amphibian ark

Keeping threatened amphibian species afloat

Newsletter

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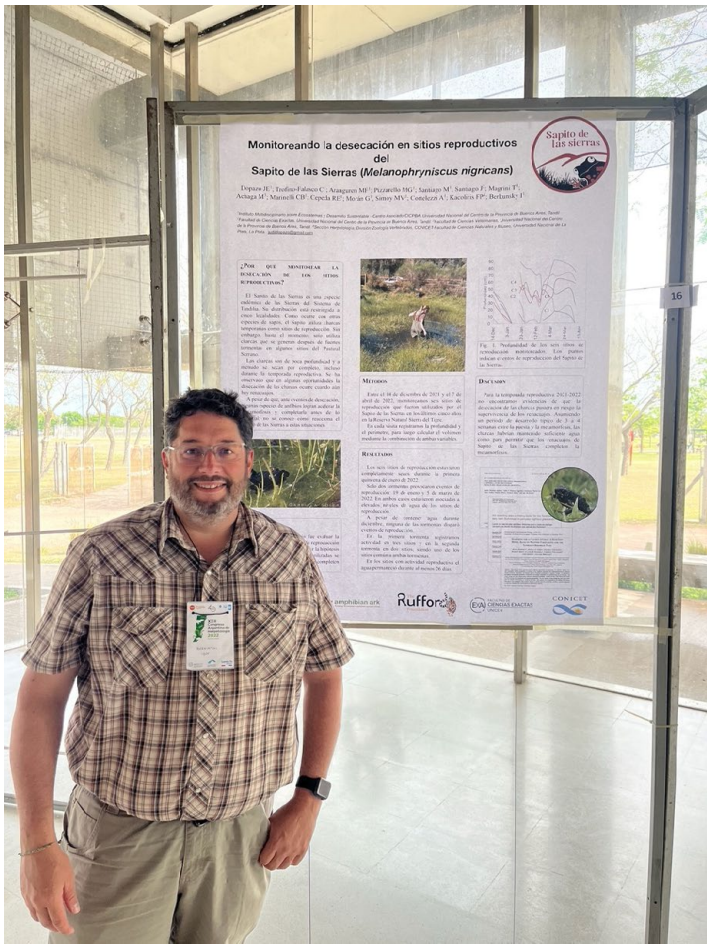
Conserving the Darwin's blackish toad in Argentina

Igor Berkunsky, National University of the Center of the Province of Buenos Aires UNICEN – CONICET, Argentina

Darwin's blackish toad (*Melanophryniscus nigricans*) is a threatened species, and since 1970 wild populations of this species have dramatically declined by more than 70%. At least two well-known populations have become extinct, and a third is probably extinct. The remnant populations face a combination of threats: habitat loss due to forestry, invasive woody species, and quarries; overgrazing and trampling by livestock; chytrid fungus; and desiccation caused by climate change.

In 2017, we began a conservation initiative to identify the main threats and explore practical conservation actions to recover the population of Darwin's blackish toad. Currently, only one protected area (the Sierra del Tigre Natural Reserve) effectively protects a wild population of this toad. In conjunction with the reserve managers, we initiated a habitat restoration project to provide more habitat for Darwin's blackish toad, however, natural recolonization by the species is unlikely due to current fragmentation and the lack of corridors between highland grassland remnants.

Since 2019, thanks to two conservation grants from the Amphibian Ark, we have established an *ex situ* facility at the university campus and established a survival colony of toads. Since then, we had two productive breeding seasons (during 2020-2021 and 2021-2022), where we successfully conducted the ranching of eggs by collecting clutches from wild breeding sites and maintaining them in captivity until they became juveniles. All individuals produced in captivity were translocated to restored and protected habitats.



Igor Berkunsky gave a presentation during the herpetological congress about the desiccation risk of the breeding ponds used by the Darwin's blackish toad. Photo: Igor Berkunsky.

During the past six months, work has continued to improve the *ex situ* facility, which should be finished in February 2023. Photo: Igor Berkunsky.

In our hemisphere, the last six months included the end of the fall, the winter, and most of the spring. We are experiencing a third consecutive year of "La Niña" events, which in our region, includes several droughts. Darwin's blackish toads need heavy storms to reproduce and consequently, the wild population has not bred since early April 2022 (seven months so far). In most humid years, breeding activity starts in late August, but in the last three years, toads started breeding in late November or even December. We hope they start soon.

Meanwhile, over the past six months, we have been working on improving the *ex situ* facility, we almost finished the building, and we plan to move all the terraria and fish tanks in February 2023. We also conducted a workshop to develop the Conservation Action Plan for the species, and we have a draft that we hope to finish by the end of the summer.

The local university is also involved in this initiative, and they provided an old house which we recycled as the main building for the Amphibian Ark initiative. They also provide us a small grant to restore the habitat for the future release individuals at the Reserve.

We have also received support and advice from Dr. Federico Kacoliris and his team from the Herps Lab of the Museo de la Plata in Buenos Aires.

During the first six months after receiving the latest AArk grant, we have achieved the following:

- We successfully maintained a few juveniles from the previous breeding season in captivity. For the first time we managed to keep juveniles alive for a period of more than seven months, however, juvenile mortality rate remains high (or at least higher than the mortality we want to have).
- We established a large enough colony of Collembola to feed juveniles. It took longer than expected, but finally we have a nice colony which produces large amounts of Collembola.

- We have almost finished setting up the Ark building. We hope to have all the work there completed by February.
- We planned and conducted a two-day workshop to work on the Conservation Action Plan. After some failed attempts, we finally were able to hold a meeting with the participation of most of the conservation stakeholders involved with the Darwin's blackish toad. This workshop was improved with the participation of Dr. Federico Kacoliris and his team from the Herps Lab of the Museo de la Plata. Federico facilitated the meeting, and after two intense days we defined the core actions, a schedule and other important and necessary details to wrap up the Conservation Action Plan. We hope to have it completed by February.

Dr Federico Kacoliris from the Herps Lab of the Museo de la Plata in Buenos Aires facilitated a two-day workshop to develop the Conservation Action Plan for Darwin's blackish toads. Photo: Igor Berkunsky.

We received a second small grant from our University (PIO grant, National University of the Centre of Buenos Aires Province) to monitor the restored habitat sites where we are releasing the captive breed individuals in the Sierra del Tigre Natural Reserve. The grant (almost US $1,000) is for one year only (2022-2023).

Our goals over the next six months are to:

1. Increase our colony by adding ten new adults.
2. Develop the rain chamber which we have designed to stimulate the breeding of captive adults.
3. Produce at least 200 juveniles from ranching wild clutches.
4. Have moved into the new facility.
5. Completed the Conservation Action Plan.

The inside of the new *ex situ* facility for Darwin's blackish toads. Photo: Igor Berkunsky.

Additional conservation grant awarded

We are very pleased to announce the award of a $5,000 Conservation Grant to Dr. Nadia Carla Bach, from the National University of San Luis in Argentina.

Conservation of *Melanophryniscus estebani*: an endemic, poorly known species from Chaco Serrano, Argentina

Central Pampean Sierras in Argentina host endemic amphibian species associated with grasslands and high altitude forests (1,600-2,100 meters above sea level). *Melanophryniscus estebani* is a microendemic anuran categorized as Data Deficient by the IUCN Red List (www.iucnredlist.org/species/78520090/101436412) and has been recommended as a species for *ex situ* rescue in a Conservation Needs Assessment (www.conservationneeds.org/summaryreport/6898). Threats in their microhabitat involve exotic species introduction, urbanization, grassland fires and mining practices which release contaminants. Researchers suggest that, considering the current status of populations, it is important to monitor and promote conservation programs to understand unknown aspects of the species' biology.

In accordance with the Argentina Amphibian Conservation Plan (Akmentis et al., 2018), this project for *M. estebani* conservation aims to develop *ex situ* management techniques, preserve the species in captivity, develop an Action Plan for the long term conservation of the species, study currently unknown aspects of the biology of *M. estebani*, establish normal parameters of health via *in situ* studies, and monitor the actual status of the populations in the wild. Expected results are to collect founder pairs, achieve *ex situ* reproduction, understand aspects of the species' reproductive biology, estimate parameters in captivity related to physiological stress indicators, know more about the conservation status of *M. estebani* in San Luis, and implement the Action Plan for the conservation of the species.

In this context, this *ex situ* conservation project aims to:

1. Develop *ex situ* protocols in order to maintain founders and progeny of *Melanophryniscus estebani*.
2. Establish an *ex situ* survival colony of *M. estebani* for conservation purposes.
3. Develop an Action Plan for the long term conservation of this species, including both *ex situ* and *in situ* management actions.

Melanophryniscus estebani in amplexus.
Photo: Juan Manuel Perez Iglesias.

The team from the National University of San Luis in Argentina who are working to conserve *Melanophryniscus estebani*.
Photo: Nadia Bach.

4. Study aspects of the biology of *M. estebani* which are currently unknown and establish normal parameters of health for the species via *in situ* studies.
5. Monitor the actual status of populations of the species in the wild.

The proposed outcomes are:

1. Know the characteristics of the microhabitat in which *M. estebani* lives, as well as aspects of *M. estebani* reproductive biology (nuptial vocalization, type of amplexus, size and type of clutch, characteristics of where the male vocalizes and where amplexus takes place, etc.)
2. Gain further information about the conservation status of *M. estebani* in several microhabitats of San Luis.
3. Collect founder pairs and achieve *ex situ* reproduction.
4. Estimate parameters in captivity related to physiological stress indicators for *M. estebani*.
5. Mitigate possible threats.
6. Protect microhabitats where *M. estebani* inhabits.
7. Implement an Action Plan for the conservation of the species.

Reference

Akmentis, M.S., Agostini, G., Bach, N., Duré, M., Lavilla, E.O., López, J.A., Ghirardi, R., Maruscak, N., Pereyra, L.C., Pérez-Iglesias, J.M., Salgado-Costa, C. and Vaira, M. (2018). Componente 6. Herramientas de información, divulgación y participación. En: *Plan de Acción para la Conservación de los Anfibios de la República Argentina*. Cuadernos de Herpetología 32 (supl. 1): 51-56. doi: 10.31017/CdH.2018.(2018-s1).

