2023 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: *Atelopus glyphus* the Pirre harlequin frog is a species that has experienced precipitous chytridiomycosis-related declines. In 2023, surplus individuals were used to investigate an exposure, clearance re-exposure vaccination protocol.

In January 2023, we convened project partners and research collaborators to develop a new strategic plan for the project. The new plan focuses around three primary goals of captive care, research and restoration and education. These goals and objectives will be used to structure this report.

Goal 1: Grow and sustain captive populations of priority amphibians

Sustaining healthy, genetically diverse captive populations of amphibians over the long term in captivity is the foundation of our program, and achieved through collaboration with our zoo partners and husbandry team. We have experienced improvements in our production and numbers of lemur leaf frogs *Agalychnis lemur* through prophylactic treatment of juveniles for worms. Crowned treefrogs *Triprion spinosus* were provided more naturalistic artificial tree holes for them to breed in, successfully increasing the number of reproducing pairs in the collection. Geminis' dartfrogs *Andinobates geminisae* are continuing to breed well in captivity and our numbers are increasing. We continue to produce offspring of *Oophaga vicentei*, but have not increased our overall holdings of this species.

Table 1: 2023 Population management report card for the 12 priority conservation species maintained by the Panama Amphibian Rescue and Conservation Project.

2023	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)
Agalychnis lemur	13	6	7	3	0	165
Andinobates geminisae	35	16	21	3	0	220
Atelopus certus	11	2	10	1	0	134
Atelopus glyphus	12	6	5	0	0	117
Atelopus limosus	15	3	10	0	0	200
Atelopus varius (lowland)	28	13	11	0	0	222
Atelopus zeteki	3	1	5	0	0	60
Craugastor evanesco	12	2	0	0	0	11
Gastrotheca cornuta	7	3	1	0	0	5
Oophaga vicentei	54	23	9	0	0	122
Triprion spinosus	16	5	10	5	2	54

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

Geminis' dart frog *Andinobates geminisae have successfully been bred for 3 generations in captivity*.

For the harlequin frogs, we are encountering a survival bottleneck at about 3 months of age, we have tried several alternate husbandry configurations and we are awaiting pathology reports for exported specimens. Nonetheless, we have increased our holdings of *Atelopus limosus* and *A. certus*, the apparent drop in *A. glyphus* numbers from last year reflects allocation of genetic surplus animals for vaccination trial research. *Atelopus varius* numbers have declined, but we have managed to produce some offspring to adulthood. We have not produced any *Atelopus zeteki* as we are experiencing delays in getting approvals from the AZA SSP in reimporting animals bred in the USA to improve the genetic stock in Panama.

We have not been successful at reproducing *Gastrotheca cornuta* or *Craugastor evanesco* in captivity, despite significant efforts. We did, however, publish our protocol for sperm collection from *Craugastor evanesco* and have begun banking sperm for this species, and we have successfully collected sperm from *Gastrotheca cornuta*.

This year we installed a remote temperature alarm system for our pods and the invertebrate production building. This hard-wired system will notify management via text and email any time that the temperatures in a pod drop below or exceed a permissible temperature range, and trigger a manual inspection by project staff or STRI security. This long-standing priority was a critical management recommendation following an air conditioner failure and high frog mortality experienced several years ago. We have also connected our -80°C freezer to the same system.

Train and engage staff to create a rewarding work environment

Our major news on this front is that Dr. Gina DellaTogna has been appointed as the executive director of the Amphibian Survival Alliance, a non-profit group affiliated with Re:Wild and Synchronicity Earth seeking to help build global capacity for amphibian conservation. Gina continues to live in Panama and is a research associate with the Smithsonian.

Jorge Guerrel, our Gamboa facility manager represented our project and presented on the Panama Amphibian Rescue and Conservation Project as part of an ex-situ session organized by Amphibian Ark at the 12th Latin American Congress of Herpetology (CLH), from October 30 to November 3, 2023, in Cochabamba, Bolivia. Sharing our progress with this key professional community provided a valuable networking and sharing opportunity with other Latin-American conservation groups working on amphibian conservation.

