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Information on *ex situ* management for the conservation of the Papallacta marsupial frog

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Introduction

For several decades, amphibians in Ecuador have experienced a serious decline in their populations, to the point that many species have almost disappeared. In response to these catastrophic events, several initiatives have been established for the *ex situ* conservation of the most endangered amphibians. This is the case with the Papallacta marsupial frog (*Gastrotheca orophylax*), which is under the SAR-RANA amphibian conservation program. SAR-RANA was created as an emergency measure to support the amphibian crisis in Ecuador which has resulted in decreased populations of several species, and in some cases, even a possible extinction (Ron et al. 2011). The program has the endorsement of the Ministry of Environment of Ecuador, through management patent No. 015-2019- FAU-DPAPCH-MA. We are pleased to report the first case of reproduction and successful maintenance of twenty-one froglets until they reached adulthood in *ex situ* conditions, to increase numbers of the species.



Gravid female Papallacta marsupial frog (*Gastrotheca orophylax*) which was found in the patio of a house, in Santa Bárbara, Ecuador. Photo: Diego P. Almeida-Reinoso.

The Papallacta marsupial frog is an olive green or emerald green frog, belonging to the family Hemiphractidae (Duellman 1987). It is a direct developer (fully-formed frogs hatch from the eggs, with no free-swimming tadpole stage), and it is distributed to the north of Ecuador in the eastern mountain range, in the provinces of Carchi, Napo and Sucumbíos (Frolich 2000); and in the Pasto knot, in the Department of Nariño and Putumayo in Colombia (IUCN 2004). This species is considered globally as Endangered by the IUCN (IUCN. 2004), but in the Red List of amphibians in Ecuador, it has been listed as Near Threatened (Ortega-Andrade. 2021). The main threats to the species are the advancing agricultural and livestock frontier, excessive use of pesticides and herbicides, accelerated loss of their habitats, cutting and burning of forests for charcoal production and climate change in their distribution areas (Chasiluisa et al. 2018).

Several historical cases of the collection of gravid females have been reported. Two females collected in June 1977 which laid eggs in August-September (Duellman et al. 1980). Del Pino and Escobar carried out studies on the development of the embryos, after the collection of gravid females (Del Pino et al. 1981). In both cases, only the development of the eggs until the birth of the young was recorded.

This study reports the maintenance of a gravid adult female under controlled conditions, within the framework of the *ex situ* conservation program for amphibians. The female was found in the patio of a house, in Santa Bárbara, province of Sucumbíos, suffering from advanced dehydration. Once rescued, it was hydrated by placing it in water and later transferring it to a plastic container with moist sphagnum moss. It remained in good condition until arriving at the SAR-RANA facilities, located in Quito, Ecuador, two days after her rescue. During that time an enclosure was prepared, adapting it in such a way to provide appropriate conditions for long-term housing of the frog.

The vertical container is 45cm wide, 45cm deep and 80cm high, and features an open irrigation system (simultaneous inflow and outflow of excess water) with a reservoir of water covered by egg-crate and mosquito mesh that form the false floor, on which the internal environment was created, enriching it with leaf litter, sphagnum moss, plants and trunks that create various strata with different temperature and humidity gradients. The top of the terrarium includes a ventilation system made with metal mesh which allows the passage of natural UV-B light and rain or artificial irrigation when required.

The frog, a pregnant female, entered SAR-RANA on 20 July 2019 and from that day on, she has been monitored daily, keeping track of her behavior and adaptation to captivity. The frog regularly stayed perched on a branch during the day, taking in the rays of the sun that penetrated the layer of natural shade from the trees and entered the container. Almeida et al. reports this same behavior of a gravid female marsupial shell frog turtle (*Gastrotheca testudinea*) (Almeida, et al. 2014). Forty-two days after her rescue, on 31 August 2019, at dawn, she gave birth to twenty-one fully formed froglets, of a total length of 17-17.2mm on average, and that were observed the next day. They were immediately separated from the mother's terrarium to avoid predation. They were placed in another outer container with the same conditions and enrichment, as the female's terrarium. This event thus becomes the first record of birth of the Papallacta marsupial frog within an amphibian conservation program in Ecuador.

From day one, individuals were fed a wide range of live insects produced in the SARGRILLO facilities (the insect breeding program) including crickets, tenebrios, galleries, moths, earthworms, etc. All insects are bred under the patent of operation, handling and commercialization which is

granted by the Ministry of the Environment Ecuador and renewed every year.

