amphibian ark

Keeping threatened amphibian species afloat

Newsletter

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Using radio-telemetry to track survival and disease outcomes in the Mountain Yellow-legged Frog to inform *ex situ* management

Talisin Hammond and Debra Shier, Institute for Conservation Research, San Diego, USA

In June 2019 we used funds from an Amphibian Ark Conservation Grant to purchase radio-transmitters from Advanced Telemetry Systems (model R1655). In August 2019 radio-transmitters were successfully implanted into twenty-one Mountain Yellow-legged Frogs (*Rana muscosa*) by veterinarians at San Diego Zoo Global, in the USA. We had aimed to implant transmitters into thirty frogs, however, some of the individuals weighed less than the minimum required for an implant (and transmitters were slightly more expensive than initially anticipated). After approximately one week of recovery, the frogs were transported to a release site in the San Jacinto Mountains, USA and were reintroduced into the wild along with eighty-seven tadpoles.

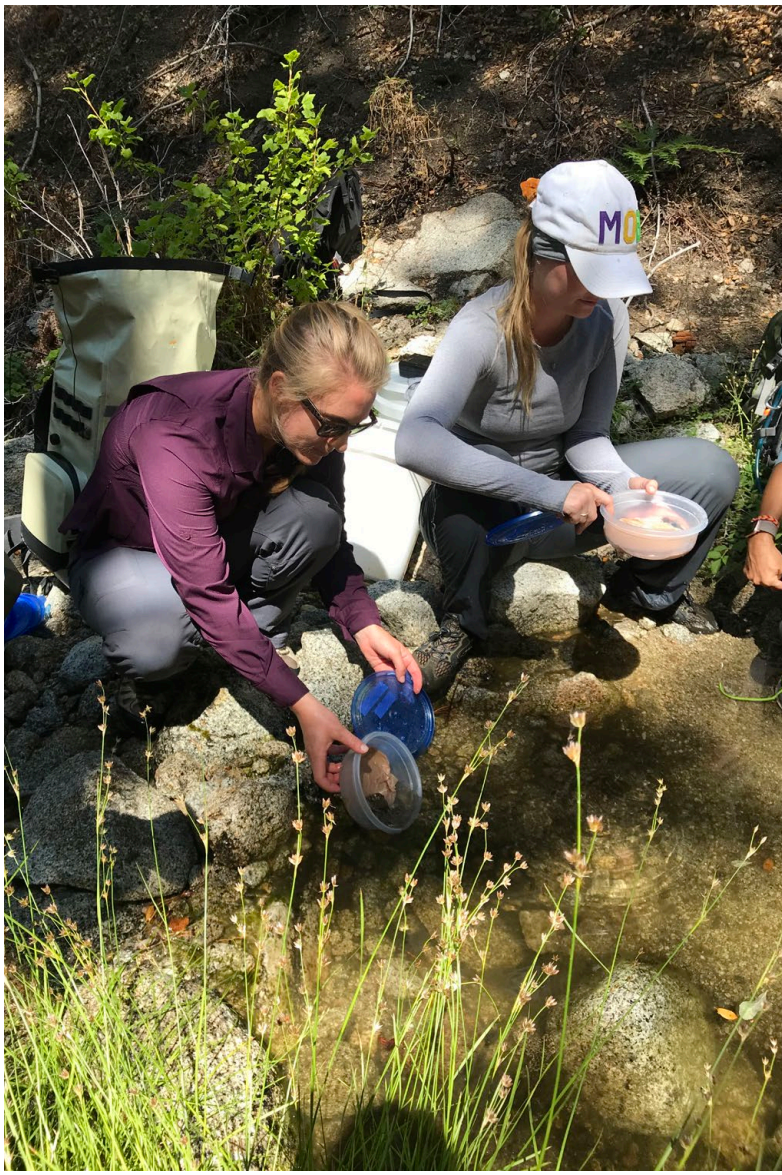
Post-release monitoring took place approximately bi-weekly for the first month after release, and then approximately weekly until late-November, when snow was on the ground and water temperatures were below 5°C (at which point this species is thought to enter hibernation. At that point the access road shut down for the season, however, the site was accessed using snow-shoes, and surveys took place approximately bi-monthly.

Almost every transmittered individual was re-located on each post-release survey (see Table 1). In some cases, due to accessibility and timing issues, not every individual could be located again. Advanced Telemetry Systems estimated that the radio-transmitter batteries would last for approximately seven months at a pulse rate of eight pulses per minute. By January 2020, however, we believe that two transmitters have died (there is an extremely weak signal, indicating that the transmitter/frog may still be in the area, but we are unable to target in to a specific location).

Despite re-locating all or most frogs on each survey, we were rarely able to visually locate frogs. The released animals exhibit highly cryptic behavior and in cases when we have visually confirmed their presence they usually appear to be wedged between rocks or hiding in rock caves. Because frogs do exhibit movement between most surveys, we believe they are still alive, despite not being able to visually confirm their status. Before water temperatures dropped, we were able to visually detect and capture one or two frogs per survey; every captured frog was measured, weighed, and swabbed for chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*). Their surgical incision site was also inspected - all individuals healed very well. *Bd* swabs will be processed in the near future.

Date	Average water temperature (°C)	Total frogs found
7 Sep 2019	14.5	21
11 Sep 2019	10.3	20
16 Sep 2019	13.5	21
20 Sep 2019	10.5	21
25 Sep 2019	12.6	19
28 Sep 2019	11.6	21
3 Oct 2019	9.1	15
10 Oct 2019	9.3	21
17 Oct 2019	9.1	21
25 Oct 2019	8.8	21
28 Oct 2019	8.3	21
6 Nov 2019	5.9	21
14 Nov 2019	7.6	20
24 Nov 2019	3.9	20
11 Dec 2019	3.5	20
5 Jan 2019	1.6	19

Table 1. Post-release telemetry surveys and temperatures.



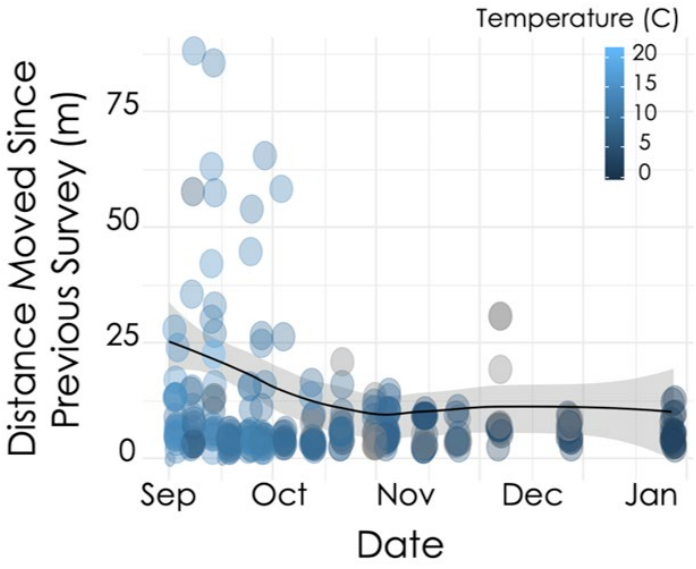
Releasing Mountain Yellow-legged Frogs (*Rana muscosa*) with radio-transmitter implants. Photo: Talisin Hammond.

Post-release movement away from the release location was limited in comparison to other release sites (average < 100 m, in contrast to ~ 500 m at another release site this year). Like other release sites, however, movements were almost exclusively upstream. Movements decreased as water temperatures decreased and date progressed, but there were still slight movements and on two occasions frogs were visually detected even as water temperatures approached freezing. Frogs appear to be hibernating/brumating in habitats that are either in the water or within approximately 2 m of the water's edge.

Conducting radio-telemetry in autumn (left) and winter (right). Photo: Michelle Curtis.

Habitat data is collected on each survey and future analyses will integrate these measures.

Amphibian Ark funding has allowed us to begin collecting valuable data using novel technology, revealing previously unknown information about the natural history of endangered Mountain Yellow-legged Frogs in the southern California distinct population segment. The data we continue to collect will help us to understand post-release movement and survival outcomes for our reintroduced individuals, and ultimately will inform management and conservation activities for this species.



Post-release movement as detected by radio-telemetry, plotted as a function of date and colored by water temperature.

An endangered Mountain Yellow-legged Frog with a radio-transmitter, immediately before release into the wild.
Photo: Talisin Hammond.

Ex situ conservation for the Critically Endangered tree-frog *Aparasphenodon pomba*

Cybele Sabino Lisboa and Cauê Monticelli, São Paulo Zoo, Brazil; and Clodoaldo Lopes Assis, Universidade Federal de Viçosa, Minas Gerais, Brazil

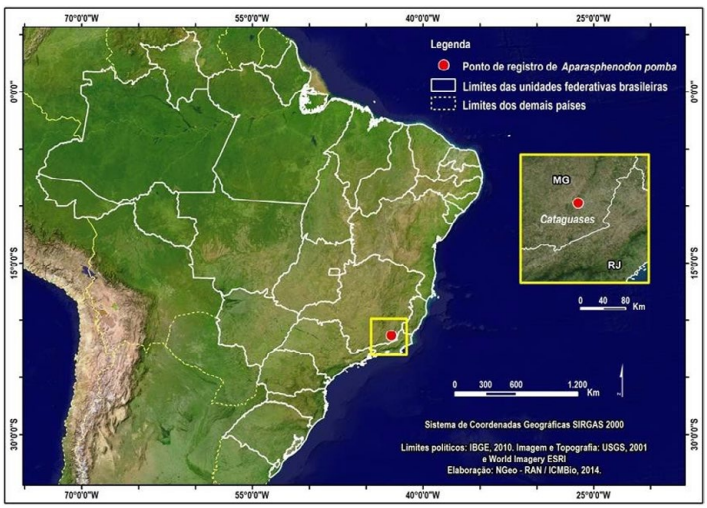
Background

Aparasphenodon pomba is a Critically Endangered tree-frog from Brazil, endemic to a small and non-protected fragment of Atlantic Forest (1.36 km²), which has legal problems and is highly impacted by farming activities. This species was described in 2013 and since then has been showing decline in its population. It is a very rare species and field researchers believe it will be extinct in a few years if nothing is done to help save it. For this reason, an *ex situ* conservation program is deemed urgent and necessary to guarantee that *Aparasphenodon pomba* is saved until threats in nature are solved. In April 2019 we were provided with US $3,000 from a private donor (through Amphibian Ark), which allowed us to undertake a field trip to search for founder animals and to buy basic lab equipment for maintaining them. We are pleased to report on our activities and the beginning of the captive population of *Aparasphenodon pomba*, which was only possible because of this generous donation.

Aparasphenodon pomba is a Critically Endangered tree-frog from Brazil. Photo: Cybele Sabino Lisboa.