Establishment of an insurance population for *Pithecopus rusticus*

Benjamin Phalan, Parque das Aves, Brazil

Based on the National Red List assessment for Brazil, *Pithecopus rusticus* is a Critically Endangered frog, known only from a few tens of individuals at a single site in Santa Catarina, Brazil. The species has not been assessed yet for the global Red List, but clearly meets the criteria for globally Critically Endangered. It faces a range of threats in its natural habitat and was identified during a Conservation Needs Assessment (https://conservationneeds.org/summaryreport/6764) as a priority for *ex situ* intervention.

Our main objectives for the first six months of our project after receiving a conservation grant from the Amphibian Ark were to expand the *ex situ* population (if an evaluation of the wild population permitted us to collect an egg mass and/or a second pair) and to begin to develop a larger facility to accommodate what we hope will soon be a growing number of froglets. We also aimed to continue supporting work in the field to search for additional populations and understand the species' requirements better.

The project is a collaboration with various partners:

- Research partners at the Federal University of Santa Maria were the first to recognize the existence of this species and describe it as a new taxon, and have continued to work on this frog since. Led by Professor Elaine Lucas, they lead work in the field, carrying out capture-mark-recapture studies to estimate the population size, visiting additional sites in the hopes of finding more individuals, installing autonomous sound recorders and studies of tadpoles.
- The São Paulo Zoo, notably Cybele Lisboa, has helped us with detailed advice on the construction of facilities and care of the frogs under human care, including aspects such as diet, water quality monitoring, and screening for disease.
- The governmental biodiversity agency, ICMBio, has identified this species as a priority in the National Action Plan, and organized transport of the frogs from the field sites to Parque das Aves by helicopter without cost to us, through a partnership that they have with a commercial helicopter company. The National Action Plans are organised by RAN-ICMBio, the Centre within ICMBio for research and conservation of reptiles and amphibians, and Tiago Vieira has been especially supportive.
- The Amphibian Specialist Group (Brazil), with the support of Amphibian Ark, has coordinated conservation planning efforts, including the Conservation Needs Assessment and the more recent planning workshops with Planos Estratégicos de Conservação de Anfíbios (PECAn, Strategic Conservation Plans for Amphibians), for strategic conservation planning for this and another threatened amphibian species.

We participated in the PECAn workshops for strategic conservation planning for *Pithecopus rusticus*, organised by the Amphibian Specialist Group (Brazil) in August, September and October 2022. This process was organised to identify priority conservation actions for the species, and a written plan is forthcoming.

Three members of the Parque das Aves/Instituto Claravis team participated in a field expedition in November 2022, together with researchers from partner institutions. The expedition searched the nearest protected area to the known site (Refúgio da Vida Silvestre dos Campos de Palmas) but without success. The team captured and marked individuals of *Pithecopus rusticus* at the known site, with eighteen individuals captured, including recaptures of individuals marked in previous years. Swabs and biometrics were collected. They also collected suitable plants for reproduction under human care, of the two known plants used for egg-laying (species of *Paepalanthus* and *Senecio*), installed autonomous sound recorders (Audiomoths) provided by Instituto Claravis, and conducted an experiment to understand the extent to which egg masses retain humidity when laid on different species of plants, using agar as a model for eggs.

It was decided that the number of frogs found was sufficient to allow the collection of a second pair of *Pithecopus rusticus* to serve as additional founders for the *ex situ* population. They were transported to Parque das Aves by helicopter, arranged by ICMBio at no cost to the project, where we had prepared a second aquaterrarium to receive them. Both aquaterraria were landscaped with plants from the region where the species occurs, which had not been possible previously. Amplexus and vocalizations have been observed by both pairs, but so far, no eggs have been laid.

Searching for *Pithecopus rusticus*, in Santa Catarina, Brazil. Photo: Victor Barreto/Parque das Aves.

We refined our plans for the new amphibian facility, and ordered a new door, air conditioning unit, and water filter. This facility will be completed with additional aquaterraria before the end of the grant period, and in time to receive any froglets should the two founder pairs reproduce successfully.

Together with our partners, we also established a social media presence for the project (www.instagram.com/projetoperere-carustica/). Please follow us for updates on the progress of our conservation work with *Pithecopus rusticus* (the posts are in Portuguese)!

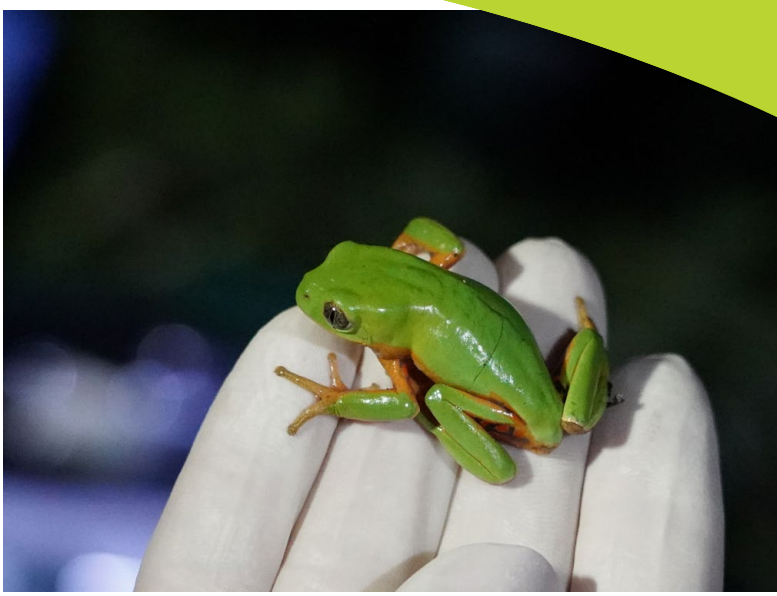
A species action plan is in production, as an output of the PECAn process led by the Amphibian Specialist Group (Brazil), and this document will be available in 2023 for our final report.

We have received in-kind contributions from ICMBio (helicopter transfer of frogs from Santa Catarina to Foz do Iguaçu) and part of the costs of this project continue to be covered by other sources.

We plan to finish the new amphibian facility, with air-conditioner and water filter installed, shelving, and new aquaterraria ready to accommodate what we hope will be an expanding population. We will continue to collect information on feeding, videos of behaviour, sound recordings and swabs from the four frogs under human care and monitor their weight and health.

We are hopeful that within the next twelve months we will see egg-laying in the aquaterraria, but of course, this depends to a large degree on the frogs!

We would also like to acknowledge Lauren and Donny, who were planning their wedding in Ottawa, Canada, earlier this year, and in lieu of giving their wedding guests a gift or favour, they decided that they would instead like to make a donation on behalf of their guests. The AArk grant which was supported by Lauren and Donny will help us to build more aquaterrariums to accommodate what we hope will soon be an expanding population. Huge thanks to Lauren, Donny and their wedding guests for their support of our project. Um grande abraço from Brazil!



A *Pithecopus rusticus* is examined in the field, in Santa Catarina, Brazil. Photo: Victor Barreto/Parque das Aves.

The amphibian room at Parque das Aves with two aquaterraria for the current *ex situ* population of four *Pithecopus rusticus*. Photo: Ben Phalan/Parque das Aves.

AArk Husbandry Document Library

The Husbandry Document library on the AArk web site (www.amphibianark.org/husbandry-documents) currently has over 295 documents in it, with additional documents being added regularly.

Eleven new documents have been added recently:

Wyoming toad *Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri* Revised Recovery Plan, May 2015 (English)

This recovery plan's structure articulates both short and long-term strategies that together comprise the conditions under which the Wyoming toad may be delisted. An adaptive management approach, which allows for the continual inclusion of updated research and information, will be the main strategy guiding the management of the species. The captive program maximizes genetic diversity in its annual breeding and continuously develops husbandry strategies to maximize the health of captive populations.

Author: U.S. Fish and Wildlife Service
Publication: 2015
www.researchgate.net/publication/312372803_First_Revised_Recovery_Plan_for_Wyoming_Toad

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Inventory and Monitoring Techniques for Amphibians of the Tropical Andean Region (Spanish)

This manual collects the experience of several researchers who have dedicated a good part of their professional lives to the development of monitoring techniques and to the arduous task of testing them for long periods to see their benefits in the results generated. Similarly, these experiences have been put into practice in the three field courses on amphibian inventory and monitoring developed by the Atelopus Initiative for Conservation International and the Darwin Initiative in Peru, Venezuela and Bolivia. The final result of this purification process is the product that is presented today to the academic community and in general to all those interested in amphibians. Likewise, this manual represents a small but strategic part of the global effort to face declines and extinctions as mentioned in the Amphibian Conservation Plan (ACAP), a document developed during the Amphibian Conservation Summit that met in Washington DC in September 2005, and which is the guide for amphibian conservation actions to be implemented, at a global level, during the coming years. We hope that by promoting research with initiatives like this, we can increase our knowledge.

Editors: Angulo, A., Rueda-Almonacid, J.V., Rodríguez-Mahecha, J.V., and la Marca, E.
Publication: Conservación Internacional. Serie Manuales de Campo Nº 2. 2006
www.amphibianark.org/wp-content/uploads/2022/09/Monitoreo-de-anfibios-baja-final.pdf

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Batrachochytrium dendrobatidis* infection and treatment in the salamanders *Ambystoma andersoni*, *A. dumerilii* and *A. mexicanum (English)

In order to better understand the impacts and treatment of infection with *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) it is important to document host species, the effect of infection and response to treatment protocols. Here we report asymptomatic *Bd* infection detected through duplex qPCR screening of three Mexican ambystomatid salamanders; *Ambystoma andersoni*, *Ambystoma dumerilii* and *Ambystoma mexicanum* at three zoo collections, and *A. andersoni* and *A. mexicanum* in a private collection. *Bsal* was tested for but not detected. We also report the effectiveness and side effects of five treatment protocols in these species. Using the antifungal agent itraconazole, *A. dumerilii* were cleared of infection without side-effects using the granulated preparation (Sporanox). Morbidity and mortality occurred when *A. dumerilii* and *A. andersoni* were treated using a liquid oral preparation of the itraconazole (Itrafungol); infection was successfully cleared in surviving specimens of the latter species. *A. mexicanum* was successfully cleared without any side-effects using Itrafungol. Mortality and morbidity were likely caused by toxic effects of some component on the liquid preparation of itraconazole, but aspects of water quality and husbandry cannot be ruled out.