It is evident that the growth rate of frogs is slow, with marked differences among the siblings; the youngest died within two months of their birth and were cryo-preserved for future analysis of Chytridiomycosis, caused by the fungus *Batrachotrichyllum dendrobatidis* (Bd), which has been a major cause of the wave of decline and worldwide extinctions (Lips, et al 2006). Preventative treatments were carried out with a 0.1% solution of Itraconazole, using the modified drip technique in a terrarium, without manipulation of individuals (Almeida et al. 2017), to rule out this possible cause of death. Currently twelve adult individuals remain. The first male was heard calling in October 2021, which is an indicator that they reached sexual maturity twenty-six months after birth. However, we need to replicate the same conditions for a second reproductive event within the *ex situ* program to corroborate the data presented at this stage.



Adult male *Papallacta* marsupial frog born under *ex situ* conditions. Photo: Diego P. Almeida-Reinoso.

The SAR-RANA program for the conservation of amphibians at risk of extinction focuses on the rescue, maintenance and reintroduction of species, and we are planning the reintroduction of five adults that were born in our facilities to their natural habitat, with the environmental authorities, following the IUCN protocols for management and reintroduction (IUCN-SSC 2013).

Acknowledgements

To Javier Romo, for rescuing the frog and delivering it to SAR-RANA; staff of the Ministry of Environment for providing for the *ex situ* conservation of the frog; Dr. Leonardo Arias for his support and checking the health status of the frogs.

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Juvenile *Papallacta* marsupial frogs which were born in captivity as part of the SAR-RANA amphibian conservation program. Photo: Diego P. Almeida-Reinoso.

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Amphibian Ark George and Mary Rabb Research Fellowship

This fellowship supports early or mid-career scientists and conservationists from all countries in research-based professional development that furthers amphibian conservation. This fellowship is not intended to help support graduate-student research. 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) will be given preference. If applicants are applying for funds to support an internship or externship at another institution, they must be formally associated with a mentor or lead scientist at a relevant university, NGO, zoo/aquarium, or other established institution; the mentor must supply a letter of support with the application. The fellowship carries a \$5,000 stipend and is open to applicants from all countries to which funds may legally be distributed from the United States of America.

Applications must include: current CV, a letter from the mentor with whom the applicant is intending to work, the names and contact information (email addresses) for two additional references who may be contacted by the Amphibian Ark, and a Statement of Purpose. This Statement should be no longer than two pages and should provide specifics of the proposed research as well as the applicant's academic and professional experiences relevant to the research. Proposals to work directly with live animals must be accompanied by copies of appropriate governmental and institutional animal-care and collecting permits as relevant, and invitations or acceptance letters from hosting institutions or programs. Fellowships are for one year, with potential for an additional one-year renewal depending on need and progress towards research goals.

Funds will be distributed to the successful applicant as a single payment. No financial reporting or receipts for expenses are required by Amphibian Ark. Because this is a fellowship that may be used for any purposes, including travel and living expenses, no detailed budget is required in the application, but please do describe fully how the funds will be used.

Application materials and the letter of support from the mentor should be e-mailed to: Kevin Johnson, grants@amphibianark.org. Submission deadline: 1 September 2022. A committee appointed by the Amphibian Ark will review all nominations and then submit their choice for award recipient to the Amphibian Ark Executive Director for endorsement.

About George and Mary Rabb

This fellowship honors Dr. George B. Rabb and his life-long partner Mary Rabb. From his boyhood days studying herps in Charleston, South Carolina through the remainder of his life, George was passionate about amphibians, conservation, and providing encouragement to developing scientists. A noted scientist, long-time Director of the Chicago Zoological Society, Chair of the Species Survival Commission of the International Union for Conservation of Nature (IUCN SSC), and recipient of numerous awards, George was a thoughtful, quiet, humble person. He never sought the limelight, and never cared about being right, only that the right thing was done. He played essential roles in stimulating, provoking and initiating new directions in amphibian conservation, ranging from the Declining Amphibian Populations Task Force (DAPTF), the Amphibian Conservation Action Plan (ACAP), the Amphibian Ark and the Amphibian Survival Alliance (ASA). George was a steadfast supporter and advisor to the Amphibian Ark from its inception, and this fellowship recognizes that which George held dear.



AArk Conservation Grants, 2022

In 2020 and 2021, we did not receive many applications for our Conservation Grants, most likely due to the various restrictions imposed by the global COVID-19 pandemic. This year however, we received sixteen program outlines, which resulted in eleven full applications being received. It was very reassuring to see a number of high quality applications being submitted again. This year we were targeting applications from Argentina and Brazil, to work with species identified for *ex situ* rescue during the recent Conservation Needs Assessments in those countries, and we received five applications from these countries.