Introduction

Aparasphenodon pomba is endemic to Brazil, and the only place where it is known is a 1.36 km² non-protected forest fragment, in the municipality of Cataguases, Minas Gerais. The region was relatively well sampled and the species was not found outside the type locality. The extent of occurrence is already quite modified (there is only 4.6% of native vegetation remnant by 2009) and there is recent loss of forests. The main threat to the species is the conversion of forested area to industrial area (strong activity in the region), which combined with farming activity, cause decline in habitat quality. For these reasons, *Aparasphenodon pomba* was considered Critically Endangered (CR) by criteria B1ab (iii) in the National List of Threatened Animals. Furthermore, the type locality of the species belongs to a sugar production and processing company, which filed for bankruptcy and pledged this property to a bank. At the same time, an employee, who has lived in this place for over seventy years, has applied for the property in court through "extended possession", a right that Brazilian citizens have to claim ownership of a property as a result of its use over a determined period. This legal process has made it difficult to establish partnerships and raise funds for the creation of a protected area in this property, since it does not have a defined owner.



Municipality of Cataguases, Brazil: type locality of *Aparasphenodon pomba*.

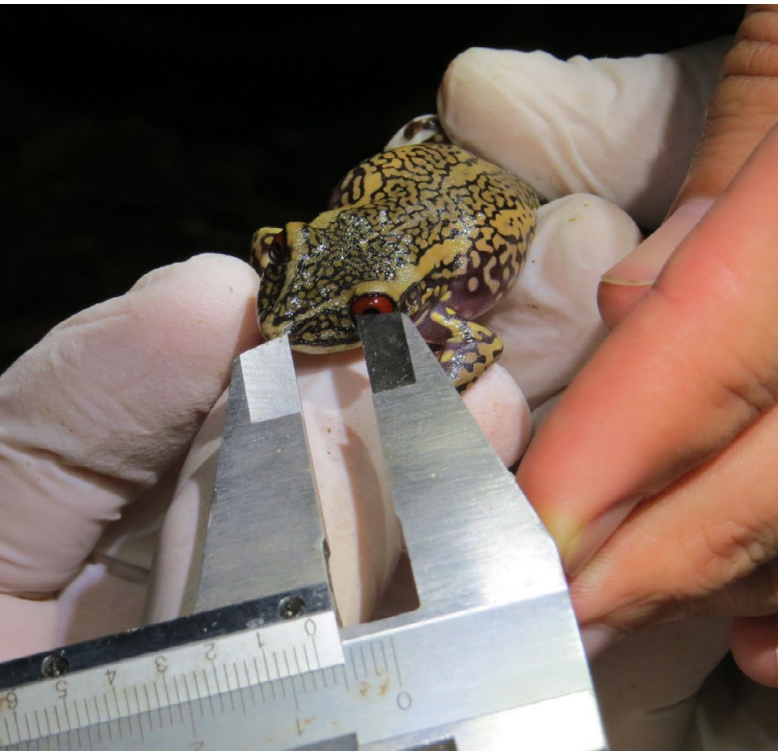
Due to the threats facing the species, *ex situ* management, as well as other conservation actions, is highly recommended and urgent until the threats in nature can be solved. Thus, *Aparasphenodon pomba* was included as one of the twenty-five priority species of the "*Ex Situ Management Program of Threatened Species*" of the Association of Zoos and Aquariums of Brazil (AZAB) in partnership with the Chico Mendes Institute for Biodiversity Conservation (ICMBio). In this program, São Paulo Zoo was listed as the studbook keeper for the species, so we can manage captive populations. To establish this program, a partnership was formed between researchers from the Federal University of Viçosa and technicians from São Paulo Zoo to develop a captive protocol of maintenance and reproduction and to form an *ex situ* back-up population of the species in case there is a need for supplementation of the wild population.

Programa de conservação
PERERECA-PINTADA

Founder acquisition and fieldwork

The first two founders of the *ex situ* population (one male and one juvenile) were transferred to the São Paulo Zoo in September 2019. The animals were being kept at the Federal University of Viçosa and had been previously collected by the partner researcher of this program.

In November 2019, a team or four people conducted a six-day expedition to Cataguases to search for additional founder animals. Photo: Cybele Sabino Lisboa.



All animals that were captured were measured and photographed. Photo: Cybele Sabino Lisboa.

In November 2019, a team or four people conducted a six-day expedition to Cataguases (around 470 miles from São Paulo). Due to the nocturnal habits of the species, our field activities were performed at night, which consisted of auditory and visual encounter surveys in the area of occurrence of the species. We sampled two main points, which are formed by bamboo and swamp with running water. We searched with the aid of lanterns and playing recorded calls of the species, hoping to attract the animals. We also searched for tadpoles in the marshes, using sieves during the day.

Aquariums for *Aparasphenodon pomba* have been set up at the Wildlife Conservation Center of the State of São Paulo. Photo: Cauê Monticelli.

During the field survey we found seven individuals, all male. We also registered a re-capture, and we were able to recognize the individual from its photo identification, a method that has become very useful for monitoring the *in situ* population. All captured individuals were measured, and photos were taken for identification purposes, and some animals were swabbed to investigate the microbiota and the presence of chytrid fungus (*Bathrachochytrium dendrobatidis*) in the wild population. Of the seven males captured, only two were retained as part of the *ex situ* population and the others were released back into the encounter area. We opted to release these individuals since our permit allows the collection of only twelve adults and fifteen tadpoles, and we still need to find females. Another juvenile collected in October by the researcher was added to the group, bringing the captive population to a total of five individuals at the São Paulo Zoo (three males and two juveniles).

Ex situ management

The laboratory for managing the species was set up at the Wildlife Conservation Center of the State of São Paulo (CECFAU), one of the São Paulo Zoo's units, located in Araçoiaba da Serra-SP. We equipped the lab in July 2019 with two aquariums and accessories which were purchased with funds from the Amphibian Ark donation.

We furnished the aquariums with gravel, trunks, bamboo and live plants, and circulating water treated by a canister filter. We installed an artificial rain system and fogger to raise enclosure humidity and to stimulate breeding. We have also provided UVB lighting by using a single 18 watt Exo Terra® Repti Glo 2.0 Compact Fluorescent bulb fixture in each enclosure. To feed the animals, we offered crickets, cockroaches and meal worms supplemented with Repashy Superfoods Calcium Plus ICB® vitamins.

Photo identification is a method that has become very useful for monitoring the *in situ* population. Photo: Cybele Sabino Lisboa.

The food was provided in plastic pots inside the aquariums twice weekly.

The natural markings of each individual enabled us to use photo ID to monitor them in captivity. During the quarantine period all individuals were examined by a veterinarian, who swabbed their skin and collected feces to collect information about the pattern of their normal microbiota and to investigate possible diseases. All individuals were weighted monthly.

Final considerations and next steps

So far, management of the captive population has been shown to be good - all individuals look healthy and have not presented any problems. We evaluated the field campaign positively, in general, as there are previous reports that finding individuals of this species is very difficult, as the species is rare and there is very little information about biology. But we still need to find females to allow the development of breeding protocols and increase the captive population, so we plan to conduct another field trip in January 2020 to look for them, using the remaining funds. We also intend to conduct bi-monthly field trips to monitor the species and to obtain additional biological data, which are key points to contribute to the conservation actions of the species.

AArk Husbandry Document library

The Husbandry Document library on the AArk web site (www.amphibianark.org/husbandry-documents/) currently has over 150 articles in it, with additional articles being added regularly. A new search engine has recently been installed on the Husbandry Documents page, which can now search for particular words or phrases within all pdf files. This results in much more accurate results when searching the document library for particular topics.

Three new documents have been added recently:

Husbandry Guidelines for *Ambystoma dumerilii* (Spanish)
Author: Biól. Manuel Antonio Pérez Rodríguez, Coordinador de proyectos de Investigación y Conservación de la Fauna, Zacango Ecological Park, Mexico
Publication: February 2020
www.amphibianark.org/wp-content/uploads/2020/02/Guia-para-el-manejo-de-Patzcuaro-Salamander.pdf

Action plan for the recovery of the Lake Pátzcuaro axolotl (*Ambystoma dumerilii*) (Spanish)
Author: Biol Manuel Antonio Pérez Rodríguez, Coordinador de proyectos de Investigación y Conservación de la Fauna, Zacango Ecological Park, Mexico.
Publication: February 2020
www.amphibianark.org/wp-content/uploads/2020/02/Plan-de-Acción-para-la-recuperación-del-ajolote-del-que-habita-en-el-Lago-de-Pátzcuaro.pdf

Action plan for the conservation of the Laguna Blanca Frog (*Atelognathus patagonicus*) in Laguna Blanca National Park (Spanish)
Author: Kacoliris, F.P., Cuello, M.E., Úbeda, C., Buria, L., Pastore, H., Rodrigo Calvo, y Chazarreta, L.
Publication: February 2020
www.amphibianark.org/wp-content/uploads/2020/02/Plan-de-Acción-para-Atelognathus-patagonicus.pdf

Amphibian Ark Conservation Grants – We're calling for proposals!

AArk has offered grants since 2009 and in the past, these have been predominantly seed grants, for newly created *ex situ* programs for species that have been assessed as in need of urgent *ex situ* rescue.

In 2018 our grants program was expanded, to include a wider range of programs types that are eligible for funding, as well as some new guidelines and requirements for grant recipients. Download the complete guidelines from www.amphibianark.org/grants/AArk-Conservation-Grants.pdf.

We will be accepting Project Outline funding applications (see below) for the following types of grants from 17th March 2020:

- **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. We will not fund projects that are already well-established or have significant funding, although we will consider projects with funding in place for complementary components (such as fieldwork or education). One-time grants of up to US$5,000 are available. Recipients are able to apply for second and third year extension grants.
- **Start-up grant extensions** – additional funds are available to provide continued support for AArk seed or start-up grant projects that a) have met their stated objectives for year one, and b) can demonstrate that additional supplemental funds have been secured for years two and three. Recipients of funding from the AArk in 2017-2019 are eligible to apply for these extensions. Grants of up to US$4,000 for year 2 and US$3,000 for year 3 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.

All applicants are required to submit a brief Project Outline, prior to submitting a full application. Your Project Outline should be less than 200 words in length and should contain information under the following headings: Species, Organization, Project Manager, Goals, Proposed Outcomes and Other funding Sources (both requested and received). 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:

- Workshops
- 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 of 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: 17 April 2020
- Grant application deadline: 22 May 2020
- Grant decision/notification date: 12 June 2020
- Successful applicants must provide bank account details, signed MOU and 3-4 photos of species and/or facilities by: 15 June 2020
- Grant payment date: 1 July 2020
- Initial progress report and species action plan provided by 1 December 2020
- Final progress report, species action plan and husbandry guidelines due 1 June 2021.

