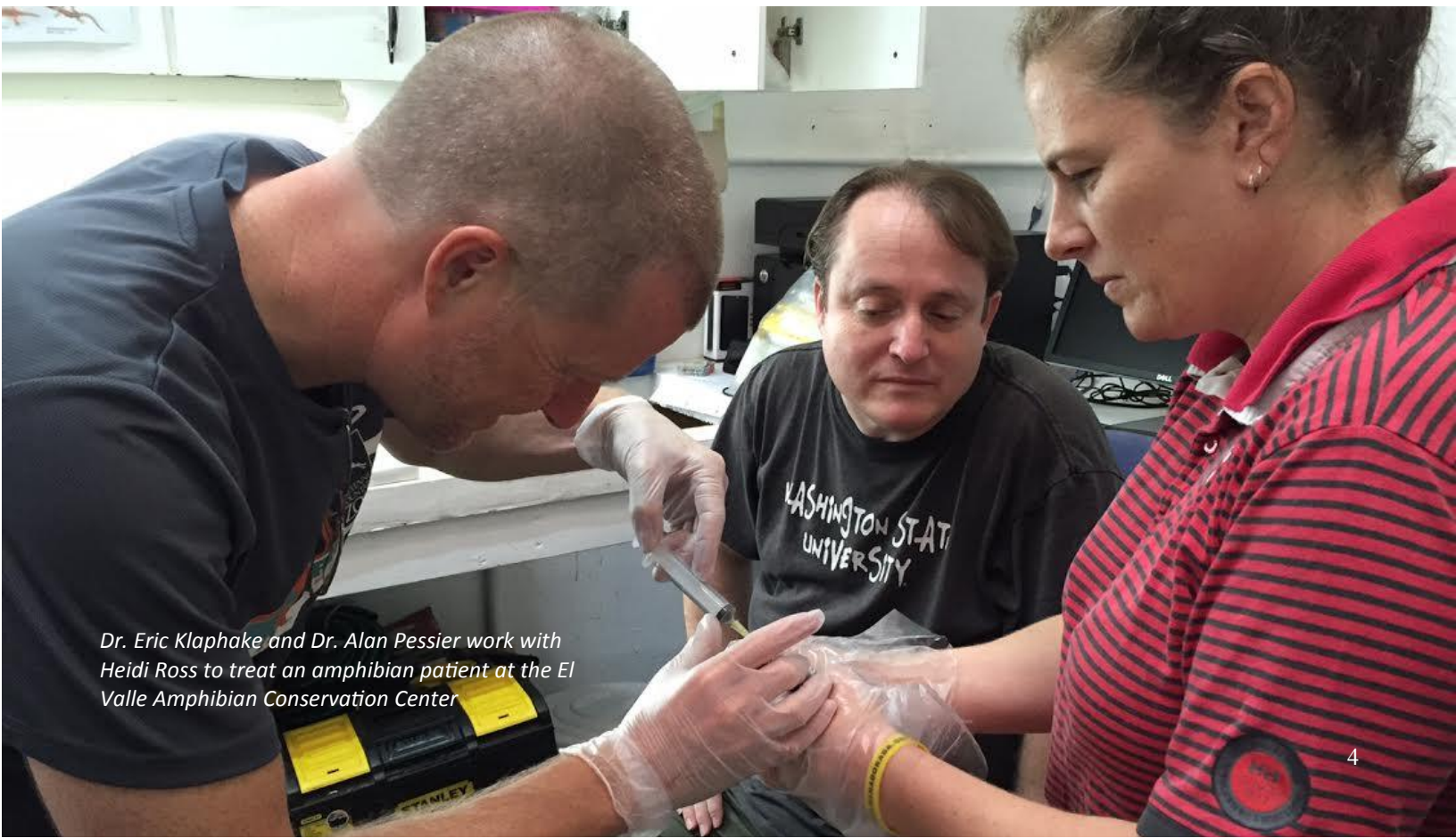
We coordinated eight field trips to continue collecting founding populations of the *Gastrotheca cornuta*, *Atelopus varius*, *Craugastor evanescens*, *Andinobates geminisae* and *Oophaga vicentei* located in the Minera Panama S.A. mining concession area. The field team also collects swabs from other amphibians present in the concession area to continue screening the area for the presence of the chytrid fungus.