After a thorough review by our Grants Review Committee, at this time, we have decided to award six grants, with a further three grants requiring additional information before a final review and decision. The successful applicants to date are:

Startup Grants

- *Ex situ* insurance population of the Rancho Grande harlequin frog (*Atelopus cruciger*) in Venezuela
Margarita Lampo, Fundación para el Desarrollo de las Ciencias Físicas (FUDECI), Venezuela
- Establishment of an insurance population for *Pithecopus rusticus*
Benjamin Phalan, Parque das Aves, Brazil
- Establishing captive breeding and husbandry protocols for *Atelopus hoogmoedi*, an analog species for the threatened Manauense harlequin toad (*A. manauensis*) in Brazil
Cybele Lisboa, Reserva Paulista - São Paulo Zoo, Brazil

Startup Extension Grants

- Towards the conservation of the Darwin's blackish toad (*Melanophryniscus nigricans*)
Igor Berkunsky, CONICET, Argentina
- Continuing captive breeding efforts for La Culata frog (*Aromobates duranti*) and the Mucuchies frog (*A. zippeli*)
Enrique La Marca, Rescue of Endangered Venezuelan Amphibians (REVA) Conservation Center, Venezuela

Mentorship Grant

- Centro de Conservación de Anfibios Amaru and Detroit Zoo Mentorship Grant
Fausto Siavichay, Centro de Conservación de Anfibios Amaru (CCA-Amaru), Ecuador; and Blake Klocke, Detroit Zoological Society, USA

Here are the executive summaries from each of the successful applicants.

Ex situ insurance population of the Rancho Grande harlequin frog (*Atelopus cruciger*) in Venezuela

Margarita Lampo, Fundación para el Desarrollo de las Ciencias Físicas (FUDECI), Venezuela

The Rancho Grande harlequin frog (*Atelopus cruciger*) is the only harlequin toad with known viable populations in Venezuela. Although it disappeared in the late 1980s, presumably due to chytridiomycosis, this species was rediscovered in 2003. Only two nearby subpopulations have been detected, despite continuing efforts to find these toads in their former habitats.

A. cruciger is currently listed as Critically Endangered in the IUCN Red List and recommended for *ex situ* rescue. Relict populations have maintained few hundred mature individuals for almost one decade, despite the presence of chytrid fungus. Chytrid transmission is low and juvenile recruitment sufficiently high to compen-



The Rancho Grande harlequin frog (*Atelopus cruciger*) is the only harlequin toad with known viable populations in Venezuela.
Photo: Margarita Lampo.

sate for losses due to Bd-induced mortality. This means that scenarios that increase Bd transmission rates could rapidly push these populations to a collapse, and possibly the species to extinction, if backup captive colonies are not available.

Our goal is to establish an *ex situ* facility for *A. cruciger* at the Leslie Pantin Paya Zoo, as a stopgap measure for an integrated long-term conservation plan that includes its reintroduction into former habitats in lowland habitats (i.e. thermal refuges). During a pilot study with very limited resources, we were able to obtain one egg batch, but low tadpole survival and high incidence of spindly leg syndrome due to calcium deficiency resulted in a low success in metamorphosis. Nonetheless, we have demonstrated that *A. cruciger* can breed in captivity.



The team which will lead the *ex situ* rescue program for the Rancho Grande Harlequin Frog in Venezuela.
Photo: Margarita Lampo.

With the start-up grant, we expect to obtain F1 juveniles. Funds will be applied to the acquisition of equipment for establishing one large reproduction unit and twenty small maintenance tanks. Required permits have already been obtained and proposals to obtain further funds for scaling up have been submitted to the UK Embassy in Caracas and the Prince Bernard Nature Fund.

Establishment of an insurance population for *Pithecopus rusticus*

Benjamin Phalan, Parque das Aves, Brazil

Pithecopus rusticus was described in 2014 and is a Brazilian endemic, restricted to a single site, with a maximum number of thirty-four individuals observed. It has not yet been evaluated on the global Red List, but at the national level in Brazil it is evaluated as Critically Endangered. The species is vulnerable to a range of threats including drainage, pesticide pollution, alteration of habitat by cattle, and possibly climate change and drought. Extensive searches for the species in other apparently suitable sites in the region have not located any additional populations. Individuals have been recorded with *Batrachochytrium dendrobatidis* (chytrid fungus).



A female *Pithecopus rusticus*. Photo: Parque das Aves.