We would like to acknowledge the generous support of AArk funders and donors who have helped to establish and support these grants.

Captive reproduction of the Titicaca Water Frog at the Huachipa Zoo, Lima, Peru

Lizette Bermúdez, José Flores and Michael Tello, Huachipa Zoo, Peru

The Titicaca Water Frog (T*elmatobius culeus*), is a species of aquatic frog, endemic to Lake Titicaca in Peru and Bolivia. It is currently listed as Critically Endangered in the IUCN Red List, Critically Endangered in Peru and included in CITES APPENDIX I at CoP17. Among its main threats is the hunting of adults for use in healing shakes for human consumption. There is also a potential problem with introduced species that prey on larvae and compete for food with adult frogs. Another major threat is the contamination of lake waters by organochlorines and organophosphates.

In 2010 the Huachipa Zoo began a conservation project for the Titicaca Water Frog in agreement with the Denver Zoo in USA, and the project includes four components of which the Huachipa Zoo manages the educational component and captive management.

The first specimens that arrived at the Huachipa Zoo were two pairs delivered as temporary custody of a seizure carried out by the Forest and Wildlife Service - Ministry of Agriculture (SERFOR) in 2010, with these specimens forming two reproductive pairs, however only one of them prospered (P1).

Arrival date	Sex	Origin	Situation	Date of death	Group
23/07/2010	Female	SERFOR	P(RM0281/13)	9/02/2014	P1
10/12/2010	Male	SERFOR	P(RM0281/13)	4/03/2014	P1
26/07/2010	Male	SERFOR	Custodia	21/08/2010	P2
10/12/2010	Female	SERFOR	Custodia	15/04/2011	P2

Table 1: The first four founder animals at the Huachipa Zoo.

A pair of Titicaca Water Frogs (*Telmatobius culeus*) in amplexus. Photo: Lizette Bermúdez.

The pair (P1) was placed in a 900-liter cement tank measuring 1.9 m x 0.68 m x 0.70 m, with gravel as a substrate and without plants. The tank included a biological filter and cooling system (chiller).

In 2011, the first batch of eggs was laid at the bottom of the tank, however we observed that the eggs were crushed, when the

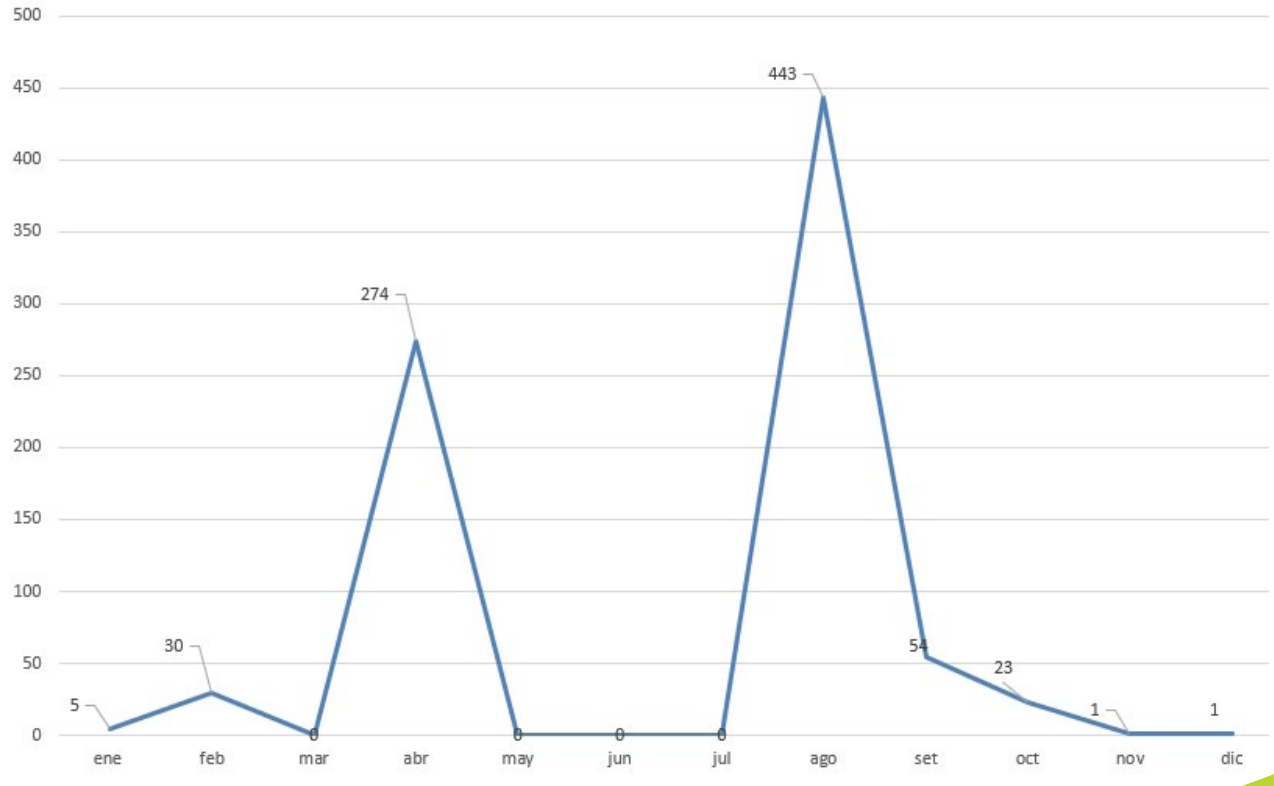


Figure 1: Number of births of Titicaca Water Frogs per month (2011-2019).

The Titicaca Water Frog is a species of aquatic frog, endemic to Lake Titicaca in Peru and Bolivia. It is currently listed as Critically Endangered in the IUCN Red List, and Critically Endangered in Peru. Photo: Lizette Bermúdez.

Generation	Total births	Date of birth	Number of individuals	Alive in October 2019	Parents
F1	183	January 2011	5	0	P1
		April 2011	154	0	
		October 2012	23	0	
		December 2013	1	0	
F2	564	November 2014	1	0	F1 (October 2012)
		April 2015	120	11	
				20 (Exported)	
		August 2015	443	0	
F3	84	February 2019	30	23	F2 (April 2015)
		September 2019	54	54	
TOTAL	**831**				

Table 2: Birth history of Titicaca Water Frogs at the Huachipa Zoo (2011 - 2019).

= Live animals.

adults moved the gravel, so the eggs were removed. After five days a total of five individuals hatched, although they did not survive. Modifications were made to the tank, with the substrate being removed and artificial plants were added to the tank. In April of the same year a second batch of eggs was laid, with a total of 154 hatched eggs, these were kept with the parents in the tank. This first nucleus produced a total of 183 individuals in three years, with the highest numbers produced in 2011.

The Zoo has produced three generations of individuals, as shown in Table 2, with a total of 831 individuals hatched. In November 2015, twenty second-generation individuals born in April 2015 were transferred to the Denver Zoo in the USA. Currently the Zoo has eighty-eight living individuals belonging to generations F2 and F3.

According to the data analyzed about the reproduction of frogs at the Zoo we can infer that frogs are reproductive at the approximately 2-3 years of age; a pair can produce up to 154 eggs with a maximum of two batches of eggs laid per year. There are two reproductive peaks, one in April and one in August, but they do not always reproduce in the two periods. It has also been observed that from May to July eggs are not laid. The maximum lifetime recorded in captivity was 4.6 years.

Throughout these nine years we have achieved successful reproduction of this species and produced standardized captive management protocols. Being a colony from confiscated animals and a single reproductive nucleus, we do not consider the animals produced as suitable for reintroduction, but for studies that provide relevant information for the maintenance for this and other related species.

The team from Huachipa Zoo on a field trip in Peru. Photo: Luis Castillo Roque.

Implementation of behavioural enrichment for the Pickersgill's Reed Frog

Candice K. Ward, Behavioural Enrichment Officer; Ian du Plessis, Curator: Reptiles, Fish and Amphibians; and Piet Malepa, Animal Welfare Manager, Johannesburg City Parks and Zoo, South Africa

Background

The Amphibian Research Project of the Johannesburg City Parks and Zoo in South Africa was initiated in 2006 to assist with the conservation of endangered South African amphibian species. The increase of habitat loss and pollution, as well as climate change, has made it necessary for South Africa to start plans to prevent and to counter the loss of amphibian biodiversity in the country. This project focused on Pickersgill's Reed Frog (*Hyperolius pickersgilli*), the species of highest national concern, which was classified on the IUCN Red List as Endangered, because of the small area of occurrence which is severely fragmented, and the decline of suitable habitat (IUCN, 2016).

The Pickersgill's Reed Frog is a small reed frog endemic to KwaZulu-Natal, South Africa; with males measuring up to 22 mm and females, 28 mm in length (Raw, 1982). While KwaZulu-Natal's coastline is 2,303 km^2, the area of occurrence of the Pickersgill's Reed Frog is calculated to be only 9 km^2 (Measey, 2011) in which the Pickersgill's Reed Frog can only be found in a few wetlands and estuaries that are all located within 20 km of the coastline.

The Johannesburg City Parks and Zoo's Amphibian Research Project (JCPZ-ARP) implemented an enrichment program to support the insurance population's survival once released back into their natural environment. To date the Johannesburg Zoo has successfully released 250 captive-bred specimens back into their natural habitat.

Objective

The purpose of behavioural enrichment is to improve animal welfare by providing opportunities for the animals to express species - typical behaviours and eliminate stereotypical behaviour.

A female Pickersgill's Reed Frog (*Hyperolius pickersgilli*) at Johannesburg City Parks and Zoo. Photo: Candice Ward.

Geographic range of Pickersgill's Reed Frog (*Hyperolius pickergilli*). IUCN (International Union for Conservation), Conservation International and South African Frog Re-assessment Group (SA-FroG), 2016. The IUCN Red list of Threatened Species. Version 2019-2.

Environmental enrichment is the component of daily animal husbandry focused on how animals interact with their physical and social environment. Behavioural enrichment allows captive animals a wider choice of natural behaviours in which to engage (Young, 2003). This is a critical component in achieving the highest level of animal husbandry practices particularly in modern zoos.

Motivation towards the study

Behavioural and environmental enrichment is a vital part of modern captive animal management. By supplying enrichment; animals are provided with mental stimulation, and are encouraged to display natural behaviours, make use of their entire enclosure and make choices about the activities they partake in, thus enabling them to have some control over their own environment (Young, 2003). Behavioural and environmental enrichment programs go a long way to contributing to the animals' overall welfare by:

- Increasing positive utilization of space
- Increasing the range of expressed innate behaviour
- Enabling animals to exhibit species-specific behaviours
- Influencing behavioural repertoires and stress levels positively
- Preparing frogs for release.