Authors: Christopher J. Michaels, Matthew Rendle, Cathy Gibault, Javier Lopez, Gerardo Garcia, Matthew W. Perkins, Suzetta Cameron & Benjamin Tapley
Publication: Herpetological Journal Volume 28 (April 2018), 87-92
www.amphibianark.org/wp-content/uploads/2022/09/Batrachochytrium-dendrobatidis-infection-and-treatment-in-the-salamanders.pdf

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Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga pumilio*) (English)

Amphibians are currently experiencing the most severe declines in biodiversity of any vertebrate, and their requirements for successful reproduction are poorly understood. Here, we show that supplementing the diet of prey items (fruit flies) with carotenoids has strong positive effects on the reproduction of captive strawberry poison frogs (*Oophaga pumilio*), substantially increasing the number of metamorphs produced by pairs. This improved reproduction most likely arose via increases in the quality of both the fertilized eggs from which tadpoles develop and trophic eggs that are fed to tadpoles by mothers. Frogs in this colony had previously been diagnosed with a Vitamin A deficiency, and this supplementation may have resolved this issue. These results support growing evidence of the importance of carotenoids in vertebrate reproduction and highlight the nuanced ways in which nutrition constrains captive populations.

Authors: Matthew B. Dugas, Justin Yeager, Corinne L. Richards-Zawacki
Publication: Zoo Biology, 30(6), 611–622 (2011) doi: 10.1002/zoo.20358
https://onlinelibrary.wiley.com/doi/full/10.1002/zoo.21102

Testing the effect of dietary carotenoids on larval survival, growth and development in the critically endangered southern corroboree frog (English)

The success of captive breeding programs for threatened species is often limited due to a lack of knowledge of the nutritional conditions required for optimal growth and survival. Carotenoids are powerful antioxidants known to accelerate vertebrate growth and reduce mortality. However, the effect of carotenoids on amphibian life-history traits remains poorly understood. The aim of our study was to use a manipulative laboratory experiment to test the effect of dietary-carotenoid supplementation during the larval life stage on the survival, growth and development of the critically endangered southern corroboree frog (*Pseudophryne corroboree*). Larvae were fed either a carotenoid supplemented diet or an unsupplemented diet and the survival, growth and development of

individuals was monitored and compared. There was no significant effect of dietary treatment on larval survival, growth rate, time taken to reach metamorphosis, or body size at metamorphosis. Our findings provide no evidence that carotenoid supplementation during the larval life stage improves the growth and development of southern corroboree frogs. However, because the carotenoid dose used in our study did not have any detrimental effects on *P. corroboree* larvae, but has previously been shown to improve adult coloration, immunity, and exercise performance, carotenoid supplementation should be considered when evaluating the nutritional requirements of *P. corroboree* in captivity. Carotenoid supplementation studies are now required for a diversity of anuran species to determine the effects of carotenoids on amphibian survival, growth and development. Understanding the effects of dietary carotenoids on different life-history traits may assist with amphibian captive breeding and conservation.

Authors: Byrne, P.G., & Silla, A.J.
Publication: Zoo Biology, 36(2), 161–169. doi: 10.1002/zoo.21352
https://onlinelibrary.wiley.com/doi/full/10.1002/zoo.21352

The relationship between spindly leg syndrome incidence and water composition, overfeeding, and diet in newly metamorphosed harlequin frogs (*Atelopus* spp.) (English)

Spindly Leg Syndrome (SLS) is a persistent animal welfare issue associated with the rearing of amphibians in captivity. We conducted two experiments to investigate the effects of diet, water composition and overfeeding on prevalence of SLS in newly metamorphosed harlequin frogs (*Atelopus* spp.). In our first experiment, we offered 400 full-sibling tadpoles of *Atelopus certus* isocaloric diets in treatments of 31%, 37%, 42% and 48% crude protein respectively. Tadpoles fed higher protein diets metamorphosed faster, but the incidence of SLS exceeded 80% in all treatments leading to the conclusion that variation in dietary protein was not responsible for causing SLS. We used 720 full-sibling *Atelopus glyphus* tadpoles in a second experiment to examine the effects of diet type, water composition and diet ration on SLS. We found that an overall incidence of 58% spindly leg in tadpoles reared in tap water, but reduced to about 10% in water treated by reverse osmosis and then reconstituted. It is possible that the reverse osmosis treatment removed some factor that caused the SLS, or that the reconstitution may have added a mineral lacking in the original tap water. Within tap water treatments, overfeeding tadpoles in tanks increased the incidence of SLS. We recommend further experimental research into this condition to identify the causative factors in the water. Additional research into the nutritional composition of food available to wild tadpoles would be useful in formulating captive diets, that have to date been solely based on surrogate species.

Authors: Julio Federico Camperio Ciani, Jorge Guerrel, Eric Baitchman, Rigoberto Diaz, Matthew Evans, Roberto Ibáñez, Heidi Ross, Eric Klaphake, Bradley Nissen, Allan P. Pessier, Michael L. Power, Caitlin Arlotta, Donna Snellgrove, Brad Wilson and Brian Gratwicke
Publication: PLoS ONE 13(10): e0204314. (2018)
https://doi.org/10.1371/journal.pone.0204314

Developments in amphibian captive breeding and reintroduction programs (English)

Captive breeding and reintroduction remain high profile but controversial conservation interventions. It is important to understand how such programs develop and respond to strategic conservation initiatives. We analyzed the contribution to conservation made by amphibian captive breeding and reintroduction since the launch of the International Union for Conservation of Nature (IUCN) Amphibian Conservation Action Plan (ACAP) in 2007. We assembled data on amphibian captive breeding and reintroduction from a variety of sources including the Amphibian Ark database and the IUCN Red List. We also carried out systematic searches of Web of Science, JSTOR, and Google Scholar for relevant literature. Relative to data collected from 1966 to 2006, the number of species involved in captive breeding and reintroduction projects increased by 57% in the 7 years since release of the ACAP. However, there have been relatively few new reintroductions over this period; most programs have focused on securing captive-assurance populations (i.e., species taken into captivity as a precaution against extinctions in the wild) and conservation-related research. There has been a shift to a broader representation of frogs, salamanders, and caecilians within programs and an increasing emphasis on threatened species. There has been a relative increase of species in programs from Central and South America and the Caribbean, where amphibian biodiversity is high. About half of the programs involve zoos and aquaria with a similar proportion represented in specialist facilities run by governmental or nongovernmental agencies. Despite successful reintroduction often being regarded as the ultimate milestone for such programs, the irreversibility of many current threats to amphibians may make this an impractical goal. Instead, research on captive assurance populations may be needed to develop imaginative solutions to enable amphibians to survive alongside current, emerging, and future threats.

Authors: Harding, G., Griffiths, R.A., & Pavajeau, L.
Publication: Conservation Biology, 30(2), 340–349. doi: 10.1111/cobi.12612 (2016)
https://conbio.onlinelibrary.wiley.com/doi/10.1111/cobi.12612

Re-establishment of an extinct local population of the Valcheta Frog, *Pleurodema somuncurense*, in a restored habitat in Patagonia, Argentina (English)

In March 2017 and March 2018, we reintroduced 196 and 50 Valcheta frogs *Pleurodema somuncurense*, respectively (tadpoles and juveniles). The individuals were translocated from an *ex situ* colony to a restored habitat at the hot springs of the Valcheta stream (Rio Negro, Argentina). The aim was to re-establish a local population of this species that had gone extinct at this site. After the individuals were released, we monitored them using night visual encounters to register the number of individuals and other relevant records that suggested acclimatization (feeding, escaping and reproduction). In addition, we performed a Capture-Mark-Recapture study to estimate the density of the reintroduced population using POPAN models. By September 2018, the estimated density was 62 ± 27 SD in a stream area of 50 m^2. This does not differ from density estimates of wild populations of the Valcheta Frog. Additionally, reproduction of reintroduced frogs was recorded in September 2018 and January 2019. Egg clutches, tadpoles and juveniles were all observed at the reintroduction site. These results suggest that the reintroduction of captive bred individuals to the wild might be an effective management action to restore local populations of this species that had gone extinct.

Authors: Martínez Aguirre, T., Calvo, R., Velasco, M.A., Arellano, M.L., Zarini, O., and Kacoliris, F.P.
Publication: Conservation Evidence, 16, 48–50 (2019)
https://www.conservationevidence.com/collection?download/48

Frogs in Glass Boxes: Responses of Zoos to Global Amphibian Extinctions (English)

To begin the preparations for a global zoo response to safeguard amphibians, the IUCN Conservation Breeding Specialist Group (CBSG) convened an international meeting to produce initial guidelines and protocols (Zippel, Lacy,

and Byers 2006). These guidelines emphasized pathogen biosecurity and development of in-country programs and encouraged the innovative use of commercial shipping containers as affordable, somewhat portable, pod units for housing amphibians in the absence of existing zoo infrastructure. Maintaining extensive proactive survival assurance colonies in captivity as a conservation strategy was included in the IUCN Amphibian Conservation Action Plan (Gascon et al. 2007) and was communicated widely (Mendelson and Rabb 2005; Mendelson et al. 2006; Zippel and Mendelson 2008).

Author: Mendelson III, J.R.
Publication: In: G. Rabb (Ed.), *The ark and beyond: The evolution of zoo and aquarium conservation* (pp. 298–310). Chicago, USA: University of Chicago Press. 2018
https://www.researchgate.net/publication/327079024_Frogs_in_Glass_Boxes_Responses_of_Zoos_to_Global_Amphibian_Extinctions

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Breeding and rearing the critically endangered Lake Oku clawed frog (*Xenopus longipes* Loumont and Kobel 1991) (English)

The Lake Oku Clawed Frog *Xenopus longipes* is a Critically Endangered, dodecaploid anuran endemic to Lake Oku in Cameroon. An *ex situ* population of this species was established at Zoological Society of London (ZSL), London Zoo in 2008, as well as at several other institutions, with the intention of providing data on the biology and husbandry of this species. We report the first captive breeding of the species. Adult frogs maintained under environmental conditions designed to mimic field data produced clutches of 7–300 eggs; eggs measured 1.23 mm in diameter, and were laid singly after a period of 6.5 hours in axial amplexus. Spawning took place only during the day. Tadpoles hatched in 2–3 days and development was very long compared to congeners, lasting 193–240+ days until metamorphosis. Tadpoles grew very large (maximum 79 mm total length), particularly compared with the relatively small adult size (maximum 36 mm Snout to Vent Length [SVL]). Tadpoles proved to be highly sensitive to total dissolved solids (TDS) in the water and only thrived when low levels (20 mg/L) were used. Metamorphosis concluded with an SVL of 19–25 mm and F1 animals began first sexual activity at 5–6 months post metamorphosis. These data will inform future husbandry in captivity as well as illuminating facets of biology previously unknown and difficult to determine in the field.