Brian Gratwicke presented our work on release trials at the Joint Meeting of Ichthyologists and Herpetologists in Norfolk VA this summer, and our work on disease mitigation at the Amphibian Microbial-Immune Interface Symposium in Washington DC and Annual Amphibian Diseases Meeting in Nashville Tennessee this fall.

Meeting of the Atelopus Survival Initiative at the 12th Latin American Congress of Herpetology (CLH), from October 30 to November 3, 2023, in Cochabamba, Bolivia

Improve availability of local veterinary care

We addressed a longstanding backlog of amphibian pathology cases in collaboration with the National Zoo Pathology team, who agreed to process prioritized cases for each species for us at no cost. We are awaiting pathology reports and have established a procedure for prioritizing and exporting specimens for examination. We hope this will help us to inform why we have been unable to rear large numbers of *Atelopus* juveniles to adulthood.

We facilitated the participation of two staff members Igli Arcia and Nancy Fairchild, who have an interest or expertise in veterinary care, and Dr. Diorenne Smith our veterinarian collaborator at the Summit Municipal Park to attend an Amphibian Ark Amphibian Veterinary training course in Argentina with 30 other participants from Latin America.

Ensure that animals thrive, not just survive

In consultation with amphibian care experts at the Cheyenne Mountain Zoo and the National Zoo we experimentally attempted to change our juvenile rearing setups to include more naturalistic setups with automated misting, and alternate false-bottom substrates to prevent any ammonia build up from juvenile enclosures. However, we did not find any improved survivorship from these modifications and have resumed our regular husbandry setups for rearing juveniles in critter keepers with paper towel changes three times per week and full cage changes every other week.

Goal 2: Restore wild populations of priority species

Plan and execute new release trials

We developed a 5-year $1M species conservation plan for *Atelopus limosus* and submitted it to the Indianapolis Zoo Saving species grant program. Unfortunately, the proposal was not selected for funding. Nevertheless, our proposal lays out a comprehensive strategy for working with the Mamoni Valley Preserve, a non-profit conservancy, to restore wild populations of this species, and we will continue to seek additional funding from other sources to implement elements of this comprehensive plan.

We are collaborating with Dr. Gonçalo Rosa and Dr. Phil Jervis at the Zoological Society of London, who are developing species conservation plan for *Agalychnis lemur* this involves captive breeding of surviving individuals that may have evolved resistance to the amphibian chytrid fungus and translocating the offspring to artificial ponds constructed at sites where the animals have been extirpated. We supported their field work last year and screening of skin peptides to understand the differences between captive populations created pre-Bd declines and recovering populations in Costa Rica and Panama.

Dr. Roberto Ibáñez and his student Kelsey Wilson from McGill conducted experimental research on Bd in *Atelopus* tadpoles to try and understand how Bd might be affecting *Atelopus* tadpoles in a multispecies system. We have identified releases of tadpoles as a potential area of investigation for future release trials, and understanding some of the disease challenges these animals will face is an area of active research.

We published the final two papers on our release trial work with *Atelopus* this year, in the journals Oryx and Frontiers in Amphibian and Reptile Science (see publications list below).