With the successful breeding of Pickersgill's Reed Frog on an annual basis, behavioural enrichment is an important pillar to assist and prepare the specimens for a reinforcement, introduction or reintroduction of captive-bred specimens back into their natural habitat. It is also a way to stimulate them in such a way that their natural instincts will be enhanced to equip and enable them to survive after translocation to the natural environment.

Goal setting	Implementation	Observation	Measures of success and welfare
Natural and Individual behaviour			
1. Learned behaviour / classical conditioning	Introducing stressors (increase and decrease in temperature/ changes in food and water quantity and quality.) Stressors need to be controlled to avoid chronic stress	Fear in juveniles vs. desensitization and habituation in adults	Stress reaction (acute vs chronic) Body condition
2. Innate behaviour	Competition calls from visible males in adjacent enclosures Alternative food items, breeding stimulation (spraying that mimics rain) Spraying enclosure with water mimicking rainfall and optimal temperatures Providing vertical furniture (reeds, grass, bamboo,)	Communication Feeding Reproduction Migratory behaviour	Vocalizations, colour change, visual Normal food consumption Vocalizations Amplexus Egg laying and fertilization Vertical migration on reeds; determined by age, season and courtship
3. Environmental perception	Enclosure design- size, landscaping, plants, water quantity, hiding spaces, appropriate substrates	Enclosure utilization	Normal development (juveniles) Maintenance of physical health and normal behaviour in adults
Hedonic budget			
4. Behavioural repertoire	Alternative food presentation (feed puzzle) Changes in furniture, substrate and amount of water (increase or decrease accordingly)	Time engaged in hunting and feeding Sitting, hopping, swimming, inactivity	Body condition Time active and inactive (too much vs. too little)
Interactions			
5. Intraspecific	Optimal sex ratios Housing together or in adjacent enclosures Optimal number of specimens being housed together during life stage (juvenile vs adults)	Mimic natural social structure Reproduction Competition Breeding Change in social structure and resultant behaviour during development e.g. immature males lower down vs. adult males are calling higher up furniture Seasonal changes in social structure	Healthy competition Vocalizations Mate selection Breeding success
6. Interspecific	Human interaction; during enclosure servicing and feeding	Reaction to humans	Avoidance behaviour Jumping/hiding/ swimming/ changing colour

Table for evaluation of enrichment implementation.

Pre-release: using enrichment as a tool

Although research on environmental enrichment has been more focused on mammalian species, reptiles and amphibians can also benefit when afforded the choice to engage in more innate behaviours. An environmental and behavioural enrichment program that is used during the *ex situ* phase, particularly while preparing the frogs for release, may aid in the success of the conservation and translocation process of the Amphibian Research Project.

The Johannesburg City Parks and Zoo enrichment program is based on a framework developed by Disney's Animal Kingdoms, Science and Environmental Animal Enrichment Program. The SPIDER (Setting goals, Planning, Implementation, Documentation, Evaluation and Re-adjustments) framework allows the Zoo to review, refine, and modify this model to fit its own needs (Mellen & MacPhee, 2001).

Setting goals

The goal of behavioural enrichment is to enhance a captive animal's living conditions beyond the primary husbandry require-

ments needed for survival. There are a number of factors that need to be considered in detail when setting enrichment goals. These include the natural history and individual history of the species as well as the exhibit in which the animal is to be housed. What behaviours should be encouraged or discouraged through enrichment activities. There are several categories of enrichment; however, when looking into the requirements of amphibians, the focus will be on feeding behaviour, social behaviour, sensory behaviour and enclosure design.

Planning

Planning involves the formation of an enrichment program to achieve desired behavioural goals. The enrichment activities should allow animals to have choice and control over their environment.

Plan development decisions

- Which behaviours to encourage or discourage?
- What resources are needed to create the enrichment activity?
- Who will be involved?
- Are there any safety concerns?

Implementation

Implementing an enrichment plan is provided for physically carrying out the enrichment activities. Implementation requires:

- Scheduling enrichment
- Ensuring the enrichment items are available
- Providing activities in order to create novel presentation methods
- Varied schedules.

Enrichment activities

- Leaf variation (reeds, Cyprus)
- Rocks – to encourage movement
- Feeding variations (crickets / flies)
- Variety of feeding containers
- Alternative or additional substrates
- Enclosure furniture rotation
- Hiding spots (inside bamboo)
- Exhibit design.

Documentation

By documenting the enrichment provided the institution can evaluate the success or failures of the enrichment activities and make decisions on whether to continue, discard or make adjustments to the activities.

Animal collection staff are required to make use of the enrichment schedule and observation record documents detailing:

- The date enrichment was provided
- What species were being recorded?
- What enrichment activity was provided?
- Description of observations.

Additional tools

- Video recordings
- Photos.

Evaluation

Evaluating the results of the documentation to determine the effect of the enrichment activities. Evaluating the success or failure of activities, to repeat successful activities and discard or adjust failed activities.

Evaluation of the results needs to happen on a regular basis by enrichment officer, curators and keepers:

- Observations
- Meetings
- Individual evaluation of enrichment activities from records.

Re-adjustments

Re-adjustments need to occur throughout the enrichment program. Alterations to the enrichment plan are required during or after implementation of enrichment activities, and after evaluation of enrichment records have been completed. If results are poor, identify the cause and revert back to the goal-setting phase.

Conclusion

The implementation of enrichment for the Pickersgill's Reed Frog plays a role in ensuring the survival of captive bred-specimens *in situ*. A pre-release protocol was drafted and implemented whereby activities were based on the behavioural repertoire of the species observed in the wild. This enrichment program contributed to the success of the *ex situ* breeding program as well as ensuring the sustainability consistent within the insurance population *in situ*.

References

Mellen, J., & MacPhee, M. S. (2001). Philosophy of environmental enrichment: Past, present, and future. Zoo Biology, 20, 211–226.

Young, RJ, (2003). Environmental enrichment for captive animals. Blackwell Science Ltd.

First steps towards the conservation of the Darwin's Blackish Toad

Igor Berkunsky, National University of the Centre of Buenos Aires Province, Argentina

The Darwin's Blackish Toad (*Melanophryniscus nigricans*) is a threatened, recently described species. In the past, this toad was considered as an unnamed taxon belonging to the *Melanophryniscus stelzneri* group. Since 1970, the wild populations of this species have dramatically declined by more than 70%. At least two well-known populations have become extinct, and a third one is probably extinct. The remnant populations are facing a combination of threats which include habitat loss by forestry, invasive woody species, and quarries; overgrazing and trampling by livestock; chytrid fungus; and desiccation caused by climate change.

In 2017 we started a conservation initiative aimed at identifying the main threats and exploring effective conservation actions to recover the populations of the Darwin's Blackish Toad. Natural recolonization by the species is unlikely, due to current fragmentation and the lack of corridors between highland grassland remnants.

This project hopes to increase the area of occupancy of Darwin's Blackish Toad. With the help of an Amphibian Ark Conservation Grant in 2019, during the last six months we built a small *ex situ* facility at the campus of the National University of the Centre of Buenos Aires Province and we safely established a breeding group of twelve individuals (six males and six females). The breeding stock have not yet laid eggs, however, we collected eggs from wild clutches on two separate occasions. In November 2019 we bred a clutch of 150 eggs, and we maintained tadpoles in captivity until they become juveniles. In February 2020, we had a second clutch of eggs.

Individuals produced in captivity will be translocated to a restored and protected habitat in the Sierra del Tigre Natural Reserve by the end of Autumn.

Currently, only one protected area (the Sierra del Tigre Natural Reserve) provides effective protection for a wild population of this toad, and the managers of the reserve have initiated a habitat restoration project aimed at providing more habitat for the Darwin's Blackish Toad. The local university is also involved in this initiative. They provided an old house to be rennovated as main building for the Amphibian Ark initiative. They also provided us with a small grant to restore the habitat for the future release individuals at the Reserve.

A pair of Darwin's Blackish Toads (*Melanophryniscus nigricans*) laying eggs in captivity. Photo: Igor Berkunsky.

Biologists and veterinarians looking for fresh clutches of wild breeding pairs of Darwin's Blackish Toad in Argentina. Photo: Gerardo Irirarte.

Darwin's Blackish Toad tadpoles at sixteen days old. Photo: Igor Berkunsky.

After the first six months, we have achieved the following successes:

- We have maintained adults in captivity during this period with zero mortality and an appropriate diet which has resulted in no weight loss in individuals. The main food item for adult frogs is termites. Termites and ants are the main item reported by studies conducted in wild individuals. We prepared a small space at the university to set up the terraria and fish tanks. We provided the appropriate photoperiod in the room, UV lamps, filters and other equipment.
- We successfully collected egg clutches from wild individuals and we successfully reared the tadpoles in captivity. During two reproductive events we collected two pairs of toads that were in amplexus. We provided them with a clean fish tank containing artificial vegetation, and the breeding pairs laid eggs just a few hours later. We released the breeding adults, and we kept the eggs, which had really high rates of fertility. Nearly 95% of the eggs were fertile and most of tadpoles survived the first week. In our first clutch we had two mortality events. The first one was associated with a change of water, and the second one was due to an electricity failure during a weekend, which resulted in no air pumps and no filters for a 48-hour period. After these events we ended up with approximately thirty juveniles from an original clutch of 180 eggs. The second clutch has now hatched (approximately 120 tadpoles), and no tadpole mortality has been recorded at this stage.
- We were able to maintain some juveniles, after many attempts to find the right diet. Most of the first thirty juveniles we produced died from starvation, and it took us many attempts to find the right diet. Juveniles are very small (between 2 and 2.5 mm) and it's really hard to find arthropods small enough to be eaten by juvenile toads. We finally found a solution by using wire mesh sieves. We collect leaf litter and we sieve it to obtain small arthropods. We have now managed to keep four juveniles alive, which are now forty-five days old, and they are large enough to eat small termites. We are still providing them with arthropods from leaf litter.
- We participated in a local science event.

We are now waiting for the acceptance of the manuscript which describes the species. The manuscript is in second review, and we hope it will be accepted soon. After that, we will begin to write a species action plan. We plan to conduct a workshop in March to produce an advanced draft of the plan, and the final version will be submitted to the provincial authorities, once the species is formally identified and gets is name.

We are also working on developing husbandry guidelines with details about requirements and diet of tadpoles, juveniles and adults. We have been documenting every step, but we have not yet completed a draft of this document.

Since receiving the conservation grant from the Amphibian Ark we have also received a small grant from our university, the National University of the Centre of Buenos Aires Province, which will be used to restore the site where we plan to release the captive-bred individuals at the Sierra del Tigre Natural Reserve. The grant of almost US $1,000 is for one year only.