Veterinary care

We continue to coordinate with the veterinary team from remote locations. Dr. Allan Pessier from Washington State University and Dr. Eric Klaphake from the Cheyenne Mountain Zoo worked to collect and prepare histological samples of polycystic kidney disease and spindly leg syndrome for detailed pathological examination. Both conditions appear to be associated with captivity and are persistent issues in the collection. Dr. Eric Klaphake conducted an orientation for two Panamanian wildlife veterinarians who are interested in helping our project, Drs. Stephanie Forero and Diorene Smith. They can act as on-call in country veterinarians for examining animals, and conducting emergency specialized treatments, this was very helpful as Dr. Forero successfully performed an emergency leg amputation in one injured collection animal. Intern Julio Camperio conducted an experiment using 720 *Atelopus glyphus* tadpoles in 36 tanks to identify the causes of the spindly leg syndrome in recent post-metamorph frogs. We found that the condition was greatly reduced in treatments where water was filtered using a reverse osmosis system and then reconstituted.

We have not had any further incidents of the *Fusarium* outbreak we dealt with in 2015 in quarantined animals. We did modify our quarantine husbandry protocols to only treat Bd infected frogs, sterilized misting water and began using false bottoms in quarantine tanks, but it is unclear whether any of these factors was responsible for solving the problem. One key question is whether the animals had the *Fusarium* infection in the field, or whether they contracted it in the quarantine room. We are continuing our attempts to develop a *Fusarium* primer that would allow us to screen swabs for *Fusarium* spores from existing swabs using qPCR.



Dr. Eric Klaphake and Dr. Alan Pessier work with Heidi Ross to treat an amphibian patient at the El Valle Amphibian Conservation Center



Dr. Gina Della Togna and Dr. Roberto Ibáñez work on stimulating unrepresented founder frogs to reproduce using hormone treatments, resulting in viable eggs and tadpoles (below).

Assisted reproduction and sperm cryopreservation

Dr. Gina DellaTogna visited the project as a post-doctoral fellow and in initial trials, she successfully stimulated oviposition in 1 pair of *Atelopus certus*, 2 pairs of *Atelopus glyphus* and 1 pair of *Atelopus limosus*. She also began collecting and cryopreserving sperm from *Atelopus certus*, *A. limosus*, *A. glyphus*, *A. varius* and *Strabomantis bufoniformis* starting the first living cryopreserved amphibian gamete collection in Panama. Dr. Della Togna has since returned to Panama and started her own research and teaching lab at the InterAmerican University, where she plans to continue collaborating with us on assisted reproduction trials and producing viable offspring using cryopreserved sperm.