Authors: Michaels, C.J., Tapley, B., Harding, L., Bryant, Z., and Grant, S.
Publication: Amphibian & Reptile Conservation, 9(2), 100–110. 2015
https://www.researchgate.net/publication/281968522_Breeding_and_rearing_the_Critically_Endangered_Lake_Oku_Clawed_Frog_Xenopus_longipes_Loumont_and_Kobel_1991

The use of visible implant elastomer to permanently identify caecilians (Amphibia: Gymnophiona) (English)

Identifying individual animals is important for studying populations and for the optimal management of individual animals in captivity. In the absence of natural markings that discriminate individuals, such identification may require animals to be marked by researchers. Amphibians are challenging subjects to mark due to their small size and sensitive, permeable and frequently shed skin. Visible Implant Elastomer (VIE) has been widely used to mark amphibians, but no long-term study has validated this technique in caecilian amphibians. We anaesthetised and attempted to VIE mark seven *Herpele squalostoma* and one *Microcaecilia unicolor* held at ZSL London Zoo. No specimens suffered ill effects of anaesthesia or VIE injection, but mean persistence of marks was 191 days in *H. squalostoma* suggesting that this marking technique is not suitable for identifying individuals of this species in the long-term. We were unable to inject VIE into the *M. unicolor* and/or the elastomer was not visible through the darkly pigmented skin. Further research is required to develop methods for long-term marking of a diversity of caecilians.

Authors: Tapley, B., Michaels, C.J., Gower, D.J., and Wilkinson, M.
Publication: Herpetological Bulletin, 150, 18–22. doi: 10.33256/hb150.1822 2020
https://www.thebhs.org/publications/the-herpetological-bulletin/issue-number-150-winter-2019/2010-05-the-use-of-visible-implant-elastomer-to-permanently-identify-caecilians-amphibia-gymnophiona

2023 Amphibian Ark Conservation Grants

Amphibian Ark is pleased to announce the 15th annual call for proposals for our grants program. Some new guidelines and requirements for grant recipients have been included, so please be sure to read these guidelines carefully. Download the complete guidelines from www.amphibianark.org/grants/AArk-Conservation-Grants.pdf.

While applications are welcomed from programs in all countries, this year we are especially keen to see applications to work with *Atelopus* species, which support the goals of the Atelopus Survival Initiative (www.atelopus.org). We are also keen to see applications for extension grants from any existing *ex situ* amphibian conservation program.

We will be accepting Project Outline funding applications (see below) for the following types of grants **from 1st March 2023**:

Start-up grants – initial funding to help newly-launched projects get started at the very beginning of their life, to help them attract larger and/or long-term funding for the duration of the program. One-time grants of up to US$5,000 are available. Recipients can apply for second and third year extension grants.

Start-up grant extensions – additional funds are available to provide continued support for existing *ex situ* amphibian conservation programs that a) have met their stated objectives for previous years, and b) can demonstrate that additional supplemental funds have been secured since the original grant was provided. All existing programs are eligible to apply for these extensions however it is expected that husbandry guidelines and a species action plan have been completed. Second-year grants of up to US$4,000 and third-year grants of up to US$3,000 are available.

Workshop attendance – partial funding to assist attendance at *ex situ* amphibian conservation-related workshops, especially those which focus on amphibian husbandry, planning and reintroduction. Applicants must have already secured partial funding to attend the workshop. You must already be actively involved in an amphibian conservation project or have well-developed plans and funding in place to implement a new program. Grants of up to US$750 are available.

Mentorship grants – support for organizations which have previously received an AArk seed or start-up grant, to bring in a designated outside expert to assist with an aspect of their amphibian conservation efforts (e.g. veterinary training, environmental control etc.). Grants up to US$1,500 are available.

Workshop support grants - support for organizations which are planning an in-person amphibian conservation-related workshop or symposium, especially those which focus on amphibian husbandry, planning and reintroduction. Applicants must have already secured partial funding for the workshop, and the dates and location for the workshop should have been publicly announced. Grants up to US$2,500 are available.

All applicants are required to submit a brief Project Outline, prior to submitting a full application. Ideally, your Project Outline should be in English or Spanish, but it can be submitted in any language. Your Project Outline should be less than 300 words in length and should contain information under the following headings: Species, Organization, Project Manager, Previous amphibian experience, Goals, Proposed Outcomes. Other funding Sources (both requested and received) and the status of a Species Action or Recovery Plan for the species (including authors of the plan). Project Outlines for start-up grants from institutions with limited amphibian expertise must include a copy of the report from the Institutional Program Implementation Tool (www.amphibianark.org/program-implementation-tool/) for the species at your institution. Your final application should address any shortfalls highlighted within the tool.

Project Outlines will be reviewed, and successful applicants will then be invited to submit a full application. Full applications will not be accepted without a Project Outline having been submitted, reviewed and approved by the review committee.

Our grants are intended to support conservation projects for amphibian species that cannot currently be saved in the wild, with a focus on *ex situ* actions, and in partnership with appropriate field activities. Preference will be given to projects for species which have been assessed as in need of *ex situ* rescue or research work, either as a recommendation from a Conservation Needs Assessment or a similar, national assessment process.

Start-up grants and start-up extension grants are not intended to fund:

- Educational exhibits
- Project overheard or indirect costs
- Field projects without a strong *ex situ* component.

All applications must reflect AArk values. Please pay careful attention to the grant guidelines and address all the appropriate items.

Need some help?

AArk staff are available if you need assistance in formulating your proposal. Please do not hesitate to contact us with any questions. Each year several proposals have been rejected due to issues that could have been prevented with a little extra guidance! We also have several past seed grant recipients who are willing to act as mentors, to help with your application – please let us know if you would like us to put you in contact with one of them. Email us at grants@amphibianark.org.

Important dates

- Project Outline deadline: **1 April 2023**
- Applicants notified about review of Project Outlines: 15 April 2023
- Grant application deadline: **6 May 2023**
- Grant decision/notification date: 20 May 2023
- Successful applicants must provide bank account details, signed MOU and 3-4 photos of species and/or facilities by: **3 June 2023**
- Grant payment date: 16 June 2023
- Initial progress report and species action plan provided by 1 January 2024
- Final progress report, species action plan and husbandry guidelines due 30 June 2024.

We would like to acknowledge the generous support of AArk funders (www.amphibianark.org/our-funders/) and donors (www.amphibianark.org/our-donors/) who have helped to establish and support these grants.

Ex situ rescue of the Rancho Grande Harlequin Frog in Venezuela

Margarita Lampo and Jaime Nestares, Foundation for the Development of Physical, Mathematical and Natural Sciences (FUDECI), Venezuela; Ingrid Márquez, Central University of Venezuela, Caracas, Venezuela; Onil Ballestas, Venezuelan Institute of Scientific Research, Caracas, Venezuela; and Federico Pantin, Leslie Pantin Zoo, Venezuela

Harlequin toads (*Atelopus* species) are the most threatened group of amphibians in the world, with 83% of the species at risk of extinction. In Venezuela, only one out of ten known species, the Rancho Grande harlequin toad (*Atelopus cruciger*), has been sighted in the last seventeen years. It disappeared in the late 1980s presumably due to chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*). Despite continuing efforts to find these toads in their former habitats, only two populations have been discovered. The Rancho Grande harlequin toad is currently listed as Critically Endangered and recommended for *ex situ* rescue by the IUCN (www.iucnredlist.org/species/54502/198626366). Our immediate goal is to establish captive breeding colonies for *A. cruciger* as a stopgap measure for a long-term conservation plan that includes its reintroduction into former habitats in lowland habitats. Our ultimate goal is to restore self-sustaining populations that will thrive naturally without the need for intensive conservation and to inspire other conservation actions by using the Rancho Grande harlequin toad as a flagship species.

A male Rancho Grande harlequin toad (*Atelopus cruciger*) at the Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo in Venezuela. Photo: Margarita Lampo.

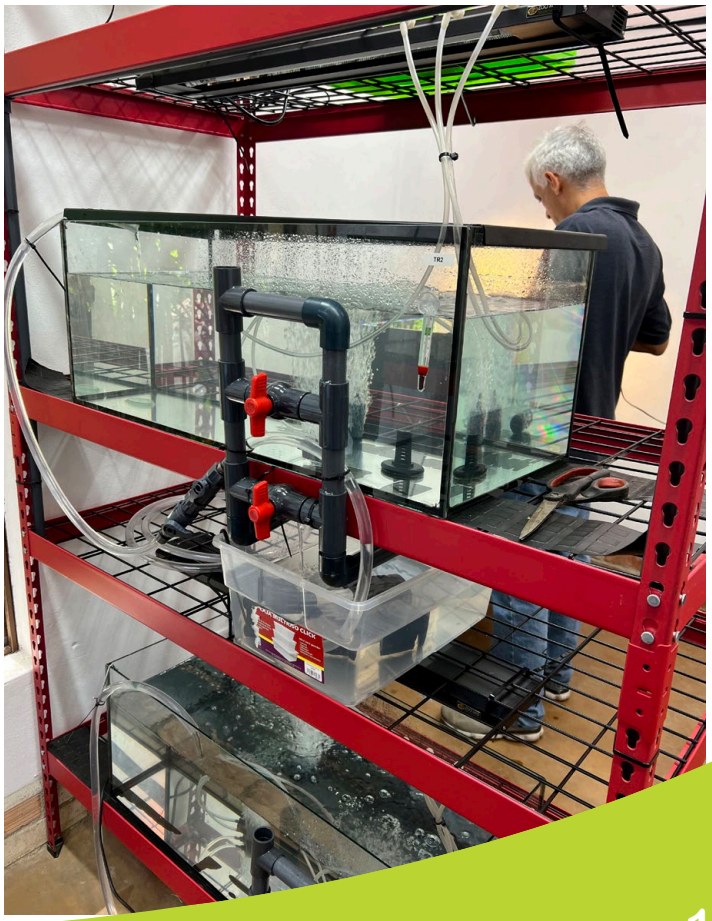
During the first phase (six months) after receiving an Amphibian Ark Conservation Grant, we aimed to:

- Secure an *ex situ* facility capable of housing twenty founders and producing up to 150 juveniles per year at the Leslie Pantin Zoo.
- Build the capacity for husbandry and breeding the Rancho Grande harlequin toad at the Zoo.