The need for an *ex situ* population was identified in a Conservation Needs Assessment in 2020 (<https://conservationneeds.org/Assessment/assessment?pageType=results&AssessmentID=6764&SpeciesID=7538&CountryID=101>). This insurance population was started with the capture of two adult individuals in early 2022 and these were transported to Parque das Aves, in collaboration with Instituto Claravis, São Paulo Zoo, ICMBio and the Federal University of Santa Maria.



Arrival of the first *Pithecopus rusticus* at Parque das Aves, in Brazil. Photo: Parque das Aves.

The purpose of this proposal is to construct additional facilities that will enable the expansion of this *ex situ* population into a functional insurance population, which serves as a backup in

case of the possible extinction of the species in the wild, and to keep options open for future reintroduction or population reinforcement.

This is the first amphibian species maintained at Parque das Aves, but we are fortunate to have the close support of São Paulo Zoo, an institution with extensive experience with amphibian husbandry.

Establishing captive breeding and husbandry protocols for *Atelopus hoogmoedi*, an analog species for the threatened Manauense harlequin toad (*A. manauensis*) in Brazil

Cybele Lisboa, Reserva Paulista - São Paulo Zoo, Brazil

The Manauense harlequin toad (*Atelopus manauensis*) is a recently named species with a very small geographic range in the vicinity of Manaus, the largest city in the Brazilian Amazon. It has few remnant populations under strong pressure from the fast and irreversible deforestation for urban and agriculture expansion. For these reasons, it will be listed as globally Endangered in the updated IUCN Red List of Threatened Species (J. Luedtke, pers. comm.).

To manage the threats, the species was included in the Harlequin Toad (*Atelopus*) Conservation Action Plan, created collectively by the Atelopus Survival Initiative (ASI), a massive collaborative effort to conserve harlequin toads. One of the strategies included in the plan is the establishment of captive breeding programs for *Atelopus* species and maintenance of backup populations for future supplementation in the wild. In the Conservation Needs Assessment conducted in 2020, *Ex Situ* Rescue was recommended as a conservation action for *A. manauensis*, since identified threats cannot be reversed in time to prevent its extinction.

In the current project, we intend to take the first step before creating a backup population of *A. manauensis*. As this species has never been maintained in captivity, we aim to establish husbandry and breeding protocols using an analog and widespread species, the Hoogmoed harlequin toad (*A. hoogmoedi*, Least Concern) that occupy habitats similar to *A. manauensis*. We will collect five pairs of *A. hoogmoedi* and bring them to the Reserva Paulista - São Paulo Zoo. Protocols will be tested and established based on the previous experience of the Project Leader and her team with *Scinax alcatraz*, *Nyctimantis pomba* and other Brazilian amphibians.



The Hoogmoed harlequin toad (*Atelopus hoogmoedi*) will be used as a husbandry analog to develop protocols for a conservation program for the Endangered Manauense harlequin toad (*Atelopus manauensis*). Photo: Samuel Gomides.



Darwin's blackish toads (*Melanophryniscus nigricans*) which were reared in captivity were translocated to restored and protected habitats in the Sierra del Tigre Natural Reserve in 2021-2022. Photo: Igor Berkunsky.

Towards the conservation of the Darwin's blackish toad (*Melanophryniscus nigricans*)

Igor Berkunsky, CONICET, Argentina

Darwin's blackish toad (*Melanophryniscus nigricans*) is a threatened, recently described species from Argentina. 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 is probably extinct. The remnant populations face a combination of threats: 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 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 guards a wild population of this toad. With the reserve managers, we initiated a habitat restoration project to provide more habitat for Darwin's blackish toad. However, natural recolonization by Darwin's blackish toad is unlikely due to current fragmentation and the lack of corridors between highland grassland remnants.

In 2019, and thanks to a start-up grant from the Amphibian Ark, we conditioned an *ex situ* facility at the university campus and established a survival colony. During the last two breeding seasons (2020-2021 and 2021-2022), we successfully conducted head-starting of eggs by collecting clutches from wild breeding sites and maintaining them in captivity until they become juveniles. All individuals produced in captivity were translocated to restored and protected habitats in the Sierra del Tigre Natural Reserve. This project achieves the first effective conservation tool for Darwin's blackish toad, connecting the current populations, and increasing the viability of the species.

With this extension grant, we hope to achieve the following outcomes:

- Have an Action Plan for Darwin's blackish toad signed by participants, including the managers of Sierra del Tigre Natural Reserve, which is the legal manager of main area for the species.
- Improve the *ex situ* facility on the campus of the University, and an increase in the production of individuals for translocation.
- Create the third sanctuary for Darwin's blackish toad.
- A translocation strategy included in the Species Action Plan is promoted to start in 2022.