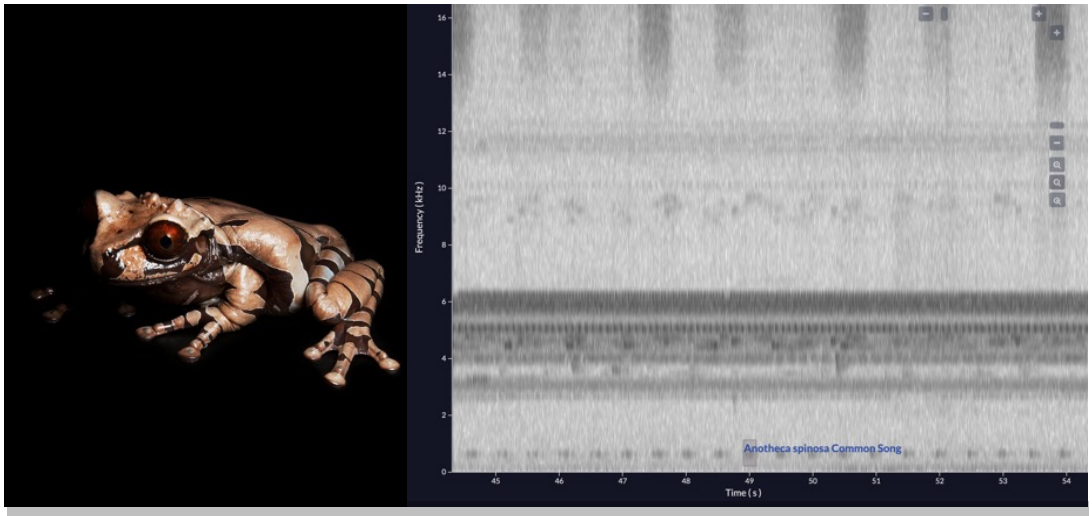
Broadly contribute to in situ amphibian scientific research and innovation that supports conservation objectives

With funding from Revive and Restore we attempted a genetic rescue project that involved *Atelopus varius* and *A. zeteki* however, we had trouble rearing the animals beyond 3 months of age and were unable to complete the experiment. We shifted our focus to conducting the first vaccination trial on *Atelopus* by exposing the animals to the chytrid fungus, clearing them using itraconazole and then re-exposing them. While this approach was successful in several other published studies using partially susceptible species. However, in the highly susceptible harlequin frogs the survival curves of vaccinated and unvaccinated animals were not statistically different. We are currently determining if the 'vaccinated' frogs elicited an adaptive immune response, which would indicate immune priming and the potential to modify this vaccination protocol to be more likely to be successful in reducing mortality in future trials.

Jorge Guerrel and Estefany Illueca collect mucusome samples from surplus-bred *Atelopus glyphus* used in our amphibian chytrid vaccine trial.

We deployed AudioMoth acoustic loggers at multiple sites in each of three general locations in an attempt to search for lost frogs such as Rabb's treefrog *Ecnomiohyla rabborum* that are thought to have disappeared as a result of the amphibian chytrid fungus. We recorded about 80,000 1-minute recordings and have begun analyzing them for calls. While we were unable to find the Rabb's treefrog during our surveys, we did find a frog that is the same genus at the Rabbs' frog – *Ecnomiohyla veraguensis*. This represents a large range extension for the species, and we reared the juvenile male specimen in captivity with the hope that we would be able to record its call for the first time to use in pattern-matching analyses for our artificial intelligence analysis of soundscapes. We did successfully get some calls from this animal and will be able to describe the call for the first time, it sounds very much like a barking dog. We were also able to identify the calls from our audiomoth recordings, providing a good proof of concept for using these types of analyses to monitor rare frogs.

We are still analyzing the data for other lost frog species, but we did pick up several potential calls of the Crowned treefrog *Triprion spinosus* that is considered to have drastically declined in Panama and was last heard in the wild in 2015. Our work on mucosomes of this species seems to indicate that they produce chytrid inhibitory compounds in their skin unlike Atelopus species, which would make them a great candidate for release trials and population supplementation work at the places they still occur.



Our pattern-matching analysis detected several calls of the crowned treefrog *Triprion spinosus* that persists in Mexico and Costa Rica, but was last heard in the wild in Panama in 2015.