Future plans

Our goals for the next twelve months are:

1. To successfully breed captive adults. We are designing a rain chamber to stimulate breeding activity. Our first goal was to keep them alive, so now we are looking to stimulate breeding behavior.
2. Explore the collection of eggs and headstarting young toads as an alternative solution, at least for the first couple of years.
3. Produce at least 200 juveniles.
4. Release at least two founder populations of 100 juveniles in two restored sites and monitor them to confirm their progress.
5. Recycle and restore the old house provided by our university to work as the main building for our *ex situ* initiative.

A sign at a local science fair to recruit Darwin's Blackish Toad guardians. It says: "I am a guardian of the Darwin's Blackish Toad!". Photo: Igor Berkunsky.

News from the Patagonia Frog rescue center and conservation project in Laguna Blanca National Park, Argentina

Federico Pablo Kacoliris, Coordinator, Wild Plateau Initiative La Plata Museum; María Elena Cuello, Conservationist; and Leonardo Buria, Administración de Parques Nacionales, Argentina

The Patagonia Frog (*Atelognathus patagonicus*), is an endemic species that is known to inhabit twenty-three isolated lagoons scattered over the volcanic tablelands of Neuquen province, in Argentina. The Patagonia Frog was very abundant in the White Lagoon (Laguna Blanca National Park), the only permanent waterbody within the range of this species. Other subpopulations of this species were common at smaller and temporary lagoons, that become totally dry once a year, during the dry season. This seasonality did not usually affect the Patagonia Frog since this species has evolved to face the cycle of droughts that characterize the area. This frog has evolved to change itself between two morphs as a way of facing different and extreme weather conditions. When lagoons contain good quality water, the frogs adopt an aquatic

In February 2020, an additional seventeen sub-adult Patagonia Frogs (*Atelognathus patagonicus*) were collected from the wild and brought into the captive population. Photo: R. Calvo.

morph that is characterized by free skin which facilitates the exchange of oxygen when underwater, and a complete interdigital membrane to enable their swimming abilities. When the dry season arrives, the lagoons become dry, and the aquatic morphs of Patagonia Frog reabsorb the excess skin and the interdigital membrane. This terrestrial morph leaves the water to hide under-rocks until the wetter season comes again.

Sadly, some decades ago, the White Lagoon was infested with invasive and exotic predatory fish for tourism purposes. The perch (*Percichthys trucha*) and trout (*Oncorhynchus mykiss*) rapidly thrived in this new habitat, predating on the frogs. The effect of these top predators was devastating, causing extreme decline of the major subpopulation of Patagonia Frog, pushing it to its limit and finally causing its total extinction that was formally established in 2004. The loss of the subpopulation that used to live in the White Lagoon represented a loss of almost half of the entire population of this species. Luckily, the invasive fishes did not reach the smaller lagoons and for some years the frogs still thrived there. However, since 2010, an unusual and prolonged drought in the region caused an extreme decline of up to 95% in these remaining small subpopulations of Patagonia Frog.

Concerned about this situation, the authorities of the Laguna Blanca National Park, developed a management plan that

The White Lagoon, in Laguna Blanca National Park in Argentina, is the only permanent waterbody within the range of the Critically Endangered Patagonia Frog.
Photo: Federico Kacoliris.

successfully reduced the invasive fish, favoring the restoration of aquatic habitat. However, the natural recovery of the White Lagoon subpopulation of the Patagonia Frog is unlikely due to the current weather scenario of increasing droughts that makes corridors unviable for the movement of the frogs. This situation was highlighted in the Conservation Needs Assessment for this species (https://conservationneeds.org/Assessment/assessment?pageType=results&AssessmentID=4594&SpeciesID=2332&CountryID=99), which recommended a rescue program for the Patagonia Frog. In 2018 we began a project aimed at recovering the Patagonia Frog subpopulation that once inhabited the Laguna Blanca. This objective is based on the fact that the re-establishment of the bigger subpopulation of this frog is needed as a way to ensure the long-lasting viability of the species.

With the help of an Amphibian Ark Conservation Grant, in February 2019 we created the first *ex situ* center for the Patagonian Frog and built several aquariums inside the visitors' center, called Nomades, in the Laguna Blanca National Park, near the habitat that was once occupied by this frog. The next step was to conduct a series of workshops aimed at developing a conservation planning document for this species. Several stakeholders involved with the conservation of the species participated in these workshops, including park rangers, biologists and researchers from La Plata Museum (Buenos Aires Province) and the University of Comahue (Río Negro Province), and biologists from the National Parks administration. As a product of these workshops, we developed the Conservation Action Plan for Patagonia Frog (www.amphibianark.org/wp-content/uploads/2020/02/Plan-de-Acci%C3%B3n-para-Atelognathus-patagonicus.pdf). In this document, we identified conservation needs and the current state of ongoing management on this species. Based on these results we started extensive and exhaustive fieldwork to rescue individuals to be translocated to the *ex situ* rescue center with the aim of establishing a survival and reproductive colony of this species.

However, things were not easy. The dramatic decline that this species had suffered was still ongoing. The drought that is affecting the area is longer than usual and it was very difficult to find frogs. Between 2019 and 2020 we conducted numerous field trips with a team of several people (including park rangers, biologists, researchers, and university undergraduate students), surveying lagoons within the range of the species and distributing traps for tadpoles. After a high human effort, based on long daily walks covering a huge area and under extreme weather conditions, we had some luck and rescued a total of seven sub-adult Patagonia Frogs, but no tadpoles.

The individuals were rapidly transported to the *ex situ* facilities to start a survival and breeding colony of this species where they still are today. These captive frogs allowed us to learn and improve our husbandry skills. Based on the research made by Ms. Cuello, one of our team members who developed a thesis studying ecology and natural history of this frog, we were able to

In early 2020, we will start building the fences for one of the three identified habitats, that will act as the first sanctuary for the Patagonia Frog in the White Lagoon. Photo: Federico Kacoliris.

build terrariums which replicate the habitat preferred by frogs and we provided specific food items to maintain them in good shape. We had some success and after some months, we can say that the frogs became acclimatized to their new artificial habitat.

In the meantime, we continue conducting surveys searching for new individuals, with the aim of increasing the survival colony up to numbers that increase the chances of reproduction. However, so far we have failed to find additional frogs, even in sites where they were abundant in the past (i.e. in sites where dozens of frogs could be seen in a single day a decade ago, no frogs or tadpoles were found). The absence of frogs even after exhaustive searches, alerts us to a scenario similar or even worse than was highlighted in the last assessment of this species in the IUCN Red List. Because of this, we decided to increase our fieldwork efforts, and in February of 2020, with a team of six people, we surveyed several lagoons and in two of them, we found seventeen new sub-adult Patagonia Frogs that were added to the *ex situ* population, increasing the sustainability of the reproductive colony.

Another activity conducted during this period was the search for a "good" habitat for frogs in the White Lagoon. As part of the Action Plan for the Patagonia Frog, we plan, among other actions, to:

- establish a viable survival colony of Patagonia Frog
- reproduce frogs *ex situ* in order to produce individuals to be part of a translocation program for this species
- create "sanctuaries" free of threats in the White Lagoon
- re-establish populational cores of Patagonia Frogs in these sanctuaries as a way to help this species to return to the White Lagoon.

In order to create these sanctuaries, we had to find suitable habitats in the White Lagoon. The absence of roads makes it difficult to access the different shores, so we had to survey the different places by boat. After an exhaustive search, we found at least three suitable habitats to become sanctuaries. The next step is to fence these habitats in order to avoid the access of livestock and invasive fish (i.e. terrestrial and aquatic fences). Although fish are being decimated through the removal program conducted by the park rangers, some of them are still there, and it is unlikely that we will achieve complete eradication of them due to the large size of this lagoon. In the coming months, we will start building the fence for one of these good habitats, that will act as the first sanctuary for the Patagonia Frog in the White Lagoon. In the meanwhile, we are adding rocks to the shores as a complement to fill this habitat with enough shelter.

After a year of hard work, we obtained some success but we also realize that the situation for the Patagonia Frog is more concerning than we had initially thought. Our next steps are to complete building the first sanctuary for this species in the White Lagoon, to continue rescuing wild individuals to add them to the *ex situ* survival colony of this species, and to promote reproduction among the captive frogs. We are confident that if we achieve this, we will start the first steps toward the reestablishment of the Patagonia Frog in the lagoon, as a prerequisite to enable the long-lasting conservation of one of the most incredible amphibians from Patagonia.

Summary of activities and outcomes of the first year of the project

- Stakeholders (park rangers, biologists, technicians and conservationists) are engaged in the conservation of the Patagonia Frog
- Participative workshops conducted and an Action Plan for Patagonia Frog developed in the frame of these meetings
- Several field campaigns conducted that allowed us to update the conservation status of the Patagonia Frog
- Creation of the first *ex situ* center for Patagonia Frog in the Laguna Blanca National Park

Work has started to build aquatic fences which will prevent access to the protected frog sanctuaries by predatory fish, in the event that the main lagoon floods. Photo: R. Calvo.

- Rescue and establishment of the first individuals of Patagonia Frog in the *ex situ* center
- Husbandry and management of captive frogs including gathering natural history data and information about the presence of chytrid fungus in these individuals
- Three portions of the shore of the White Lagoon identified as good habitats for the creation of sanctuaries for this species
- Start of the creation of one sanctuary for Patagonia Frog in one of the identified good habitats, including fencing to avoid the access of livestock and invasive fish, and supplementation of shelter for frogs.

Short term objectives (1 – 3 months)

- Finish the sanctuary on the shore of the White Lagoon
- Continue exhaustive searches for new individuals to be added to the survival colony of this species
- Continue gathering data on the status of wild subpopulations and natural history in captivity
- Promoting reproduction of captive frogs.

Medium term objectives (4 – 10 months)

- Develop workshops with stakeholders and start an awareness-raising campaign among the local community
- Continue exhaustive searches for new individuals to be added to the survival colony of this species
- Continue gathering data on the status of wild subpopulations and natural history in captivity
- Promoting reproduction of captive frogs
- Starting the creation of new sanctuaries in the shore of the White Lagoon
- Start the first translocation of Patagonia Frogs to the sanctuaries in the White Lagoon.

An update on the head-starting program for Critically Endangered White-bellied Frogs at Perth Zoo

Kay Bradfield and Cathy Lambert, Perth Zoo, Western Australia; and Kim Williams, Parks & Wildlife Service, Western Australia

White-bellied Frogs (G*eocrinia alba*) are small frogs (maximum snout-vent length 25 mm) that are endemic to a small area near Margaret River in the south-west of Western Australia, where they live in swampy seepages along small creeks. The males dig and call from burrows during Spring, and mating occurs in these burrows. Females produce small clutches (usually only ten to twelve eggs), and the endotrophic larvae stay in the burrow until they metamorphose.