GOAL 2: Re-establish healthy wild populations of Panamanian amphibians

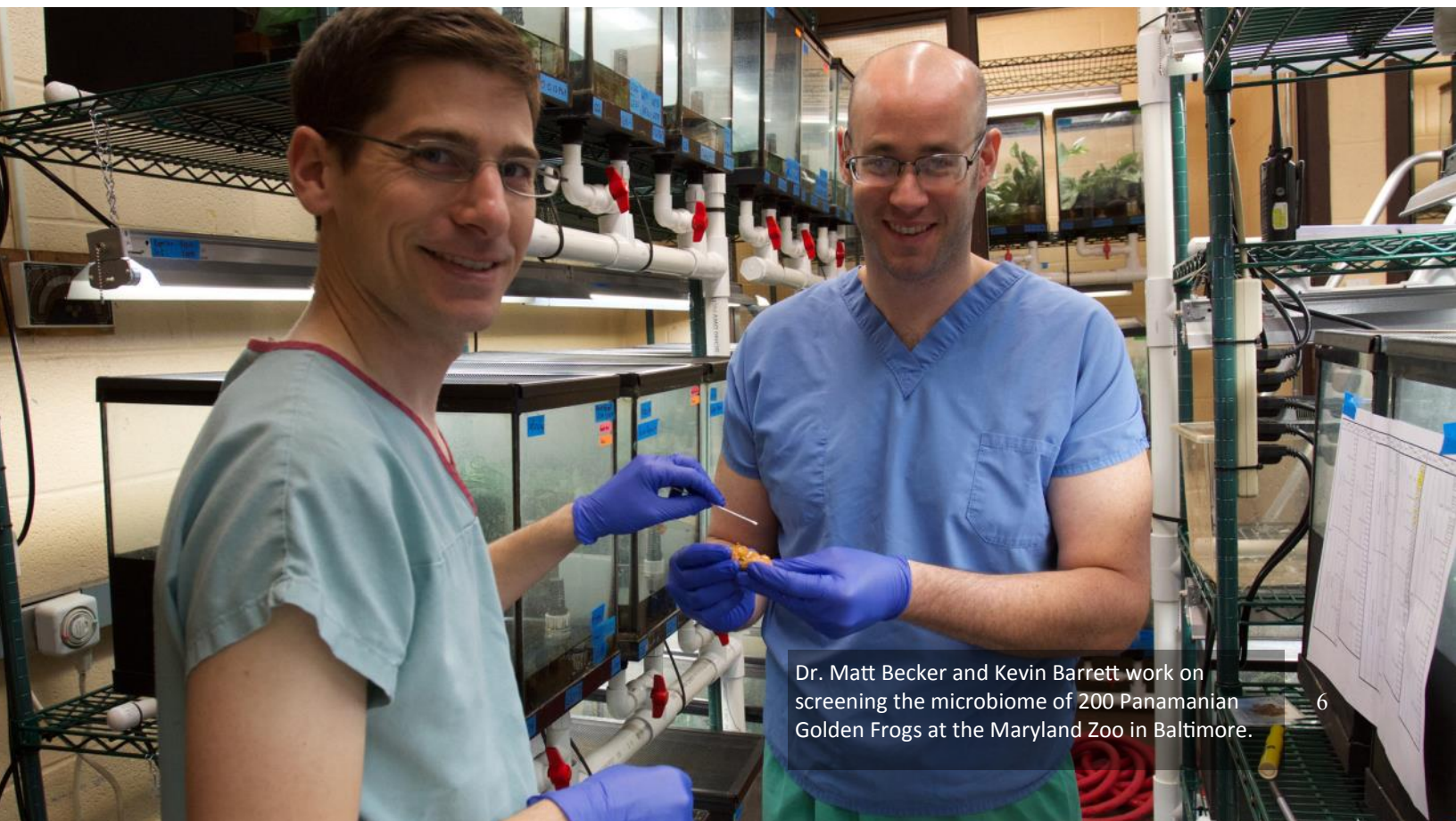
Developing tools to inform reintroductions in Bd-positive areas

Smithsonian Post-doctoral fellow Dr. Matthew Becker completed his cure experiment using surplus captive-bred golden frogs provided by the Maryland Zoo in Baltimore. He worked on 3 main approaches, the first to predict resistance based on a pre-existing microbiome, the second to promote resistance by applying a cocktail of anti-Bd probiotics that were isolated from captive golden frogs, and the third was to genetically modify a common core skin bacterium to produce antifungal compounds (this was done by collaborators in the Voight lab at MIT), and reapply it to frog skin. All frogs inoculated with Bd succumbed to the infection, so there was no apparent survival benefit, but we continue to analyze skin swab microbiomes and transcriptomes to understand the effects of each treatment on the microbiome.

We analyzed results of a separate study conducted by Smithsonian post-doctoral fellow Dr. Anna Savage examining differential disease outcomes in lowland leopard frogs that are moderately susceptible species to the chytrid fungus. We were hoping to identify over-expressed genes that might be associated with resistance, but were surprised to find that frogs that were more tolerant of Bd infections under-expressed most genes in the skin and spleen. We have submitted our findings for publication.

Reintroduction planning

One of the goals of our strategic planning meeting was to begin working on reintroduction trials. We recognize that the Bd remains a continued, unmitigated threat to reintroductions, but carefully planned reintroductions offer valuable learning opportunities to study both disease and non-disease-related causes of mortality in released frogs in a systematic way that will provide valuable information for future reintroductions. We also know that temperature is a strongly limiting factor for Bd, and we are collaborating with Carrie Lewis, a PhD student at George Mason University to try and identify potential climatic refuges to prioritize future release trials. Systematically collected climatic and survivorship data from initial releases can be used to improve the predictive power of the climate refuge models.



Dr. Matt Becker and Kevin Barrett work on screening the microbiome of 200 Panamanian Golden Frogs at the Maryland Zoo in Baltimore.