Several partners are now involved in this project:

- Venezuelan Institute of Scientific Research (IVIC), the largest research infrastructure owned by the government. The Center for Ecology at IVIC will support us with the *Bd* diagnosis by providing the necessary equipment for rt-PCR. Dr. Onil Ballestas will be conducting all *Bd* testing once funds for buying reagents are received.
- Ministry of Popular Power for Ecosocialism, a government office in charge of issuing the required permits for collecting live specimens and regulating captive breeding of fauna species.
- Rio Verde, a private company specialized in audio-visual production. Rio Verde is currently assisting us with communications (social media) and audio-visual resources.
- Atelopus Survival Initiative (ASI), a regional NGO dedicated to the conservation of harlequin toads. ASI is assisting us with networking, fund raising and coordination of stakeholders, and is funding Margarita Lampo's training on assisted reproduction in Quito, Ecuador.

The Centro para la Reproducción e Investigación para Arlequines (Center for the Reproduction and Research for Harlequins, CRIA) at the Leslie Pantin Zoo was completed and began operations in November this year. It consists of a ~12 m^2 facility equipped with air conditioning, four-stage filtering systems for water conditioning, holding tanks for water treatment before disposal, three shelves and one working table. It is currently equipped with five 10-gallon tanks with capacity for five to seven individuals each, two 20-gallon breeding tanks for amplectant pairs and tadpoles, and ten small plastic mouse cages for rearing metamorphs. All



Federico Pantin installing breeding tanks at the Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo. Photo: Margarita Lampo.

tanks have UVB light with automatic dial. Each breeding tank has three air diffusion stones, an external water storage container with a filter and a pump, and a water levelling system that allows for switching from low to high water level and easy replenishing of tanks. Maintenance tanks have an automatic misting system and wastewater collectors. All automatic systems can be monitored and controlled remotely through intelligent phones and alarms are deployed if any system fails. Also, a small space was refurbished and equipped for maintaining live food colonies of crickets, flies and collembola. To raise awareness among the community of Leslie Pantin Zoo visitors, the *ex situ* facility has an exhibit tank facing outside and a window through which visitors can peak into lab's activities.

The opening of the Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo. Our team: Federico Pantin, Ingrid Márquez, Onil Ballestas and Margarita Lampo. Photo: Jaime Nestares.

In addition to completing the Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo, we obtained additional permits for FUDECI's lab in Caracas to operate as a second facility. Therefore, the Center now has two facilities. Due to its proximity with the genetic laboratory at the Venezuelan Institute for Scientific Research in Caracas, testing for *Bd* and treatment of all animals prior to their arrival at the Leslie Pantin Zoo will take place in Caracas, in a separate space designated for quarantine, with small cages for housing single individuals during quarantine or treatment. The Center´s facility in Caracas was equipped under the same standards of Leslie Pantin Zoo with a capacity for twelve 10-gallon maintenance tanks, five breeding tanks and ten small plastic mouse cages for rearing metamorphs. This lab is fully operating with four females and four males. In September, one female died with signs of an infection (possibly bacterial). As we could not diagnose *Bd* (waiting for funds for PCR reagents), all animals were treated with itraconazole and enrofloxacin.

The Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo began operation after an initial training session on adult husbandry and the arrival of the first two adult toads. Training on breeding, egg and tadpole management will continue in December. We have an Action Plan for the Rancho Grande Harlequin Toad, which is continuously updated as we gather information and gain experience.

We have drafted husbandry and breeding guidelines, however these need to be adjusted as we gather information and gain experience. For example, sections describing breeding, spawning and tadpole husbandry have not been fully developed as we are still investigating the climatic cues these toads use for triggering spawning and the water parameters that maximize the percent of tadpoles successfully completing metamorphosis.

The estimated cost of the Center for the Reproduction and Research for Harlequins at Leslie Pantin Zoo was approximately US $8,000. The Amphibian Ark Conservation Grant supported US $5,000 of this, with the additional expenditure being covered in-house by Federico Pantin, the Zoo Director, and Margarita Lampo.

Our goals during the next twelve months include:

- Completing the collection of twenty founders
- Obtaining our first egg batches
- Raising healthy tadpoles to metamorphosis
- Rearing juveniles
- Completing the husbandry and breeding protocols
- Updating the Action Plan
- Securing funds for 2023-2024 operation.

To achieve these goals the following activities have been planned:

- Attend the assisted reproduction training course at Centro Jambatu in Quito, Ecuador (December)
- Conduct two field trips to collect adults and complete the twenty founders (December and January)
- Conduct a training course on breeding and egg and tadpole management at Leslie Pantin Zoo (January)
- Transfer new animals to Leslie Pantin Zoo after quarantined at CRIA-Caracas (January-February).
- Continue to manage captive individuals to obtain egg batches and raise tadpoles to metamorphosis in Leslie Pantin Zoo and Caracas.
- Install infographic dispplay on the Rancho Grande Harlequin Toad facility at Leslie Pantin Zoo (February).
- Submit proposals to Rufford, National Geography, Prince Bernard Nature Fund.
- Submit proposal for a program extension grant to AArk.

Onil Ballestas analyzing water quality at the Center for the Reproduction and Research for Harlequins in Caracas. Photo: Margarita Lampo.

Setbacks and advances at Project Palaka

Norman Greenhawk, Project Palaka, The Philippines

Conservation projects - like all endeavors - have ups and downs. As with conservationists the world over, in 2020 and part of 2021 we had to deal with the COVID-19 pandemic, navigate the subsequent lockdowns and travel restrictions, and implement safety precautions to keep ourselves safe. In spite of this, the Project Palaka team were able to conduct two field work trips to the Gigantes Islands in the Philippines to work with the Gigante wrinkled ground frog (*Platymantis insulatus*), the only frog species in the Philippines designated as Critically Endangered by the IUCN. Our travels took us to the Gigantes once in July and August of 2021, and again in January and February of 2022. We also established an assurance colony of *P. insulatus* to begin our captive breeding program.

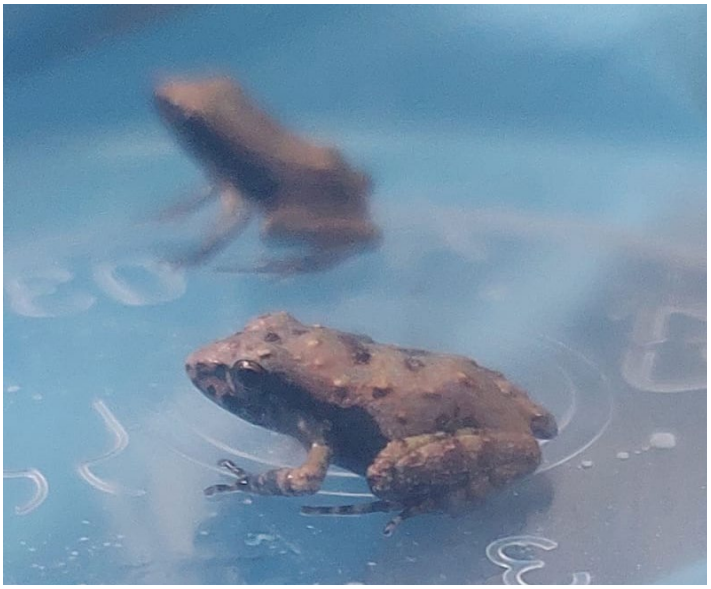
Unfortunately, in May 2022, the team became just me, and I needed to find a new in-country partner. It has been an intense seven months of caring for the frogs on my own, while also coordinating with government agencies and seeking new in-country partnerships.

Challenges like this can provide opportunities to identify weaknesses and strengthen a project. Project Palaka has reached an agreement with two new in-country partners, and we are in talks to potentially add additional organizations as well. My hope is that by having a more inclusive network of in-country partners, Project Palaka will have the solid footing it needs to weather future challenges and prevent further disruptions. Additionally, by the end of the year, the project will be incorporated independently as a Philippine nonprofit organization. We plan to have our project moved to new facilities by the end of January 2023, and to resume our field work by February 2023.

Despite the obstacles we have faced, I am elated to report that a major objective of Project Palaka has been realized. On October 17th, 2022, I was able to witness the first-ever captive hatching of Gigante wrinkled ground frogs. Over the course of five days, twenty-one F1 neonates hatched from a clutch of eggs (descended from frogs collected on Gigantes Sur). After several clutches of infertile eggs had been laid by females since the beginning of our project, it was heartening to see that breeding this species in captivity is indeed possible. The froglets are growing quickly, and eagerly consume pinhead crickets at every feeding.

There are still a lot of unknowns about the natural history of this species. We have an additional seventeen gravid females that we hope to also breed successfully. Eventually, these offspring will be used to further boost the numbers of our captive colony, as well as be released back on Gigantes to boost wild populations.

On a broader scale, I'm happy to announce that my US nonprofit



On October 17th, 2022, the first-ever captive hatching of Gigante wrinkled ground frog (*Platymantis insulatus*) occurred, with twenty-one F1 neonates hatching over five days. Photo: Norman Greenhawk.

was approved by the US IRS (Internal Revenue Service). The Harris Conservation Initiative for Reptiles and Amphibians (HCI for short) was formed in honor of my late grandparents, both of whom encouraged my interest in the natural world from a young age. We already have partnership agreements with organizations in Belize and Puerto Rico, and I am currently working on finalizing agreements with organizations in my home state of Maryland, USA, as well as in other East and South-East Asian countries.

The goal of the nonprofit is two-fold: (1) to facilitate the conservation of overlooked or understudied herpetofauna and (2) to provide opportunities for research and conservation collaboration to students and conservationists in each of the partnering countries. Although Project Palaka will be a Philippine registered nonprofit, it will also be under the "umbrella" of the HCI, which will facilitate international funding and the long-term sustainability of the project, as well as allowing Filipino students the opportunity to travel abroad to other partner projects.

The froglets are growing quickly, and eagerly consume pinhead crickets at every feeding. Photo: Norman Greenhawk.

An adult female Gigante wrinkled ground frog in the conservation program managed by Project Palaka in the Philippines.
Photo: Norman Greenhawk.

New Conservation Needs Assessment report

Kevin Johnson, Amphibian Ark

Our conservation resources are limited, and a vital first step in identifying and prioritizing species for both *ex situ* and *in situ* conservation actions is a thorough assessment of their conservation needs. The Conservation Needs Assessment process (www.conservationneeds.org) developed by the Amphibian Ark uses current knowledge of species in the wild to determine those with the most pressing conservation needs, and to generate recommendations for the most appropriate conservation actions to ensure their survival. These recommendations provide a foundation for the development of conservation action plans which combine *in situ* and *ex situ* actions as appropriate.