The major threats facing the species are habitat destruction and disturbance, altered hydrology, decreased water quality, inappropriate fire regimes and climate change. Climate change is a serious issue as the climate in south-west Western Australia has already become warmer and drier over recent decades, and these trends are predicted to continue.

Above: Recently metamorphed White-bellied Frogs (*Geocrinia alba*) are not much bigger then the tip of a pen.
Below: Over the last ten years, Perth Zoo has released more than 900 White-bellied Frogs to the wild.
Photos: Perth Zoo.

Perth Zoo, in collaboration with the Western Australian Parks and Wildlife Service, has been conducting a head-starting program for this species since 2008. Zoo staff collect clutches of eggs / larvae from the wild and rear them until approximately ten months post-metamorphosis, before releasing them back into the wild. In the wild, only approximately 20% of White-bellied Frog eggs and larvae survive to metamorphosis, largely due to predation by invertebrates and fungal infections, so head-starting them protects them through this highly vulnerable stage of their life-history. This is a very successful strategy for this species; we consistently have 96-98% survival from the time we bring them to the Zoo through until release, which is approximately five times higher than in the wild (and our survival rate includes the first eight to ten months post-metamorphosis, but we do not know how many survive that period in the wild). This means that we can release approximately 20% of each clutch back to the source population to minimise any adverse impacts of clutch collection, and still have a considerable number of animals that can be used to supplement existing small populations or establish new ones.

Over the last ten years, Perth Zoo has released more than 900 frogs to the wild. Post-release monitoring conducted annually by Parks and Wildlife staff indicates that populations at the first two release sites are doing very well; the population at the initial release site is now classed as a large population (>50 calling males) and appears to be stable, while the population at the second one is still increasing and is close to also being classed as a large population. The third release site is not faring as well, with only a few calling males present in 2019; we suspect that this site is not suitable for this species. Early signs at a more recent release site are positive, although it's still too early to draw a conclusion. In 2019, animals were released to a fifth site, but we will not have any indication how that translocation is faring until late 2020 / 2021.

Head-started juveniles have also been used to supplement two existing populations. Numbers at the first augmentation site have increased over the last few years, indicating that the population is recovering. Given the current distribution of frogs at this site, however, it looks like part of the site is no longer suitable for White-bellied Frogs. Initial signs at the second site are positive, but this is another site where it is too early to draw a conclusion.

While five out of the six release sites where post-release monitoring has occurred to date are either successful or showing early signs of success and only one appears likely to fail, it is clear from these results that the biggest challenge facing this program at present is the identification of additional release sites that are suitable for White-bellied Frogs both now and into the future. A PhD student at the University of Western Australia is currently working in collaboration with Parks and Wildlife and Perth Zoo to determine how to identify these sites, so watch this space!

Good news for the *ex situ* Titicaca Water Frog program in Bolivia

Teresa Camacho-Badani, Sophia Barrón Lavayen and Ricardo Zurita Ugarte, Natural History Museum Alcide d'Orbigny, K'ayra Center for Research and Conservation of threatened amphibians of Bolivia

In Bolivia there are fourteen species of aquatic frogs in the genus *Telmatobius*, among them is the Titicaca Water Frog (*Telmatobius culeus*), which stands out for its morphological and physiological adaptations to its particular lifestyle in Lake Titicaca and surrounding lagoons in the departments of La Paz in Bolivia and Puno in Peru. It is considered to be one of the largest aquatic frogs in the world - they can reach 145 mm in length - although there are data such as those from Jacques-Yves Cousteau (in 1970) that states its size can reach 500 mm in total length, Another of its main characteristics, in addition to its large size, is the soft and baggy skin arranged in the form of a sack with detached folds, which allows it to breathe in waters over 3,800 meters above sea level.

This species has been categorized as Critically Endangered on the IUCN Red List and by the national Red Lists in both Peru and Bolivia. Among its main threats are traffic for human consumption as a source of protein and juices; the introduction of exotic species; climate change; contamination of lake waters with human waste, mining products and pesticides, which can cause deadly diseases for frogs.

In 2015, there was a massive death in the minor lake of the Bolivian region of Lake Titicaca. Thousands of frogs were found dead, floating on the water surface, mainly due to pollution. Thanks to the support of the IUCN Save Our Species (SOS) Fund, Amphibian Ark, and other institutions, the project "Rescuing *Telmatobius culeus* Populations at Lago Menor, Titicaca Lake, Bolivia" was executed, where a second recycled shipping container was installed in the captive program breeding at the Alcide d'Orbigny Natural History Museum in the city of Cochabamba in Bolivia. This is now part of the K'ayra Center, which belongs to the same Museum.

Titicaca Water Frog (*Telmatobius culeus*) tadpoles which were bred in captivity. Photo: D. Alarcón / D. Grunbaum.

A male Titicaca Water Frog from the Luna Island population. Photo: Sophia Barrón.

A pair of Titicaca Water Frogs in amplexus at the K´ayra Center. Photo: D. Alarcón / D. Grunbaum.

In 2016, two founding populations were established: one for Luna Island that is located on Lake Maggiore, the largest and best-known section of Lake Titicaca, and another population from the town of Guaqui on Lake Menor, the smallest section and where both the habitat and the frogs are in very bad conditions. It is in Lake Minor where mass deaths were recorded in 2015, and in some areas the frogs have completely disappeared.

The main objective of having the *ex situ* program for this species is to keep populations genetically and demographically viable until conditions improve in their natural habitat, and then we hope that animals hatched in captivity can be released back to the wild in the future. However, none of the frogs brought from the two locations of Isla de la Luna and Guaqui had reproduced, until in April 2019, a pair from the town of Isla de la Luna laid a fertile batch of eggs, and later in In August, a second pair from the same town had another successful reproductive event, from which there are currently juveniles and metamorphs. Finally, in November 2019 a pair of frogs from the town of Guaqui laid about 130 fertile eggs, after spending almost four weeks in amplexus. We currently have healthy larvae from those eggs.

Although in the past some pairs in the *ex situ* program had bred, there had never been so much success and such low mortality (from zero in the last eggs from the Guaqui locality). Development is so fast that at eight months from the first egg-laying, the juveniles measure 46 mm in body length and are maintaining very good physical condition.

This advance for captive management and for the conservation of the Titicaca Water Frogs has been possible thanks to the support of the Kansas City Zoo, Amphibian Ark and Global Wildlife Conservation, who during the last year have collaborated in the maintenance of the facilities and caretakers in the K´ayra Center. This captive management program is part of conservation efforts for the Titicaca Water Frog and for four other species of water frogs in the genus *Telmatobius* that are in danger of extinction.

A captive-bred Titicaca Water Frog tadpole at the K'ayra Center. Photo: Sophia Barrón.

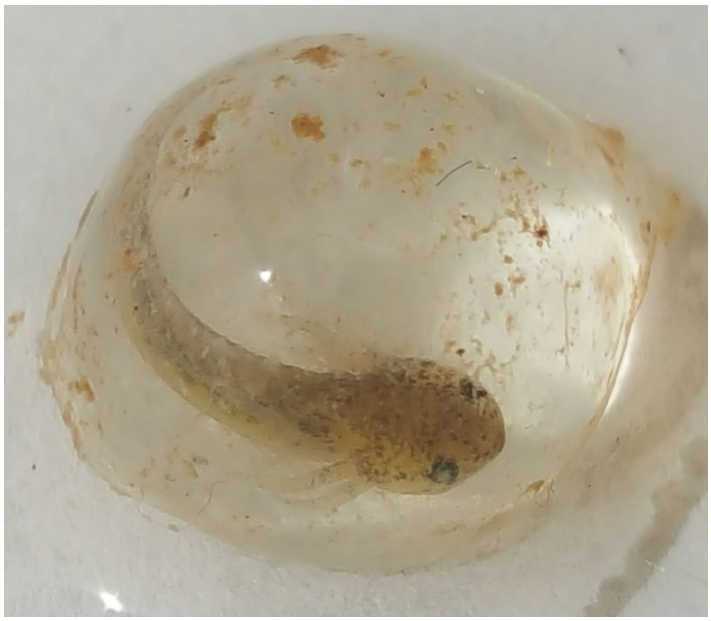
Advancing with the *ex situ* conservation strategy of the Lake Patzcuaro Salamander at the Zacango Ecological Park

Mtro. Biol. Manuel Antonio Pérez Rodríguez, Research and Conservation Area, Zacango Ecological Park, Mexico; Dr. Luis Escalera Vázquez, Laboratory of Aquatic Biology, Michoacana University of San Nicolás de Hidalgo; and Comunidad Ojo de Agua, fishermen's cooperative of Lake Pátzcuaro, Mexico

The Zacango Ecological Park in Mexico has animals of different species from around the world. Opened on December 11, 1980, it was declared a Protected Natural Area of the State of Mexico on August 29, 1981. It is 159 hectares and is built on the former 16th century Franciscan Hacienda, which later belonged to the Counts of Santiago and Calimaya. The hacienda is considered to be one of the largest and most beautiful in Latin America. The Ecological Park has been accredited by the Latin American Association of Zoological Parks and Aquariums (ALPZA) since 2019.

The Coordination of Zoos, through the Research and Conservation Area, carry out actions that allow the recovery of species that are in critical danger of extinction and of ecological importance for Mexico, such is the case of the Lake Ajolote Conservation Program of the Lake Pátzcuaro Salamander (*Ambystoma dumerilii*), which supports the development of management strategies to achieve the conservation of wild populations, including an *ex situ* adaptive management scheme.

This conservation program helps to better understand some general aspects of the current situation of the species through regional monitoring methods and the implementation of conservation, research and management strategies, developed within the Zacango Ecological Park's facilities, with the support of the Amphibian Ark Conservation Grant obtained in 2019.



Lake Pátzcuaro Salamander (*Ambystoma dumerilii*) eggs are clear and jelly-like, much like frog eggs. In fact, baby salamanders are just like baby frogs; their eggs are laid in water and the young are born without legs.
Photo: Hector Javier Castelán Ortiz.

The Lake Patzcuaro Salamander, in Michoacán, better known by the Purepecha culture as Achoque, is a species of salamander to which medicinal properties are attributed. From ancient times and still now, this species has been a source of food among the local communities living around the lake. This species has a very particular biological characteristic, having the ability to regenerate any cell, including brain cells. Today the Achoque is a species that is Critically Endangered according to the IUCN Red List, with the main threats being the destruction of its habitat.