Continuing captive breeding efforts for La Culata frog (*Aromobates durantii*) and the Mucuchíes frog (*A. zippeli*)

Enrique La Marca, Rescue of Endangered Venezuelan Amphibians (REVA) Conservation Center, Venezuela

Conservation of endangered amphibian species is a must for the Venezuelan Andes, the most biodiverse region in the country. The Rescue of Endangered Venezuelan Amphibians (REVA) continues, as an amphibian conservation center, to devote efforts to rescue some of the most threatened frog taxa in the country. Since its inception in 2018, and thanks to the support of Amphibian Ark, REVA managed to raise and breed several high Andean frogs that are in risk of extinction.

The first most successful programs at REVA were those with the Merida collared frog (*Mannophryne collaris*) and the Merida whistling frog (*Leptodactylus meridensis*), both with reintroduction programs that ended up in well-established and monitored populations in the Merida terrace. These species, along with the

Building the new facilities at Rescue of Endangered Venezuelan Amphibians for the La Culata Frog (*Aromobates durantii*) and the Mucuchíes frog (*A. zippeli*). Photo: Miguel Angel Bastidas.



recently described banana frog (*Boana platanera* La Marca et al., 2021 in Zootaxa) established well and reproduced successfully in the temperate room at REVA, with conditions closely resembling the ones prevalent in their regions of origin. All other amphibian species kept at REVA in Mérida city, most belonging to the genera *Dendropsophus*, *Pristimantis* and *Aromobates*, have been raised in the Center's cold room, assisted with air conditioners. The resulting colder conditions are more favorable for cloud forest and paramo (alpine tundra ecosystem) species.



(Above) An adult female Mucuchíes frog (*A. zippeli*). (Below) An adult female La Culata frog (*Aromobates duranti*).
Photos: Erik La Marca.



After successfully managing captive breeding and reintroduction programs for the Merida collared frog and the Merida whistling frog, as well as the banana frog which has established well and reproducing successfully in the temperate room at REVA, we are applying for an extension grant to continue the captive breeding efforts with two species' programs that received financial support from AArk since their start, namely, La Culata frog (*Aromobates duranti*) and Mucuchíes frog (*A. zippeli*). The proposal is also addressed to rescue additional specimens of these endangered amphibians through captive husbandry and breeding, as well as to plan and execute later releases and monitoring of their offspring in the wild.

Following we sum up the intended goals with the requested extension grant:

- To establish new *ex situ* conservation programs for La Culata Frog and the Mucuchíes frog in a new *ex situ* facility under more natural conditions, starting with new parental

stocks collected from the wild.

- To set up an artificial enclosure for the Mucuchíes frog in the Moconoque region, to keep specimens under controlled natural conditions, taking advantage that there is a natural water spring and a short slow-moving stream.
- To introduce or re-introduce captive-born and raised offspring of both species into previously studied suitable places within their respective ranges of distribution.
- To continue the program of involvement of the local community at the places of origin of both species, and to spread knowledge and concern on the species and the need to protect the habitats they occupy.

Centro de Conservación de Anfibios Amaru and Detroit Zoo Mentorship Grant

Fausto Siavichay, Centro de Conservación de Anfibios Amaru (CCA-Amaru), Ecuador; and Blake Klocke, Detroit Zoological Society, USA

The Conservation Center for Amphibians – Amaru (CCA-Amaru) in Ecuador and the Detroit Zoological Society (DZS, USA) have been involved with mitigating the current amphibian biodiversity crisis through *ex situ* conservation for many years. The AArk Mentorship grant will provide an opportunity to form international collaboration to strengthen both institutions in achieving their amphibian conservation goals. Fausto Siavichay has been involved with *ex situ* amphibian conservation, research, education, and outreach in Ecuador since 2007. Blake Klocke, from DZS has worked with *ex situ* populations of endangered Panamanian amphibians (primarily *Atelopus* species) since 2013, mitigation research, and has led three reintroduction trials of captive bred *Atelopus* from the Panama Amphibian Rescue and Conservation Project.



The Azuay stubfoot toad (*Atelopus bomolochos*) is one of the species managed at the Conservation Center for Amphibians – Amaru (CCA-Amaru) in Ecuador. Photo: Jaime Culebras.