There is a new Assessments and Recommendations Summary Report (www.conservationneeds.org/reports/AssessmentsAndRecommendations) in the Conservation Needs Assessment program which includes a summary of the number of completed assessments and the number of each recommended conservation actions, for user-selected regions or countries.

CONSERVATION NEEDS ASSESSMENTS

On the Reports menu, select Assessments and Recommendations Summary Report, then select Amphibia. Click Sub Region, and then select a subregion. Multiple subregions can be selected at the same time, which provides the flexibility to view summaries by continent. As an example, to view assessment and recommendation data for Latin America, you could select Central America and South America from the subregion list.

When a subregion is selected, all countries in the selected region(s) are displayed in a Country list, with all countries automatically selected, but any countries that aren't of interest can be deselected. Click Generate Report to display the results.

The results show the combined summary of recommended conservation actions for all selected countries, including the total number of assessments for each recommended action, and the unique species count for each action – different assessments might exist in different countries for each species.

A list of countries from the previously selected list for which assessments exist, is displayed, along with the number of completed assessments for each country. To display a summary of the recommendations for each country, click the View links.

As of 30 November 2022, 4,228 Conservation Need Assessments have been completed for 3,554 species in 49 countries. This represents 41.9% of the currently known amphibian species. Additional assessments will be added over the coming months. All assessments, and the recommendations generated from them are available at www.conservationneeds.org.

Recommendations Summary - Latin America

Type	Total count	Unique count
Rescue	248	232
In Situ Conservation	763	668
In Situ Research	1166	1057
Husbandry Research	143	130
Applied Ex Situ Research	90	87
Mass Production in Captivity	7	7
Conservation Education	417	367
Supplementation	8	8
Biobanking	248	232
None	204	194

Assessment Summary - Latin America

Type	Total
Approved Assessments	1648
Countries with Assessments	15
Species Assessed	1432

Country	Total	Recommendations
Ecuador	267	View
Bolivia	255	View
Brazil	229	View
Costa Rica	193	View
Guatemala	141	View
Argentina	133	View
Colombia	118	View
Honduras	97	View

Recommendations Summary for Brazil

Type	Total count	Unique count
Rescue	19	18
In Situ Conservation	37	36
In Situ Research	182	181
Husbandry Research	18	18
Applied Ex Situ Research	15	15
Conservation Education	90	89
Biobanking	19	18
None	17	17

Miguel A. Landestoy

First report of reproduction in captivity of the La Banderita marsupial frog from the Amphibian Rescue and Research Center at the Horco Molle Experimental Reserve

Elena Correa, Rojas Paz I., Gabriel Ferderico Rodriguez, Davalos N., Fuensalida E. and Juan Pablo Juliá, Horco Molle Experimental Reserve (Faculty of Natural Sciences - National University of Tucumán), Argentina; Javier Gonzalez Raffo, Neotropical Biodiversity Institute, CONICET-UNT, Argentina; and Martín Boullhesen and Mauricio Akmentins, Institute of Andean Ecoregions, CONICET-UNJu. Argentina

In 2018, the La Banderita marsupial frog (*Gastrotheca gracilis*) project was created with the aim of contributing to the conservation of this endangered species (IUCN SSC Amphibian Specialist Group 2019). This project was initiated by the scarcity of records of occurrence from its historical localities (Akmentins et al 2012), and originally aimed to supplement the populations of this species in the Reserva Provincial Los Sosa, Tucumán province, in Argentina. Given that the tadpole was identified as the most vulnerable stage to the threats detected for the species, tadpoles were collected from the field and then kept in captivity until they reached metamorphosis at the Amphibian Rescue and Research Center at the Horco Molle Experimental Reserve (CRÍA-REHM), of the National University of Tucumán. After a period of quarantine and preventive treatment for chytridiomycosis with standardised Itraconazole baths, the juveniles were returned to their natural environment at the same collection site. Given that captive breeding and research is one of the main objectives of our team and, considering the lack of information on the biology life cycle of this marsupial frog, our project represents an excellent opportunity to deepen our knowledge of the biology of the species.

A male La Banderita marsupial frog (*Gastrotheca gracilis*). Photo: Elena Correa.

Previous results from captive breeding of tadpoles are encouraging, with 90% survival rate and more than 160 post-metamorphic specimens released to date, but no attempt has yet been made to achieve F1 animals from specimens fully bred within our facilities.

Therefore, from the tadpole cohorts rescued at the end of 2020, a batch of individuals was set aside for the *ex situ* breeding attempt. The parents were kept in an aquarium measuring 40 x 50 x 130 cm, which was set up with synthetic grass, plants, moss, logs, containers with fruit flies, a pool measuring 50 x 35 x 10 cm with an aerator and stones inside. They were transferred to this enclosure when the development of external sexual characteristics (marsupium in females) and reproductive behaviour (beginning of calling in males) was observed, resulting in three females and two males.

Axillary amplexus between male 1 and female 2 La Banderita marsupial frogs. Photo: Javier Gonzalez Raffo.

Specimens were fed with live insects (crickets, fruit flies, cockroaches, and terrestrial isopods), kept under controlled conditions of temperature (18 - 22°C) and humidity, with plants, soil and

F1 La Banderita marsupial frog tadpoles. Photo: Omar Saguir.

grass kept moist by means of sprinklers. Two hours of continuous UV light was provided via lamps to ensure vitamin D availability and along with these tasks, recording and monitoring of their behavioural activities was carried out by means of two cameras (Tapo C100) that are activated by movement and have night vision.

A photographic record was taken of the dorsal part of the specimens to identify them, assigning them a number according to sex. The characteristics we considered were coloring, pattern of spots and presence or absence of the marsupium.

After more than a year and a half of growth and maintenance in captivity, on August 3, 2022, amplexus between two individuals was recorded for the first time, which lasted one hour. Subsequently, on October 14 (72 days later), female number 3 extracted fifteen tadpoles and five unfertilized eggs from her marsupium for the first time, an action she carried out inside the pool.

An aquarium where adult La Banderita marsupial frogs are housed. Photo: Elena Correa.

On November 25 (114 days later) we recorded video of the deposition of tadpoles from female number 2, which removed forty-three tadpoles and nine unfertilized eggs. Female number 1 has not yet shown any increase in the size of her marsupium.

The advances presented in this newsletter correspond to the first records of the reproductive behaviour of this species in captivity and contribute greatly to the knowledge of its life history. The mission of this project will now focus on achieving normal development and survival of the individuals from these first litters.

Female N°3 with her marsupium open on the day after deposition. Photo: Omar Saguir.

Acknowledgements

We would like to thank to Amphibian Ark for their continued support since the start of the project in 2018 through their conservation grants program. We would like to thank the Horco Molle Experimental Reserve and the National University of Tucumán for supporting our project and providing us with a place to work. We would also like to thank Vialidad Provincial for their assistance at the sampling sites.

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A gravid female La Banderita marsupial frog. Photo: Omar Saguir.

2022 George and Mary Rabb Fellowship awarded

Amphibian Ark's George and Mary Rabb Fellowship was launched in 2020, and supports early or mid-career scientists and conservationists in research-based professional development that furthers amphibian conservation. This annual Fellowship is awarded to proposals that address species research priorities indicated in the Amphibian Red List and/or the Amphibian Ark Conservation Needs Assessment (CNA), or thematic research priorities indicated in the Amphibian Conservation Action Plan (ACAP). Applicants are formally associated with a mentor or lead scientist at a relevant university, NGO, zoo/aquarium, or other established institution. The fellowship carries a $5,000 stipend and is open to applicants from all countries.

This years' Fellowship was awarded to Dr. Rose Upton, from The University of Newcastle, Australia, for her project "Retention of genetic diversity of the endangered Littlejohn's tree frog through sperm cryopreservation".

The global amphibian community is facing increasing species decline, in a resource constrained environment. For many species, captive breeding is the only viable option to ensure survival where mitigation of threats in wild populations is not possible. However, captive breeding is expensive, and it lacks the financial support of *in situ* conservation initiatives. Innovative solutions are urgently needed to reduce the cost and improve the effectiveness of such programs.

The Amphibian Conservation Action Plan has identified the complimentary conservation approach of using assisted reproductive technologies (ART), such as sperm cryopreservation, as a priority area of research. Progress in translating ART research into application has been a challenge. This grant will provide the opportunity to bridge this gap in an endangered tree frog and a co-occurring threatened ground frog, representing two of the three native Australian frog families.

To incorporate cryopreservation of sperm into the breeding programs for Littlejohn's tree frog (*Litoria littlejohni*) and giant burrowing frog (*Heleioporus australiacus*) Dr. Upton will:

1. Validate the use of a previously developed cryopreservation protocol in a related *Litoria* species in *L. littlejohni*, whilst making preliminary attempts to achieve the same for *H. australiacus* and;
2. Collect and cryopreserve sperm from at least five founder males of the University of Newcastle's *L. littlejohni* breeding program, as well from 15-20 males captured at the Blue Mountains and Central Coast populations.

This years' Fellowship was awarded to Dr. Rose Upton, from The University of Newcastle, Australia, for her project "Retention of genetic diversity of the endangered Littlejohn's tree frog through sperm cryopreservation". Photo: Gemma Wolk.

The project will also target sperm collection and cryopreservation from up to five males from co-occurring species, including the threatened *H. australiacus*. These samples will be stored at the Conservation Biology Research Group at the University of Newcastle for future use within breeding programs. The first objective will be completed during the summer period of 2022/23 when *L. littlejohni* detection is usually low, using five founder males housed in the University of Newcastle breeding program. The trials will be completed using a field-friendly cryopreservation device to ensure the second objective can be achieved in the field.

The giant burrowing frog (*Heleioporus australiacus*) is one of the focal species for Dr. Upton's research project. Photo: Rose Upton.

Littlejohn's tree frog (*Litoria littlejohni*) being swabbed for chytrid. Photo: Nadine Nolan.