Implementation of the first *ex situ* conservation facilities

As outlined in 2019 in our Amphibian Ark grant application, we proposed to collect Achoque eggs that incidentally remain attached to the fishing nets used by the local fishermen inside Lake Pátzcuaro. The Zacango Ecological Park has opened a dedicated space that allows us to maintain eggs and larvae, and a continuous process of improvement allows us to fulfil the objectives of the program to research and conserve the Lake Patzcuaro Salamander in Mexico.

Collecting live salamanders from the lake with a fish trap to evaluate the medical status.
Photo: Manuel Antonio Pérez Rodríguez.

Over the last eight months, the necessary modifications to this space have been made, placing hatching tanks, feeding tanks for fry less than 5 cm, as well as feeding and maintenance tanks for fry of between 5-10 cm. Each tank has a thermostat which maintains the water temperature at a constant 18°C and includes a constant aeration system, with a reverse osmosis filter that helps maintain water quality conditions, mimicking the conditions of Lake Pátzcuaro. All installation processes were carried out with the participation of the work team of the Research and Conservation area.

Currently, we are working on the construction of two fish tanks, with the necessary isolation for the homogenization of the characteristics of the handling, stay of eggs, fry and specimens larger than 5 cm, all in the same system, with improved devices for temperature control, filtration, vibration management and control of the fiscal chemical conditions of the water.

Two egg collections have been made so far. In the first one, 186 eggs were collected, and in the second, 1,365 eggs were collected. At Zacango Ecological Park, the eggs were placed in containers at a temperature of 19°C, and when they hatched they were separated into one-litre containers, with the temperature also maintained at 19°C. For the first and second week they were fed with daphnia, and in the third fourth, fifth, sixth and eighth week they were fed with worms.

During their stay at Zacango Ecological Park, records of water parameters, pH, salinity, hardness, and ammonia are collected once a week and water changes are made every three days, matching the parameters of Lake Patzcuaro Lake. The development of the salamanders is recorded.

The veterinary staff performs daily monitoring of the salamander larvae and keep a record of their medical condition. The biosecurity protocols that were established by park are followed rigorously by all staff who work with the salamanders. We now have larvae of various sizes, some without legs, others with two legs and some with all four legs developed.

A recently-hatched salamander in the *ex situ* facilities.
Photo: Hector Javier Castelán Ortiz.

Standardized operational and biosecurity protocols

The *ex situ* area for hatching and maintenance of Achoques is located within the artificial breeding building of the Zacango Ecological Park. The area is separated from the exhibit, research and conservation specimens found in the collection.

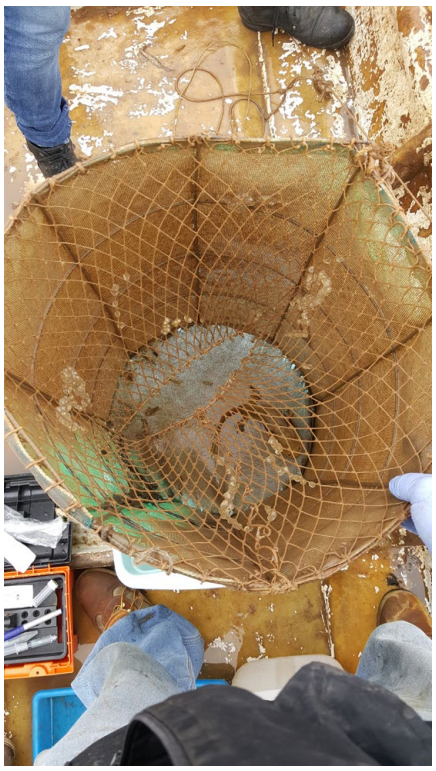
Biosafety protocols have been established for the protection of the specimens and personnel that work with them, providing the biological protection protocols such as the use of a sanitary mat in each of the entrances to the recitations. Additionally there are specific protocols for cleaning and disinfection processes for larvae, fry, food and handling utensils.

Ex situ conservation objectives and reintroduction of the species

The main objective of this conservation strategy is to be able to determine an appropriate methodology for the recovery of eggs that remain attached to fishing nets, preventing them from becoming food for the fishermen, carp and other opportunists. We are also developing standardized protocols for the *ex situ* breeding of the Achoque, which will be released at the collection site when they reach 10 cm in length. Released animals will be marked with a fluorescent color elastomer, allowing us to carry out monitoring to identify the integration and movement of the specimens in the natural environment.

The long-term objective of the program, is to carry out a second introduction, when animals reach ten months of development, an appropriate size for the microchips to be implanted, and the second population will be reintroduced in an area of the lake where there is a low incidence of Achoque being reported. We hope to be able to increase the wild populations and we will carry out an annual monitoring program to determine the mobility of the specimens within Lake Pátzcuaro.

The team from the Zacango Ecological Park, working on the salamander project.
Photo: Manuel Antonio Pérez Rodríguez.



A folding fish trap with salamander eggs, and an adult salamander.
Photo: Manuel Antonio Pérez Rodríguez.

Progress update from the amphibian program at the Amaru Amphibian Conservation Center, Ecuador

Fausto Siavichay Pesantez, Coordinator at the Amphibian Conservation Center at Zoo Amaru, Ecuador

The Amphibian Conservation Center of the Amaru Biopark in Cuenca - Ecuador, (CCA AMARU) aims to conserve several amphibian species from the south of the country. The work performed by our technicians is diverse and includes *ex situ* management, citizenship education and monitoring of wild populations. In order to achieve our objectives, we work together with institutions such as the Philadelphia Zoo and the Amphibian Ark in the USA, which have provided scholarships and donations, allowing our technicians to have essential equipment and materials to work with and care for our amphibians.

Since 2016 the Amphibian Conservation Center has been a part of the Ecuadorian Amphibian Conservation and Sustainable Use of its Genetic Resources project, which is an initiative of the Ministry of Environment of Ecuador, and which has the technical assistance of the Program of United Nations Development Units and is funded by the Global Environment Facility. The main objective of this project is to implement emerging actions that favor the conservation of the amphibian diversity of Ecuador, as well as the sustainable use of the active compounds derived from the secretions of the skins of some species, for future potential applications in biomedicine.

Similarly, we work in coordination with the Environmental Management Commission of the Municipality of Cuenca to identify and protect urban amphibians that still survive in some areas with natural vegetation within the city. The *ex situ* management of these species has been improved in recent years, and thanks to the contributions of the grants and scholarships obtained, our processes in nutrition, biosecurity and infrastructure and water quality have all been improved.

Invertebrate breeding and amphibian nutrition

Currently the Center has two new laboratories where several invertebrate food species are raised, and used as live food for the different amphibian colonies maintained in the breeding programs. These laboratories are an essential part of the new nutrition protocol, which includes quality control of the food that will be supplied to amphibians, selection of the materials to be used in the preparation of substrates and assessing the nutritional contribution provided for the amphibians The laboratories are conditioned and tempered so that the reproduction of invertebrates is a success.

The expansion of food breeding laboratories has allowed increasing the quantity and variety of invertebrate production, and then the species that are reproduced in the center's bioterium include: crickets, fruit fly, weevils, mealworms, small mealworms, arachnids, moths, colembolas and earthworms.

In the first laboratory, crickets (*Acheta domesticus*) are exclusively bred and raised and are used as live food at all stages of development. From type 0 crickets (newborns), to type 7 crickets (adults), the laboratory temperature is maintained at 24°C.

In the second laboratory, the other invertebrates are reproduced and maintained. These are arranged in separate containers, which allows greater control at the time of aging and selection of food, and the room is maintained at a temperature of 20°C.

Biosecurity and infrastructure

Our veterinary staff has intensified control procedures to ensure that all animals entering the breeding program remain healthy. The clinical evaluation consists of performing physical exams, coproparasitic examinations, preventive baths with Itraconazole, diagnosis of chytrid fungus (*Bd*) by cytology and/or PCR.

A technician at the Amphibian Conservation Center AMARU supervising the production of crickets. Photo: Fausto Siavichay Pesantez.

Development of *Atelopus balios* metamorphs. Photo: Fausto Siavichay Pesantez.

The results during 2019 have shown the presence of parasites such as strongyloides and among others, the analyses of *Bd* by conventional PCR have tested positive in some individuals, for urban species as endemic species of natural populations. The results and the opportune detection of these types of pathologies have allowed us to take priority actions in the biosafety of the Center.

The resources obtained through the donations and scholarships, have allowed us to improve the infrastructure of the maintenance and breeding room of amphibian species from the

high Andean areas, such as; *Atelopus nanay*, *Atelopus exiguus* and *Atelopus bomolochos*, which are all considered to be critically threatened.

Improvements in the water collection, use and discharge system

The water that is used in the Amphibian Conservation Center has a specific treatment from its collection to its disposal. The procedure is detailed in the management protocol, which is based on the environmental regulations in force in the Organic Environmental Code of Ecuador and in documents published on this topic, such as the Amphibian Management Guide of the Association of Zoos & Aquariums (AZA). The water system in the Amphibian Conservation Center AMARU includes:

- 2,500-liter storage tanks
- Water pipe and distribution pipe
- Reverse osmosis filter before entering the Center
- Automation of sprinklers and internal humidity
- Wastewater treatment plant.

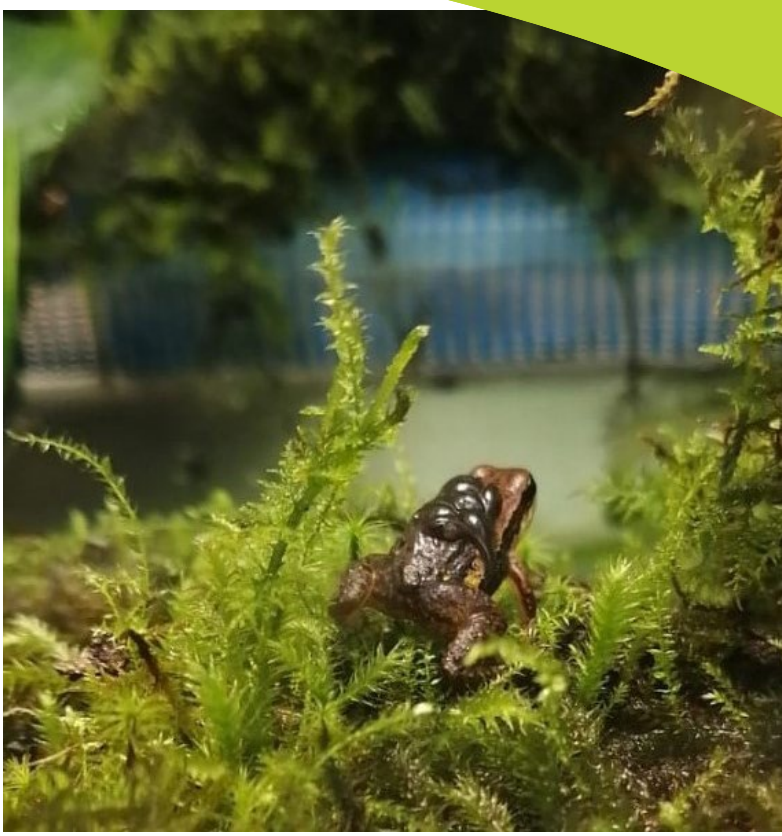
The resources obtained from the Amphibian Ark donation allowed to acquire and install an osmosis filter, which eliminate particles and residues of chlorine and other elements present in the pipes and storage tanks, vastly improving the quality of the water. A small water treatment plant was built at the Amphibian Conservation Center, which includes physical filter, chlorine, oxygenation and UV filter, to eliminate possible toxic elements from the management of amphibians in the Center.