We are wrapping up the analysis on two projects working to restore the natural skin defenses to poisonous frogs. The first project led by Dr. Luke Linhoff involved alkaloid feeding supplementation to both of our poison dart frog species over six months. The frog skin was stimulated with a mild electric current to release the contents of their granular glands and the alkaloid content of the resulting skin swab will be compared with wild frogs to understand whether this is likely to restore skin toxins to levels observed in wild frogs. In the second study, in collaboration with Zoological Society of London chemist Dr. Phil Jervis, we fed *Atelopus* frogs some moth larvae that had been injected with large doses of tetrodotoxin. We have finally gotten the samples exported to London and prepped for chemical analysis and should understand if we have been able to make these frogs 'spicy' again from the perspective of a predator. This was identified as an important conservation objective following the high mortality of released frogs and the failure of frogs to rapidly recover their skin toxicity following release into the wild.

Dr. Luke Linhoff wrapped up his postdoctoral fellowship with us this summer that was primarily focused on understanding whether skin secretions of different frogs could predict their susceptibility to disease. We did find that there was an apparent relationship between susceptibility and skin mucus inhibition. This may explain interspecies differences in susceptibility, and it does look like our lowland *Atelopus varius* population may be less susceptible to disease than other *Atelopus* species, which is a promising finding. Furthermore, the complete inhibition of Bd growth by skin secretions by *Triprion spinosus* is an important finding that suggests that release trials using this species may have a higher short-term probability of success, and will be the focus of our release efforts as we are able to breed numbers that are surplus to our captive management goals.

Conduct research on cryptic species, including genetics and taxonomy

In June 2022, a population of frogs with yellow spots resembling the polka dot poison frog (one of Panama's "Lost Frogs") was discovered in Veraguas, outside the known distribution of the polka dot frog. The researchers collaborating with our project director Dr. Roberto Ibáñez collected a few specimens and analyzed them genetically where they most closely matched Vicente's poison frog *Oophaga vicentei*. These dart frogs are known to be highly polymorphic, coming in slate gray, metallic blue, yellow or brick red with mottling. This yellow polka dot version, however, has now been confirmed to be Vicente's poison dart frog as opposed to strawberry (*Oophaga pumilio*) or polka dot (*Oophaga arborea*) poison dart frogs.

Goal 3: Foster an appreciation of amphibians.

We filmed a 15 minute documentary with a film crew from PBS for a series called "Wild Hope". The documentary will cover assisted reproduction and biobanking, the search for lost frogs and attempts to find a cure for the amphibian chytrid fungus. It should be released in March 2024 and hopefully will help us to spread the message about the importance of our work to save the frogs.

We have an active social media presence with 11,000 Facebook followers 7,000 Instagram followers and 5,000 followers on Twitter. We actively share news about our project and publications on our website and social media and have had 7 independent news articles cover our project this year.

Our new exhibition area at the Punta Culebra Nature Center in Amador, Panama City featuring the Panamanian golden frog

School kids visit an amphibian rescue pod during our open house for the Festival de la Rana Dorada.

Create novel ways to share amphibian biodiversity information

This year we conducted our Golden frog day celebrations in partnership with the Summit Zoo, and showed golden frogs and insect rearing to zoo goers, as part of a larger joint effort featuring other amphibian research efforts in Panama and we had related activities at our center in Gamboa and the Punta Culebra Nature Center. We also opened our new Golden frog exhibit at Punta Culebra, allowing visitors to our primary frog exhibition space at the Punta Culebra Nature Center to see Panamanian Golden Frogs and produced a 5th edition of frog posters (3,000) to give away to kids and teachers as part of our education and outreach work.

Gina DellaTogna also led a hands-on training workshop in Ecuador for assisted reproduction methods coordinated by the Atelopus Survival Initiative that led to successful reproduction of *Atelopus cruciger* in Venezuela.