Amphibian nutrition webinar series

Luis Carrillo, Training Officer, Amphibian Ark

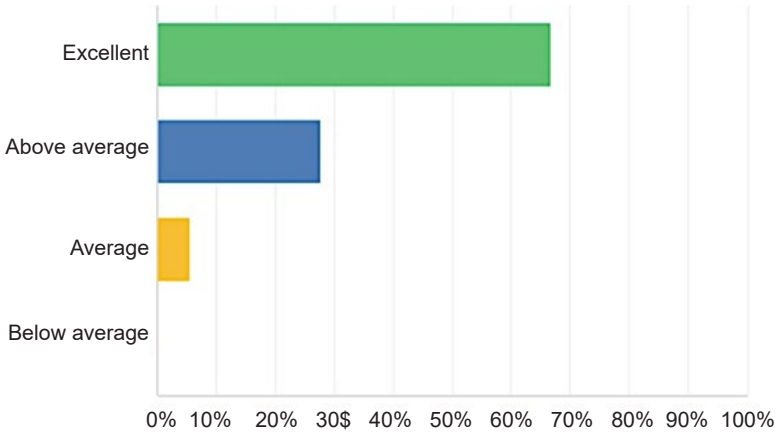
One of the key aspects of animal husbandry and welfare and preventive medicine is proper animal nutrition, and for amphibians, the existing knowledge on this subject is scarce and scattered.

Understanding the nutritional needs of the different orders and groups of species and their different life stages is essential for their well-being, thus, providing proper nutrition to amphibians kept under human care is necessary to ensure they remain healthy.

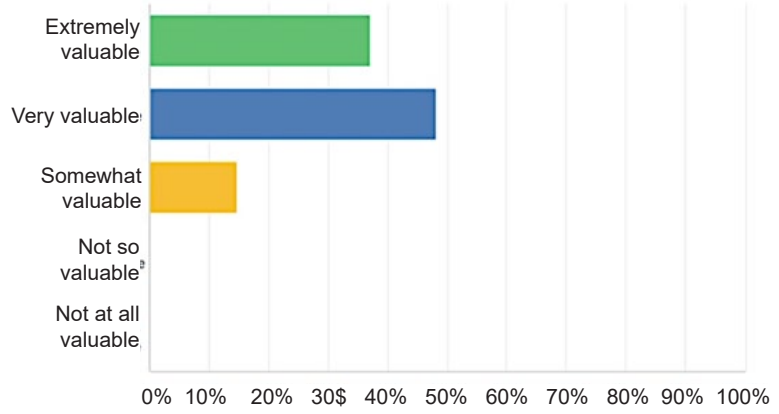
On October 10th,12th, and 14th, Amphibian Ark presented a series of webinars on nutrition and feeding of *ex situ* amphibians. Over 200 professionals, representing twenty-four countries joined these online sessions. Renowned professionals shared their experience and expertise on this very important aspect of the *ex situ* management of amphibians. Topics covered during the webinars were: Importance of nutrition, basics of amphibian nutrition and feeding, feeding ecology and applications in *ex situ* populations; Carotenoids and vitamins in amphibian nutrition; Nutrient composition of insects and gut loading; Establishing live food colonies using "native" species; Importance of UV light for amphibians; Nutritional diseases in amphibians, and Applied nutrition research.

The webinar series had a wide representation of professionals attending from zoos and aquariums, universities, NGOs, and the private sector. The webinars were well-received, with an overall positive assessment from the participants' about their satisfaction and the knowledge they acquired during the webinars.

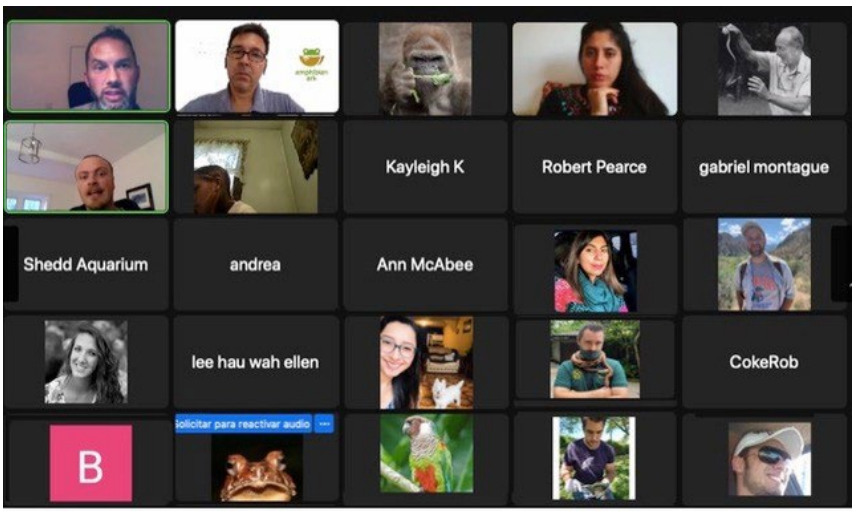
The presenters were also very well-accepted by participants. We would very much like to thank the following instructors and their organizations for sharing their knowledge and experience: Andrea Fidgett, San Diego Zoo Wildlife Alliance; Chris Michaels, ZSL - London Zoo; Arturo Muñoz, Ghent University; Richard Preziosi, University of Plymouth; Dennis Oonincx, Wageningen University and Research Centre; Ben Tapley, ZSL- London Zoo; Allan Pessier, Washington State University; and Amanda Ferguson, ZSL - London Zoo.



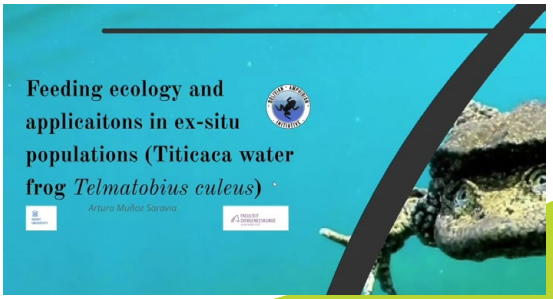
Overall level of satisfaction with the webinar series.



Knowledge acquired from the webinars by the participants.



The nutrition webinar series had a wide representation of professionals attending from zoos and aquariums, universities, NGOs, and the private sector.

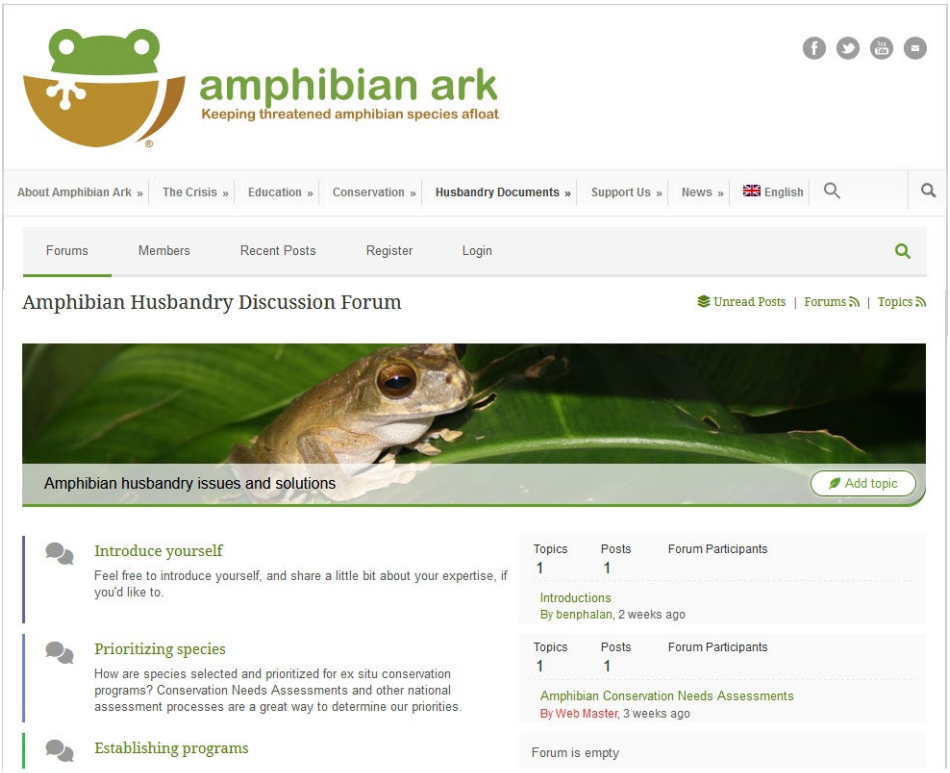


New amphibian husbandry discussion forum

Amphibian Ark has recently set up a new discussion forum on our web site (www.amphibianark.org/forums/husbandry-issues/), which we hope will be of benefit to everyone involved with *ex situ* amphibian programs. It provides a place to post questions and engage in discussions on a range of topics, and to share experiences with other program managers all over the world. As the number of questions and answers increases, and discussions grow, we hope this will become a valuable husbandry-related resource.

The following topics are currently available:

- Prioritizing species
- Establishing programs
- Enclosures
- Light and UV
- Diet and nutrition
- Water quality
- Health and diseases
- Reproduction
- Genetic and demographic management
- Reintroduction/translocation
- Field and monitoring equipment



Additional topics can be included in the forum – please let us know if you would like us to add any – webmaster@amphibianark.org.

If you create a login to the forum, you can subscribe to any of these topics, and you will then receive a notification of any new posts in each topic. You can also choose to be automatically notified when another user posts a reply to any of your posts.

On a similar subject, we have also created a new static web page called Resolving amphibian husbandry issues (www.amphibianark.org/resolving-issues/), which includes a number of published papers on various husbandry issues, and solutions for resolving some of them. This library will also grow over time, so please make use of it, and if you'd like to suggest any additional suitable papers that we can add, please let us know.

We hope you will find this new forum and document library to be useful resources. Please let us know if you have any questions.

Andrew Gray leaves Manchester Museum

Bethany Dean, Manchester Museum, UK

Andrew Gray, who has worked at the Manchester Museum in the UK for the past twenty-seven years, is moving on. During his time as Curator of Herpetology he designed and established The Vivarium on our second floor, a purpose-built amphibian captive breeding facility and public gallery dedicated to highlighting and supporting amphibian conservation. Whilst at the museum he has also been instrumental in establishing several student field courses in the tropics for the University of Manchester, re-discovering frogs thought to be extinct, and described new species to science. He's championed critically endangered frogs and raised awareness of the plight of amphibians through continually developing the Museum's live animal displays and devotedly furthering meaningful related engagement work.

Andrew Gray releasing a variable harlequin frog into a new exhibit at the Manchester Museum's Vivarium. Photo: Mathilda Nyqvist.

The Museum, which will re-open in February 2023, will open its doors to some stunning new vivarium developments that Andrew and his team have been painstakingly completing - including a spectacular harlequin toad (*Atelopus varius*) exhibit which authentically replicates their specific habitat within the Santa Fe National Park, Panama, alongside a new display highlighting the teams' conservation research contributions.