CCA-Amaru was established in 2008 to mitigate amphibian declines due to emerging diseases, habitat fragmentation, and urbanization. Collaboration between CCA-Amaru and DZS will focus on *Atelopus* husbandry, captive breeding, and developing reintroduction strategies and methodologies. CCA-Amaru and DZS have combined captive husbandry knowledge of ten *Atelopus* species. Developing strategies, providing guidance, and sharing lessons learned from *Atelopus* reintroduction

trials will provide information important for future planning. The amphibian species at CCA-Amaru include *Atelopus bomolochos* (CR), *Atelopus exiguous* (EN), *Atelopus nanay* (CR), *Atelopus* sp. 'Wampukrum' (Not Evaluated), *Atelopus balios* (CR), *Pristimantis erythros* (Not Evaluated), *Hyloxalus vertebralis* (CR), *Cenophryne aequatorialis* (LC), and *Gastrotheca cuenca* (Not Evaluated).

Funding from this mentorship grant will support DZS staff to make a one-week trip to CCA-Amaru in 2022. DZS will match the grant amount to allow an additional member to join the trip (Blake Klocke and a member from the animal care staff team). Fausto and Blake will work together to develop an itinerary before the trip for efficient time usage during the visit to CCA-Amaru. This mentorship exchange will help both institutions in their efforts to conserve the most imperilled genus of amphibians and strengthen international *ex situ* amphibian conservation efforts.



Blake Klocke, from the Detroit Zoological Society, USA, has worked with *ex situ* populations of endangered Panamanian amphibians (primarily *Atelopus* species) since 2013.
Photo: Blake Klocke.

First-time captive breeding of the Critically Endangered Sucre water frog

Teresa Camacho-Badani, Ricardo Zurita and Maria José Borda.
Alcide d'Orbigny Museum of Natural History, K'ayra Center for Research and Conservation of Threatened Amphibians in Bolivia

Aquatic and semi-aquatic frogs of the Telmatobiidae family are exclusive to the South American Andes and are one of the most endangered groups in the Neotropics. According to the International Union for Conservation of Nature (IUCN), of the sixty-one species included in the Red List, 84% face some degree of threat.

Bolivia has the second highest number of species of aquatic frogs in the world and of the fifteen species reported here, all are threatened - nine are Critically Endangered, and of these, four are possibly extinct.

The Sucre water frog (*Telmatobius simonsi*) is an endemic species of the Bolivian inter-Andean valleys in the departments of Cochabamba Chuquisaca (Sucre) and Santa Cruz and is found at elevations between 1,000 and 3,241 m above sea level. It has been categorized as Critically Endangered in the IUCN Red List, because its populations have decreased by 80%. The

Sucre water frog tadpoles hatched at the K'ayra Center.
Photo: Teresa Camacho Badani.



A Sucre water frog (*Telmatobius simonsi*) at the K'ayra Center for Research and Conservation of Threatened Amphibians in Bolivia.
Photo: D. Alarcón/D. Grunbaum.

main causes of its decline are habitat loss and diseases such as chytridiomycosis.

The Sucre water frog is one of the five species of the *Telmatobius* genus we maintain in the K'ayra Center at the Alcide d'Orbigny Natural History Museum in Cochabamba. These individuals that are part of the *ex situ* conservation program, were collected in 2011 in the Cajamarca locality in the department of Chuquisaca at 3,241 m above sea level and during the time they were in captivity, they had not produced fertile eggs, until March 2022, when a clutch of around 250 eggs was successfully deposited.

This is the first time that a Sucre water frog has bred in captivity and is the first record of parental care for this species. Once the eggs are deposited, the male stays with them for the first few days. When the male stopped caring for the eggs, the parents were removed to a new tank and the tadpoles have remained in the original tank, developing successfully.

This is an important step for the conservation of the Sucre water frog and for the *ex situ* management of the genus *Telmatobius* in Bolivia, which has been possible thanks to the support received by the K'ayra Center from different organizations such as Re:wild, Amphibian Ark and Aquazoo Loebbecke Museum.

AArk Husbandry Document library

The Husbandry Document library on the AArk web site (www.amphibianark.org/husbandry-documents) currently has over 290 documents in it, with additional documents being added regularly.

Twelve new documents have been added recently:

Captive breeding program for *Scinax alcatraz* (Anura: Hyliidae): introducing amphibian *ex situ* conservation in Brazil (English)

Scinax alcatraz is endemic to a small island ("Ilha dos Alcatrazes") and is threatened by restricted distribution and habitat loss. Here, we present the establishment of a captive breeding program for *S. alcatraz* at São Paulo Zoo and introduce *ex situ* conservation as a strategy for amphibians in Brazil. We recorded 125 breeding events with about 10,200 eggs laid. We also observed that *S. alcatraz* does not have a marked breeding season, laying eggs throughout the year, and that breeding events are positively correlated with relative humidity and negatively correlated with temperature. This program has shown great success in the maintenance and reproduction of *S. alcatraz* in captivity and has great potential for conducting research relevant to amphibian conservation and for the development of educational materials to share information about the global amphibian crisis, using *S. alcatraz* as a flagship species.