GOAL 3: Build capacity in Panama to safeguard the nation's amphibian fauna over the long-term

Strategic Planning

In April 5-7, 2016, the implementation team of the Panama Amphibian Rescue and Conservation Project and invited collaborators met at the Smithsonian Conservation Biology Institute in Front Royal, VA. During this workshop, we reviewed the achievements of the project under the 2010-2015 strategic plan and developed a new plan for 2016-2021, including goals around capacity building, population management, research, education and funding aspects of this project.

Training

Yeisson Muñoz, Nancy Fairchild, Rigoberto Diaz and Milagro González participated in the training course "Biology, Management and Conservation of Neotropical Salamanders" organized by Amphibian Ark and held at the Costa Rican Amphibian Research Center in Siquirres, Costa Rica, during March 2016. Heidi Ross was hosted by the Maryland Zoo in Baltimore in April 2016. As part of an ongoing collaboration, she toured their successful dedicated breeding facility and discussed golden frog management strategies and husbandry issues.

Facility evaluation and repairs and consolidation decision.

We obtained a professional evaluation of the EVACC infrastructure issues. After extensive consultation with our office of facilities and engineering, the implementation team and our project steering committee, we recommend consolidating the project's living amphibian collection at Gamboa. This consolidation will improve our ability to oversee the collection, protocols and staff, raise infrastructure standards, simplify veterinary supervision and streamline service costs including telecommunications, electricity, transport and record-keeping. A move of this magnitude will require a significant expansion of the Gamboa facility, and we have begun planning a new 1,600 sq ft insect production facility to make more space for the expanded amphibian collection.

New golden frog pod.

This year with funding from the US Fish and Wildlife Service we were able to order tanks and racks to outfit a new amphibian pod primarily intended for Panama's golden frogs *Atelopus varius* and *Atelopus zeteki*. Volunteer teams helped drill tanks, build false bottoms, make lids, hang lights and install misting and drainage systems. A second pod became empty after we were able to occupy the office and quarantine areas of the new building at Gamboa and will be outfitted in 2017 for poison dart frogs.



Jorge Guerrel, Dr. Roberto Ibáñez, Dr. Eric Baitchman, Dr. Alan Pessier, Heidi Ross, Matt Evans and Dr. Brad Wilson at the 2016 strategic planning meeting held at SCBI in Front Royal, VA.



GOAL 4: Cultivate and foster an appreciation for amphibians in the public mindset

We continued our online and exhibit-based offerings at the Punta Culebra Nature Center and the El Valle Amphibian Conservation Center and offered informal amphibian curriculum throughout the year.

Golden frog festival

We helped to coordinate and organize this year's golden frog festival using the following fliers for events. We had pro-bono assistance from the public relations company Stratego and the event was covered in 52 different news, TV, radio and online outlets resulting in publicity valued at \$18,000 and a public relations value of \$99,000. The Golden Frog Festival this year was marked by several major events throughout the month of August. In El Valle de Anton, the Ministry of the Environment led and organized the 2016 Golden Frog Day Parade and we also organized a family day at Paseo El Valle that drew 850 participants. Caminando Panama hosted the second annual La Dorada 5k/15k Trail Run that was sold out. Three hundred and fifty-eight runners / walkers participated, increasing the number of participants from the previous year by one third. In Panama City we hosted a frog-themed talk by Angie Estrada at the newly opened BioMuseo, an open house at the Gamboa Amphibian Rescue and Conservation Center, and the Punta Culebra Nature Center. These events were collectively attended by 475 people. This year we were fortunate enough to have a booth at the book fair donated to us at the Atlapa Convention Center our frog-themed booth drew 35,000 people.

Golden Frog matching gift campaign

We forged a generous corporate sponsorship from Golden Frog, an internet privacy company based in Texas. Golden Frog Co-CEOs Ron and Carolyn Yokubaitis were Peace Corps volunteers in Brazil during the late 1960s. While travelling through Central America after their service they stopped in Panama, where they visited the Golden Frog Coffee Bar and named their company after it. Golden Frog agreed to provide an outright gift of \$10,000 to the project and match up to \$20,000 raised in a two month long social media campaign leading up to golden frog day. The Smithsonian National Zoo public affairs department strategized a twitter, facebook social media campaign that told our story and solicited contributions from the general public. We were successful in raising \$21,800 in public donations and received the full matching pledge from Golden Frog. We are doubly grateful to Golden Frog because not only did they help us to raise significant funds for conservation, they facilitated telling our story to a wider Smithsonian audience.