Andrew specialises in Central American species, particularly phyllomedusine frogs of the genus *Cruziohyla*, for which he has an international reputation. However, he is also well known for his important environmental education work and committed *in situ* conservation efforts. His contributions have been recognised with many awards and although he has decided it is now time to leave the Museum, he has recently been awarded an Honorary Senior Lectureship in teaching and research within the Faculty of Biology, Medicine and Health at the University of Manchester. Andrew's research goes from strength to strength, and he assures us he will continue working hard connecting people with nature, especially with amphibians, for life.

First *ex situ* management actions for the conservation of Argentina horned frog in Argentina

Sofía Micaela Perrone, Conservation of Amphibians in Argentina

The Argentina horned frog (*Ceratophrys ornata*) is an endemic anuran of the South American temperate grasslands. This wide distribution has been recently reduced to the Argentine Pampas, and the species' occurrence is associated with grassland remnants. The habitats where Argentina horned frog populations still occur are at risk due to pressure from urbanization and livestock, compromising the viability of the species. The objective of our project is to implement an *Ex Situ* Management Plan (PMES, abbreviation in Spanish) for Argentina horned frogs in one of the priority areas for its conservation in Argentina. This plan includes the rescue and transfer of adult specimens from urbanized areas to sites identified with suitable habitats. Additionally, eggs and larvae from fragmented wetlands will be collected and will be raised to juvenile stages (head-starting) in the reception center prior to their subsequent release in nearby populations. In the long term and in synergy with *in situ* conservation actions, we seek to reduce the impact of habitat loss on relict populations.

The Giant of the Pampas project supplied the center's equipment, and the staff training for animal care has been rescheduled for the second stage (during the next six months). Meanwhile, the government authorities of General Lavalle will provide the center´s facilities. The communication and education strategy has been carried out to support the promotion of the *Ex Situ* Management Plan within the local communities, being fundamental to establishing a citizen network with accurate information to proceed when encountering Argentinean horned frogs. Finally, we will conduct fieldwork campaigns during the species breeding season and after heavy rainfalls.

We propose to promote collaboration between scientific institutions, NGOs, farmers and governmental authorities, and decision-makers to achieve territorial planning that guarantees the effectiveness of the *ex situ* management for Argentina horned frogs in the remnants of Argentinean temperate grasslands.

The *Ex Situ* Management Plan achieved strong links and collaborated with the two municipalities (General Lavalle and La Costa). The authorities have been beneficial and supportive in carrying out the proposed different objectives. Last September, the Education Offices of both municipalities provided us with the necessary connections to schools and coordinated the education and communication strategy. Informative meetings and workshops were held to reach a wider audience and people of different ages. In addition, we attended five local radio and TV programs.

Juvenile Argentina horned frog (*Ceratophrys ornata*) in captivity at the Faculty of Exact and Natural Sciences of the University of Buenos Aires in Argentina. Photo: Javier Gutierrez.

Camila Deutsch working in the facilities at the Faculty of Exact and Natural Sciences of the University of Buenos Aires. Photo: Gabriela Agostini.

We also worked hard with the Municipality of General Lavalle, the NGO Fundación Vida Silvestre Argentina, and park rangers of Campos del Tuyú National Park. Through several meetings, we made progress in outlining a county ordinance that will provide the legal framework to implement the land donation devoted to an educational and cultural reserve where the Reception and Transit Center facilities will be placed.

Additionally, with Fundación Vida Silvestre Argentina, we visited several private reserves and cattle ranches from the Alianza de Pastizal (Grassland Alliance in English), to detect new release sites to be included in the *Ex Situ* Management Plan. These institutions also facilitated new connections with farmers and other stakeholders, allowing the possibility of doing new communication activities with them to raise awareness about the conservation problems facing the Argentina horned frog.

The local community has a key role in implementing the *Ex Situ* Management Plan, as citizens constitute a network that is attentive to the appearance of Argentina horned frogs at risk. They will be the ones to trigger the first alarm and must know how to proceed in such situations (i.e., notify members of our team). Currently, the educational workshops carried out in primary and high school institutions, as well as the participation in fairs and local festivals, have led to an increase in species records.

A local community member, the biologist Pablo Otero, has been especially helpful in recent months. Pablo lives in the area of influence of the management plan and teaches biological science in a technical institution where he leads a laboratory for keeping and maintaining small animals. The collaboration with Pablo and his institution made it possible to deal with the main constraints we faced during this period. Since the county ordinance to formalize the Reception and Transit Center donation has been delayed, Pablo offered his laboratory facilities as a temporary transit center in case animals need it for translocation. So far, four individuals have been found in the backyards of houses and were received in those laboratory facilities. They were all in healthy condition and were marked and released in the corresponding areas. We expected, based on previous breeding season studies, to rescue a greater number of individuals. It is worth noting that the region is suffering from one of the most severe droughts of the last

thirty years, limiting the reproductive events of the species and, consequently, the appearance of specimens.

Additionally, our team has been collaborating with the animal care facilities belonging to the Faculty of Exact and Natural Sciences of the University of Buenos Aires, to receive Argentina horned frogs rescued from the pet trade. We work to provide the necessary care to keep these animals healthy, even though they cannot be released into the wild since we are unaware of the collection localities. This work has allowed several members of our team to increase their knowledge about the primary care of this species (e.g. fine-tuning aspects of feeding). We are also working on preliminary experiences in assisted reproduction. We have successfully obtained fertilized clutches and many larvae reaching metamorphosis.

The Giant of the Pampas team and academic, scientific and governmental authorities are working on a new Action Plan with a national scope for Argentina. A month ago, members of the IUCN Conservation Planning Specialist Group (CPSG) agreed to support a new process, and the first workshop for the Action Plan is scheduled for May 2023.

Over the past six months, we have achieved several goals expected according to our time-line:

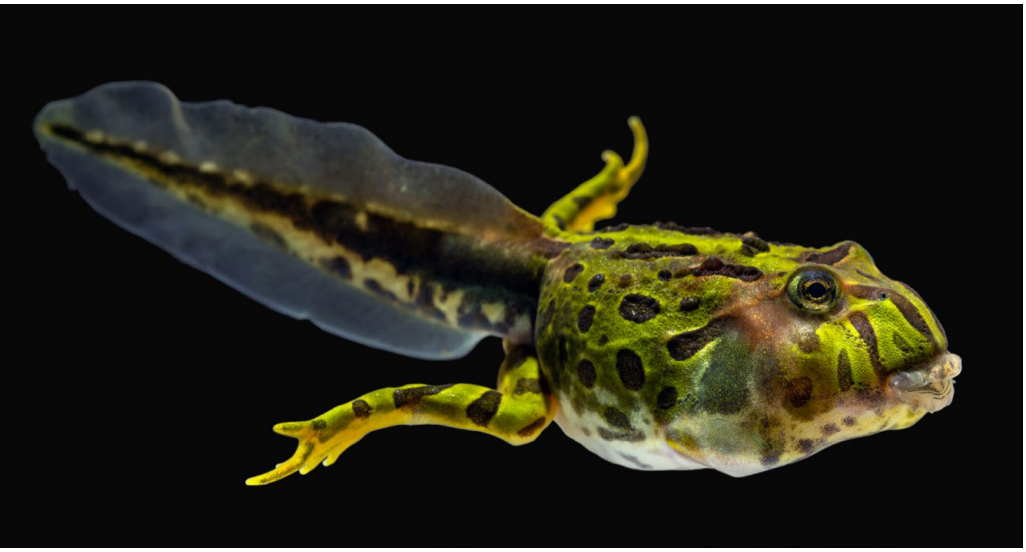
1. The equipment for the Reception and Transit Center has been bought (e.g., glass fish tanks, plastic trays, equipment for measuring water parameters, a GPS, a camera, flashlights, peat for terrariums, batteries, etc.). Part of the equipment and supplies are located in the laboratory facilities of the Institute (General Lavalle Department) and the rest in the animal care facilities at the Faculty of Exact and Natural Sciences (Buenos Aires City). All the equipment will be moved to the Reception and Transit Center once the authorities provide the facilities.
2. The communication and educational strategy have advanced incredibly both in person and in social media. Preliminary analysis on social media metrics (Instagram, Twitter and Facebook) showed rising engagement, followers, and numbers reached in response to new formats and information shared. Meanwhile, during September, educational workshops were carried out in fourteen schools from La Costa and General Lavalle, with the participation of more than 400 children overall. The strategy deployed in local media increased the audience to an estimated 4,000 people.
3. Fieldwork was reduced to identifying suitable habitats for Argentina horned frogs in some private reserves of General Lavalle to be included in the *Ex Situ* Management Plan. The reproductive behaviors of the species are associated with rainfall exceeding 50-60 mm. Sadly, the scarcity of rain in the region, as a consequence of La Niña, determined that reproductive events have not yet occurred. We expect that, as every year, high rainfall events will occur at some point during the summer, at which time the appearance of at-risk specimens is recorded and rescues are carried out. If the species is able to reproduce successfully, we will be able to work on the collection of clutches and larvae in fragmented wetlands.
4. Argentina horned frogs held in animal cares facilities of the Faculty of Exact and Natural Sciences have reproduced in captivity. As mentioned above, this achievement contributes to the necessary knowledge for the center to function successfully, in addition to helping with the correct training of team members.

We have decided to move forward to a National Action Plan for Argentina horned frog instead of the international approach, with help from the CPSG. The results obtained on the occurrence of the species during the last forty years indicate that the distribution is restricted to the Argentine territory. Moreover, the populations that receive conservation priority are in areas where our team has been working for eight years. Therefore, we consider it highly necessary to move forward with an action plan for the species which has a national scope. The first workshop is planned for May 2023 with more than thirty stakeholders from Argentina already involved in the process.

We hope that in the coming months, we will finally finish the negotiations for the declaration of the county ordinance. Then we will be able to fully equip and operate the Reception and Transit Center in Las Chacras. We will also be able to proceed with staff training.

After heavy rainfalls, fieldwork campaigns will be carried out during the species' breeding season (January and February 2023, September 2023 to March 2024). The aim will be to detect breeding choirs in areas of drained or fragmented wetlands, identify amplexus and collect the eggs. During this season, it is highly probable that a significant number of adult specimens at risk will need to be rescued.

Each released adult and froglet will be monitored in September 2023 and animals will have been tagged with PIT (Passive Integrated Transponder) tags.



Argentina horned frog larva in captivity.
Photo: Javier Gutierrez.

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Anne Baker and Robert Lacy, in memory of George Rabb

Bernard and Nancy Karwick

Synchronicity Earth

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