Authors: Cybele S. Lisboa, Renata I. Vaz, and Cinthia A. Brasileiro

Publication: Amphibian & Reptile Conservation, 14(2) [General Section]: 279–288 (e293), 2021

www.amphibianark.org/husbandry-documents/amphibian-reptile-conservation.org/pdfs/Volume/Vol_15_no_2/ARC_15_2_%5bGeneral_Section%5d_279-288_e293.pdf

Developmental life stages of the Pickersgill's reed frog (*Hyperolius pickersgilli*) in an *ex-situ* environment at Johannesburg Zoo's captive breeding facility, South Africa (English)

We initiated a captive breeding project to create an insurance population for the endangered Pickersgill's reed frog (*Hyperolius pickersgilli* Raw, 1982) at the Johannesburg Zoo from parents collected from KwaZulu-Natal Province, South Africa, in 2017. We found that this species has seven developmental life stages, each with unique management requirements... A greater understanding of Pickersgill's reed frog's developmental stages and physiological and environmental needs can improve captive breeding and subsequent release of the frogs, facilitate captive breeding elsewhere, and improve the species' conservation status.

Authors: Ian du Plessis, Adrian Armstrong, Piet L. Malepa, Arnold T. Kanengoni, Cormac Price, and Colleen T. Downs

Publication: Zoo Biology, 2022;1–11 DOI: 10.1002/zoo.21688 www.amphibianark.org/wp-content/uploads/2022/03/Developmental-life-stages-of-the-Pickersgills-reed-frog.pdf

The effects of two calcium supplementation regimens on growth and health traits of juvenile mountain chicken frogs (*Leptodactylus fallax*) (English)

The mountain chicken frog (*Leptodactylus fallax*) is among the 42% of amphibians threatened with extinction and is dependent upon *ex situ* populations to recover in the wild. Amphibian captive husbandry is not fully understood and empirical data are required to optimise protocols for each species in captivity. Calcium metabolism and homeostasis are areas of importance in captive husbandry research and have been identified as a challenge in maintaining *ex situ* populations of *L. fallax*. We trialled two frequencies (twice and seven times weekly) of calcium supplementation via dusting of feeder insects in two groups of *L.*

fallax juveniles and measured growth and health effects through morphometrics, radiography, ultrasonography and blood and faecal analysis over 167 days, followed by a further 230 days of monitoring on an intermediate diet informed by the initial dataset. We showed that supplementation treatment did not affect growth or health status as measured through blood analysis, radiography and ultrasonography. More frequent supplementation resulted in significantly more radiopaque endolymphatic sacs and broader skulls. Frogs fed more calcium excreted twice as much calcium in their faeces. The intermediate diet resulted in previously lower supplementation frogs approximating the higher supplementation frogs in morphometrics and calcium stores. Comparison with radiographic data from wild frogs showed that both treatments may still have had narrower skulls than wild animals, but mismatching age class may limit this comparison. Our data may be used to inform dietary supplementation of captive *L. fallax* as well as other amphibians.

Authors: Michaels, C., Servini, C., Ferguson, A., Guthrie, A., Jayson, S., Newton-Youens, J., Strike, T., and Tapley, B.

Publication: Herpetological Journal, 31, 18-26, 10.33256/hj31.1.1826 (2021)

www.researchgate.net/publication/348098561_The_effects_of_two_calcium_supplementation_regimens_on_growth_and_health_traits_of_juvenile_mountain_chicken_frogs_Leptodactylus_fallax

Amphibian reproductive technologies: approaches and welfare considerations (English)

Captive breeding and reintroduction programs have been established for several threatened amphibian species globally, but with varied success. This reflects our relatively poor understanding of the hormonal control of amphibian reproduction and the stimuli required to initiate and complete reproductive events. While the amphibian hypothalamo–pituitary–gonadal (HPG) axis shares fundamental similarities with both teleosts and tetrapods, there are more species differences than previously assumed. As a result, many amphibian captive breeding programs fail to reliably initiate breeding behaviour, achieve high rates of fertilization or generate large numbers of healthy, genetically diverse offspring. Reproductive technologies have the potential to overcome these challenges but should be used in concert with traditional methods that manipulate environmental conditions (including temperature, nutrition and social environment). Species-dependent methods for handling, restraint and hormone administration (including route and frequency) are discussed to ensure optimal welfare of captive breeding stock. We summarize advances in hormone therapies and discuss two case studies that illustrate some of the challenges and successes with amphibian reproductive technologies: the mountain yellow-legged frog (*Rana muscosa*; USA) and the northern corroboree frog (*Pseudophryne pengilleyi*; Australia). Further research is required to develop hormone therapies for a greater number of species to boost global conservation efforts.