Media

Our project was covered in 9 independent English and Spanish news stories in 2015, with more Spanish news articles than English ones this year. Our online constituency continued to grow steadily, we now have 4,600 twitter followers, 10,400 Facebook fans, 4,000 Instagram followers and 41,000 unique visitors to our bilingual website (about 8,400 Spanish visitors).



Newly outfitted golden frog pod in Gamboa



Golden frog baby boom at El Valle Amphibian Conservation Center



*Captive-bred *Atelopus limosus* scheduled for release trails in 2017*



Nancy Fairchild who oversees invertebrate production at the Gamboa Amphibian Research and Conservation Center



The Annual "La Dorada" Race hosted by Caminando Panama drew 350 participants.

FINANCIAL REPORT FOR CALENDAR YEAR 2016

Funding Source	Purpose	Amount (US \$)
<i>Houston Zoo and EVACC donors</i>	<i>Salaries and operating costs</i>	<i>61,500</i>
<i>Minera Panama</i>	<i>Salaries, operating costs, expedition, swab analysis</i>	<i>526,261</i>
<i>BBVA</i>	<i>Supplies, travel, meetings, education, internships</i>	<i>8,567</i>
<i>Zoo New England</i>	<i>Salaries</i>	<i>15,000</i>
<i>Cheyenne Mountain Zoo</i>	<i>Salaries</i>	<i>20,000</i>
<i>USFWS</i>	<i>Research for cure & golden frog pod</i>	<i>40,247</i>
<i>SCBI and donors</i>	<i>Coordinator salary, supplies, internships, fellows, travel</i>	<i>154,436</i>
<i>Golden Frog (Corporate sponsor)</i>	<i>Supplies, fellows</i>	<i>30,000</i>
<i>Wood Tiger Fund</i>	<i>Research into assisted reproduction</i>	<i>45,387</i>
<i>Friends of the National Zoo</i>	<i>Operating costs, strategic planning</i>	<i>3,455</i>
		904,853

*Captive-bred
Atelopus glyphus*

2016 Scientific Publications Related to the PARC Project or involving PARC staff collaboration.

Rodríguez-Brenes S, Rodríguez D, **Ibáñez R**, Ryan MJ. (2016) Spread of amphibian chytrid fungus across lowland populations of túngara frogs in Panamá. *PloS one*. 11(5):e0155745.

Pereyra MO, Baldo D, Blotto BL, Iglesias PP, Thomé MT, Haddad CF, Barrio-Amorós C, **Ibáñez R**, Faivovich J. (2016) Phylogenetic relationships of toads of the *Rhinella granulosa* group (Anura: Bufonidae): a molecular perspective with comments on hybridization and introgression. *Cladistics*. 32(1):36-53.

Rebollar EA, Hughey MC, Medina D, Harris RN, **Ibáñez R**, Belden LK. (2016) Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *The ISME journal*. 10: 1682-1695.

Rodríguez C, Rollins-Smith L, **Ibáñez R**, Durant-Archibold AA, Gutiérrez M. (2016) Toxins and pharmacologically active compounds from species of the family Bufonidae (Amphibia, Anura). *Journal of Ethnopharmacology*. 198:235–254.

Savage AE, Terrell KA, **Gratwicke B**, Mattheus NM, Augustine L, Fleischer RC. (2016) Reduced immune function predicts disease susceptibility in frogs infected with a deadly fungal pathogen. *Conservation Physiology*. 4(1):cow011.

Gratwicke B, Neff M, Mayer LR, **Ryan S**, Sevin J. (2016) Education and outreach. In Dodd, CK. *Reptile Ecology and Conservation: A Handbook of Techniques*. pp 436.



Andinobates geminiae

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 or via the Houston Zoo: Buffalo Zoo, David Castro, Dickerson Park Zoo, Fundación Eleta, Golden Frog, Lennox Company, Susan and Frank Mars, George and Mary Rabb Foundation, Seneca Park Zoo Society, Stratego, the Wood Tiger Foundation, The Shared Earth Foundation, The Anele Kolohe Foundation.