Scientific publications from our amphibian conservation program in 2023

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*, pp. 1-13. *Results of the first release trial of* Atelopus varius*, and out third release trial publication showing that animals disperse widely females dispersing further than males and don't readily regain their skin defences.*

Luedtke, J. A. et al. 2023. Ongoing declines for the world's amphibians in the face of emerging threats. Nature 622(7982), pp. 308-314. *This is the result of the second global amphibian assessment showing that 40% of amphibians are threatened or endangered. Our team evaluated some 200 species in Panama as part of the global effort*.

Lötters, S., et al. 2023. Ongoing harlequin toad declines suggest the amphibian extinction crisis is still an emergency. *Communications Earth & Environment*, 4, 412.*This paper updates our knowledge of the conservation status of the world's harlequin frogs across their range.*

Monteiro, J.P., **Ibáñez, R.,** Mantzana-Oikonomaki, V., Pröhl, H. and Rodríguez, A. 2023. Genetic diversity of *Oophaga vicentei* (Anura: Dendrobatidae) and taxonomic position of a remarkable color morph from Panama. *Salamandra*, 59(4), pp. 347-351.

Rodriguez, C**., Ibáñez, R.,** Olmedo, D.A., Ng. M., Spadafora, C., Durant-Archibold, A.A., Gutiérrez, M. (2023) Anti-trypanosomal bufadienolides from the oocytes of the toad *Rhinella alata* (Anura, Bufonidae). Molecules 29(1), 196.

Otero, Y., Calatayud, N.E., **Arcia, I.D., Mariscal, D., Samaniego, D., Rodríguez, K., Guerrel, J., Ibáñez, R.,** and **Della Togna, G.** 2023. Recovery and characterization of spermatozoa in a Neotropical, terrestrial, direct-developing riparian frog (*Craugastor evanesco*) through hormonal stimulation. *Animals*, 13(17), 2689. *This documents the hormone protocols for collecting sperm from* Craugastor evanesco*.*

Sreedharan, G., Singh Panwar, Y., Murthy, S., Klop-Toker, K., **Ibáñez, R., Illueca, E.E.,** Webb, R., Govindappa, V., Subba, B., Segu, H., and Kumar Komanduri, K.P. 2023. A universal and efficient detection of chytridiomycosis infections in amphibians using novel quantitative PCR markers. *Transboundary and Emerging Diseases*, 2023, 9980566. *We collaborated with colleagues in India who developed a new qPCR protocol for testing for Bd that could also puck up theAsian strains missed by current methods.*

Klocke, B., Estrada, A., **Mataya, M.**, Medina, D., **Baitchman, E.**, Belden, L., **Guerrel, J., Evans, M., Baughman, J.,** Connette, G., **Illueca, E., Ibáñez, R.,** and **Gratwicke, B.,** 2023. Movement and survival of captive-bred Limosa harlequin frog (*Atelopus limosus*) released into the wild. *Frontiers in Amphibian and Reptile Science*, 1, 1205938. *The second of our publications on our* Atelopus *release trials. We learned that holding frogs in mesocosms for 30 days reduced post release dispersal and mortality.*

Kaganer, A.W., Ossiboff, R.J., Keith, N.I., Schuler, K.L., Comizzoli, P., Hare, M.P., Fleischer, R.C., **Gratwicke, B.**, and Bunting, E.M. 2023. Immune priming prior to pathogen exposure sheds light on the relationship between host, microbiome and pathogen in disease. *Royal Society Open Science*, 10(2), 220810. *Here we tried to develop a chytrid vaccine for hellbenders. Hellbenders are quite tolerant of Bd, and so there was no improvement in disease outcome in vaccinated vs unvaccinated animals, but the animals did respond to the oral vaccine, in a similar way to if they had been exposed to the live pathogen.*

Campbell Grant, E.H., Amburgey, S.M., **Gratwicke, B.,** Chaves, V.A., Belasen, A.M., Bickford, D., Brühl, C.A., Calatayud, N.E., Clemann, N., Clulow, S., and Crnobrnja-Isailovic, J., et al. 2023. Priority research needs to inform amphibian conservation in the Anthropocene. *Conservation Science and Practice*, 5(9), 12988. *An analysis of global amphibian research priorities for the future.*