Reproduction of species in the Amphibian Conservation Center AMARU

Atelopus nanay

This species has been maintained and cared for since 2007, and it reproduced on one occasion in 2011. In 2019 a further clutch of eggs was obtained, although the larvae were only partially developed, and after several weeks the small larvae died.

Gastrotheca cuencana reproduced and maintained at the Amphibian Conservation Center AMARU.
Photo: Fausto Siavichay Pesantez.



A male *Hyloxalus vertebralis* carrying larvae reproduced in the center. Photo: Fausto Siavichay Pesantez.

Atelopus balios

We were able to reproduce this species after several matings (amplexus) in 2018. On this occasion approximately 400 eggs were laid, and after analyzing and controlling water variables such as temperature and oxygenation, approximately 20% of the youngsters survived.

Atelopus sp nov. (Wampukrum)

This is a species which still does not have a formal taxonomic description, however, populations in the wild have been eliminated almost entirely. Currently in the Amphibian Conservation Center AMARU we have some individuals, including several F1 generations, which have already reached sexual maturity.

Gastrotheca cuencana

This is a species that was only described in 2019 and is located only in the city of Cuenca in urban and peri-urban areas. We are currently working on a program of reintroduction of some individuals in areas with appropriate ecological characteristics.

Hyloxalus vertebralis

This species of the Dendrobatidae family is critically threatened in the city of Cuenca, although currently there is a large population being bred in the Amphibian Conservation Center AMARU. We are investigating future reintroduction of some of the animals into the wild.

Establishment of a new western population of Montseny Brook Newt in 2019

Francesc Carbonell, Torreferrussa Wildlife Center, Spain; Dani Fernandez and Albert Montori, Group of the School of Nature of Parets del Vallès, Spain; Felix Amat, Granollers Museum, Spain; and Dani Guinart, Diputacio of Barcelona, Spain

The genus *Calotriton* (Gray, 1858), includes only two species adapted to live in cold, well-oxygenated waters: the Pyrenean Brook Newt (*Calotriton asper*) and the Montseny Brook Newt (*Calotriton arnoldi*). Populations of Montseny Brook Newt were found initially in 1980 and obtained the species status in 2005.

Currently, Montseny Brook Newts have been found only in seven streams, its distribution area is less than 8 km^2. The total population is estimated to be between 1,000–1,500 mature individuals and is separated into two main areas on both sides of the Tordera River valley. There is a high genetic structure, with a significant differentiation between population sectors (eastern and western) with an absence of genetic flow between them. The Montseny Brook Newt is listed as Critically Endangered in the IUCN Red List.

The Critically Endangered Montseny Brook Newt (*Calotriton arnoldi*) is found only in seven streams in Spain, and its distribution area is less than 8 km^2. Photo: Jaime Culebras | Photo Wildlife Tours.

In 2017, a conservation program for the species was established, investigating its biology and implementing management strategies including an *ex situ* conservation program. In 2016 the project Life Tritó Montseny started with the aim of protecting this Critically Endangered species. The project is being carried out at a site of community interest, the Natura 2000 Network. The project promotes around fifty actions to ensure the conservation of this endemic species and its habitat.

Currently, there are four breeding centers (Torreferrussa Wildlife Center and the Pont de Suert Wildlife Center in Spain, Barcelona Zoo in Spain and Chester Zoo in the United Kingdom) with an annual production of about 500 larvae.

Some releases have previously been carried out between 2010-2015 in four streams, with differing results. (Figure 1).

We have found that it is very difficult to monitor this species in the field, and that their biological features and their habitat needs are very complex. Environmental conditions are a major determinant of the probability of detection of the specimens, which present a very marked surface activity seasonality.

With this previous experience, and with the aim of improving the methodologies applied, evaluating the optimal streams and deciding the number and location of releases, a multidisciplinary Reintroduction Committee was created in 2018. Four workshops were organized in order to determine the streams that met the optimal conditions to ensure the long-term survival of the released individuals. As a result of these workshops and three field surveys, one western stream was selected in spring of 2019 to establish a new population of Montseny Brook Newts as part of the reintroduction program. At the same time, characteristics of released newts were discussed (sex ratio, maturity, mark methodology, surveillance after release etc.).

New population	Total number released	Age (years)	Release years	Survival	Breeding success
W1	390	2-3	2010, 11, 12, 14	>20%	Yes
W2	106	0-1	2014,15	Yes	?
E1	166	2-4	2011,14	2017 -19?	Amplexus seen 2016
E2	62	0-1	2014,15	Yes?	?
W3	136	4-8	2019	Yes	?

Figure 1. Previous release (W1, W2, E1, E2) and 2019 release (W3).

Some of the conclusions of these workshops was the need to improve the previous knowledge of the release places, and the need for a more intensive monitoring of released specimens.

Thanks to the financial support received from Amphibian Ark, two specialists in amphibian ecology collaborated with the project. They have assisted in the choice of release locations, tagging of the specimens, and carried out the subsequent monitoring of the population's progress. Prior to the introduction, three field visits were made to the chosen torrent, four sections of the selected stream were analyzed in order to determine the exact segment, mark the sections and decide the number of newts that should be released in each section. Once the best segments were selected, a total of 120 meters of stream were marked at 10-meter intervals and twenty-one release points were identified and marked to control the displacement of the released newts.

One hundred and thirty-six newts (forty-seven males, sixty-one females and twenty-eight immature animals) were released on 30 May 2019 in the selected sectors. The number of newts released in each section was determined by the area and depth of the river section. Newts were released only in pools with low current and high number of potential refuges. All the introduced newts originated the Torreferrussa breeding center (Generalitat de Catalunya) and were all born between 2010 and 2015.

Each newt was marked with a pit-tag implanted subcutaneously. Individuals were kept under observation for several days to ensure that they did not lose the microchip and that they had recovered well from the manipulation process and presented a good biological condition. Additionally, before release, newts were also marked with acrylic polymers under the ventral and leg skin for quick identification and to control that the microchip was not lost.

After release, four nocturnal surveys (two in the Spring

and two in Autumn) were carried out in order to determine the presence of newts, body condition and migration or sedentary behavior of individuals. The number of surveys was limited by the weather conditions, a particularly dry Summer and a Spring with some torrential rain. Figure 2 shows the number of newts released and recaptured in each stream selected section. A total of twenty-one newts were captured during the post-release surveys (eight males, ten females and three immature newts). This represents 15.44% of the released newts (17% of males, 16.4% of females and 10.71% of immature newts). The percentage distribution of recaptures is similar to that of releases. Only in the case of immature individuals is there a slight difference, possibly due to some immature individuals, primarily males, having reached sexual maturity.

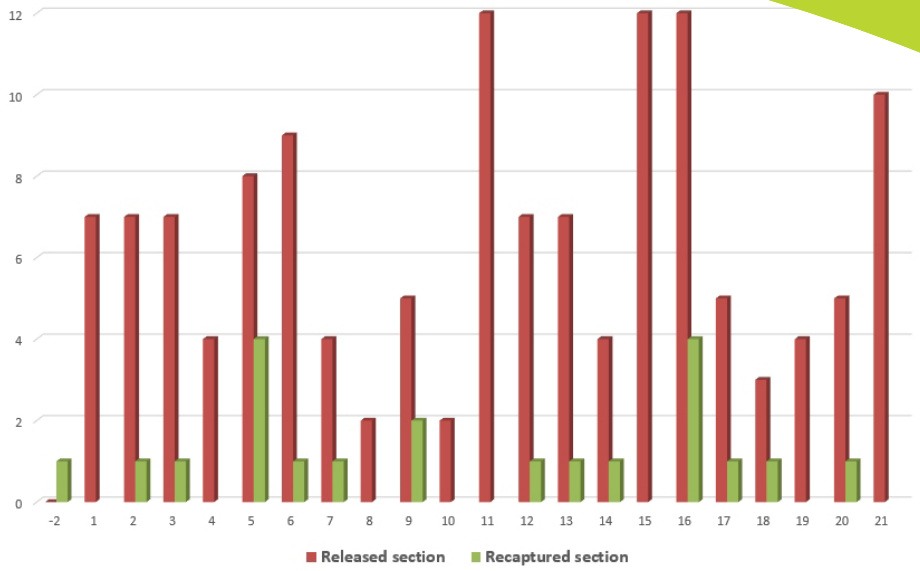


Figure 2. Number of newts released in each sections and location of recaptures.

Preliminary results indicate that the population is well established and that there have been few movements despite the floods and the summer drought. The average displacement of newts throughout 2019 was 0.76 downstream sections. Despite this, one individual was found 20 meters downstream after the great flood, outside the marked area.

Weight loss or gain is very variable between months, given the low number of individuals being captured. The average weight loss or gain of captured individuals was +0.67% with a standard deviation of 10.9%.

To prevent the transmission of pathogens and diseases between amphibians due to the onset of infectious disease caused by the *Batrachochytrium dendrobatidis* and *B. salamandrivorans* fungus, and the virus Ranavirus, strict biosafety protocols were put in place to control all the different steps of the reintroduction process.

Disease detection tests were performed in both the captive and natural population of Montseny Brook Newts and in all cases showed negative results. The same negative results were obtained for other amphibian species found living in the Montseny

Before release, newts were marked with acrylic polymers under the ventral and leg skin for quick identification.
Photo: Dani Fernandez.

Brook Newt populations (Common Midwife Toad (*Alytes obstetricans*), Fire Salamanders (*Salamandra salamandra*) and *Bufo spinosus*) caught inside and outside the selected section of the stream.

The proposal for 2020 is to reinforce the population with fifty more individuals and to continue the surveys by expanding the prospecting area downstream. Extending the stream survey upstream is not possible as there is a large waterfall precluding the access. A new introduction in the western sub-population is also proposed. This new introduction will need the same protocol of surveillance as the eastern introduced population.

One hundred and thirty-six newts (forty-seven males, sixty-one females and twenty-eight immature animals) were released on 30 May 2019 in carefully selected locations.
Photo: Albert Montori.

Amphibian Ark donors, January 2019 - March 2020

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