2016 Online Contributions

Our sincere thanks to the following individuals who contributed \$25 or more online: Michael Bafico, Pamela Baker-Masson, Thomas Baldwin, David Barker, Don Bright, Tracey Brown, Larissa Brunner, Laura Buck, Peter Butt, Julio Camperio, Julie Campoy, Steve Carter, V. Lea Cavaco, Anne Cinadr, Sharon Collins, Erin Dannecker, Keith Dennison, Alastair Dobbie, Charles Drum, Lynn Eddy, Chrissy Elick, Amy Ellis, Aaron Engstrom, Rolf Ent, Diana Evans, Kenneth Faulstich, Rich Goldsworth, Brian Gratwicke, Ryan Greenblatt, Reagan Haslam, Stephen Hazan Arnoff, Dirk Hobman, Tres Hofmeister, Celia Jansen, Randall Jenkins, Lallitha John, Izabella Jones, Michael Jordan, Brian Kanen, Claus Kjær, John Lapiana, Lisa Lotspeich, Catherine McCarthy, Isabella Mezzaroba, Lori Miller, Gaspar Modelo-Howard, Aila Morgan Cordingley, Devin Murphy, Anne Orth, Lindsay Renick Mayer, Tina Rhea, Julio Rodriguez, Edward Samuel, Jennifer Sevin, Diane Shaw, Pauline Sinnett, Eric Stubbs, Julia Thornton, Emily Tonks, Elizabeth Wade, Kathleen Wasselle Croft, Michael Weinberg, Gregory Wilson, Torsten Wolff.

Staff

Lead Scientist & International Coordinator - Dr. Brian Gratwicke
Project Director, Panama - Dr. Roberto Ibáñez

Gamboa Amphibian Research and Conservation Center Manager– Jorge Guerrel.
Technical Staff - Nair Cabezon, Lanki Cheucarama, Rigoberto Díaz, Nancy Fairchild, Estefany Illueca.
Interns – Ivette Herrera, Orlando Garcés, Jennifer Warren, Julio Camperio, Chelsea Morton.

El Valle Amphibian Conservation Center Director/ Manager – Heidi Ross.
Technical Staff – Milagro Gonzalez, Yeisson Muñoz. *Interns* – Vielka Ramirez, Yarineth Valdez, Madian Miranda, Diana Troetsch.

Steering Committee

Bob Chastain, President and CEO *Cheyenne Mountain Zoo*;
Lee Ehmke, CEO, *Houston Zoo*;
Dr. Steve Monfort, Director *Smithsonian Conservation Biology Institute*;
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 & Heidi Ross *Smithsonian Tropical Research Institute*; Dr. Eric Baitchman *Zoo New England*; Dr. Brad Wilson *Atlanta Botanical Gardens*.



Captive-bred Atelopus certus

2016 Volunteers

We are grateful to the following volunteers for their generous assistance: 30 members of the Eleta family (who helped paint EVACC!), Antonio Delgado Velayos, Jordi Maggi, Juan Antonio Licea, Mandy Wong, Joe Porter, Sara Hasenstab, Joli Stavish, Courtney Sproles, Claire Sundahl, Catherine Cussans, Oriana Edman, Erick Barria. Kristine Jepsen, Vicky Poole, Robert Hill, Edgardo Griffith, Bodhi Hostel and Lounge, Elliott Lassiter, Matt Neff, Kevin McCarthy, Tommy McCarthy and Phillippe Masson.

2016 Golden Frog Festival

Organizing Committee: Sharon Ryan, Roberto Ibáñez, Jorge Alemán, Nelly Florez, Crystal DiMiceli, Sonia Tejada, Jimena Pitty, Álvaro González, Rigoberto Díaz, Adrián Benedetti, Ana Matilde Ruiz, Ana Endara, Sean Mattson, Carlos Celis, Heidi Ross, Lanki Cheucarama.

Participating organizations: Ministerio de Ambiente de Panamá, Smithsonian Tropical Research Institute, Fundacion Smithsonian, El Valle Amphibian Conservation Center, Panama Amphibian Rescue and Conservation Project, SENACYT, US Fish and Wildlife Service, APRADAP, Stratego, North Face, Caminando Panamá.

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