Financial Report for Calendar Year 2023

Funding Source	Purpose	2022 Expenses (US$)
SCBI and donors	Salaries, supplies, fellows, travel.	259,749
Adrienne Arsht Community-Based Resilience Solutions Initiative	Salaries, operating costs.	285,336
The Woodtiger Fund	Research and program support	45,000
Zoo New England	Salaries	41,824
Cheyenne Mountain Zoo	Salaries	7,354
National Science Foundation	Research	46,000
Revive & Restore	Research	60,072
SENACYT	Research	11,532
STRI and donors*	Supplies	21,679
TOTAL		**778,546**

*These funds include direct project costs incurred in the calendar year 2023, 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 and listed funders, we are grateful to the following donors: Susan and Frank Mars, Linda Mars, Adrienne Arsht Community-Based Resilience Solutions Initiative, The Woodtiger Fund, The Shared Earth Foundation, The Anela Kolohe Foundation, Revive and Restore, Friends of the Baton Rouge Zoo, and Hugh Rand.

The Toad Mountain harlequin frog *Atelopus certus* are from Cerro Sapo in the Darien Gap, revisiting this site is a priority in order to understand how the amphibian chytrid fungus has affected wild populations of this species.

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, Lanki Cheucarama, Nancy Fairchild, Yimayri Figueroa, Orlando Garcés, Estefany Illueca, Jennifer Warren, Endy Araúz, Erick Barría, Rafael Lorenzo, Paolo Pinzón, Sharon Mc Lean.
Graduate Students - Blake Klocke, Carrie Lewis.
Post Doctoral Fellows - Luke Linhoff, Phil Jervis, Gonçalo M. Rosa, Joe Madison.
Research Associates – GinaDella 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.*

2023 Volunteers

Thank you to the following volunteers for generously providing their time to support the amphibian conservation program: Julio Alejandro Artuz, Nicolette Ávila, Alexandra Sofia Cohen, Lissette Fabiola Montenegro, Rachel Prokopius, Dionel Antonio Rodríguez, Karina Rodríguez, Diego Joel Samaniego, Barbora Thumsová, Miriam Wagner, Jasmin Wagner, Kelsey Wilson.

Acknowledgements

We are very grateful to the following people and organizations for their invaluable support and advice in the design and execution of this project: Zurenayka Alaín, Pamela Baker-Masson, Lisa Barnett, Delia Basanta, Nicole Bernat, Roberto Borrell, David Castro, Lil Camacho, Rivieth De Liones, Linette Dutari, Amy Ellison, Angie Estrada, Rob Fleischer, Leon Grayfer, Marie Francey, Luis Guardia, Arturo Higuera, César Jaramillo, Beth King, Luis Marin, Juan Mate, Daniel Medina, Annalisa Meyer, Carly Muletz-Wolz, Isis Ochoa, Yira Paz, Jimena Pitty, María de los Ángeles Pérez, Drew Portocarrero, Edwin Rangel, Corinne Richards-Zawacki, Felix Rodríguez, Karina Rodríguez, Xenia Saavedra, Nucharin Songsassen, Diorenne Smith, Raineldo Urriola, Lina Valencia, Anthony Vega, Jamie Voyles & Doug Woodhams.


CHEYENNE
MOUNTAIN
ZOO


Smithsonian


ZOO
NEW ENGLAND
Franklin Park Zoo ♦ Stone Zoo


SHARED
EARTH
FOUNDATION


revive&restore
genetic rescue for endangered and extinct species


MiAMBIENTE


SENACYT
Secretaría Nacional de Ciencia, Tecnología e Innovación


asa
amphibian survival alliance
PARTNER


ATELOPUS
SURVIVAL INITIATIVE