Authors: Aimee J. Silla, Natalie E. Calatayud, Vance L. Trudeau

Publication: Conservation Physiology, Volume 9, Issue 1, 2021, coab011

<https://doi.org/10.1093/conphys/coab011>

Substrate preference in the fossorial caecilian *Microcaecila unicolor* (Amphibia: Gymnophiona, Siphonopidae) (English)

Caecilians epitomise the complexities of maintaining poorly known amphibian taxa in captivity. Empirical data on even the most basic husbandry parameters are lacking for most species of caecilian, including the substrate used to maintain them. We used a simple choice chamber to compare two commonly used substrate types. *Microcaecila unicolor* were housed in individual choice chambers. On one side of the chamber we used Megazorb as a substrate and on the other we added moistened topsoil. Our results show that *M. unicolor* has a statistically significant preference for Megazorb as a diurnal resting site.

Authors: Whatley, C., B. Tapley, C.J. Michaels, Gower, D., and Wilkinson, M.

Publication: Herpetological Bulletin. 152. 18-20. 10.33256/hb152.1820 (2020)

www.thebhs.org/publications/the-herpetological-bulletin/issue-number-152-summer-2020-1/3097-05-substrate-preference-in-the-fossorial-caecilian-i-microcaecila-unicolor-i-amphibia-gymnophiona-siphonopidae/file%0d%0a

The importance of enrichment for advancing amphibian welfare and conservation goals: A review of a neglected topic (English)

Enrichment, broadly the provision of stimuli to improve the welfare of captive animals, is known to be important in husbandry practice and in the success of *ex situ* conservation and reintroduction programs. Practical evidence of the importance of enrichment exists for a number of taxa, yet amphibians are poorly represented. There is no reason to assume a priori that amphibians would not benefit from enrichment and, given their increasing prominence in captive programs, their requirements in captivity beyond basic husbandry should be the focus of more intense study. We review the existing body of research on enrichment for amphibians, as well as that for fish and reptiles, which may be regarded as behaviorally and neurologically broadly similar to amphibians. We also briefly discuss mechanisms by which enrichment might affect amphibian fitness and, therefore, reintroduction success. Our review supports the contention that there may be important consequences of enrichment for both captive welfare and *ex situ* conservation success in amphibians and that amphibian enrichment effects may be highly variable taxonomically. In the face of increasing numbers of captive amphibian species and the importance of *ex situ* populations in ensuring their species level persistence, enrichment for amphibians may be an increasingly important research area.

Authors: Christopher J. Michaels, J. Roger Downie, and Roisin Campbell-Palmer

Publication: Amphib. Reptile Conserv. 8(1) :7–23. (2014)

www.amphibian-reptile-conservation.org/pdfs/Volume/Vol_8_no_1/ARC_8_1_%5bGen_Sec%5d_7-23_e77_high_res.pdf

Comparison of the nutritional content of the captive and wild diets of the critically endangered mountain chicken frog (*Leptodactylus fallax*) to improve its captive husbandry (English)

It is vital to provide appropriate nutrition to maintain healthy populations in conservation breeding programs. Knowledge of the wild diet of a species can be used to inform captive diet formulation. The nutritional content of the wild diet of the critically endangered mountain chicken frog (*Leptodactylus fallax*) is unknown, like that of most amphibians. In this study, we analyzed the nutritional content of food items that comprise 91% of the wild diet of *L. fallax*, by dry weight of food items, and all food items offered to captive *L. fallax* at ZSL London Zoo and Jersey Zoo. We subsequently

compared the nutritional content of the wild diet and captive diet at ZSL London Zoo consumed by *L. fallax*. To the authors' knowledge, this is the first study to directly compare the nutritional content of the wild and captive diets of an anuran amphibian. The captive diet at ZSL London Zoo, without dusting of nutritional supplements, was higher in gross energy and crude fat and lower in ash, calcium and calcium:phosphorus ratio than the wild diet. Most of the food items in the captive diets had a high omega-6:omega-3 fatty acid ratio and in the wild diet had a low omega-6:omega-3 fatty acid ratio. We recommend a combination of modifications to the captive diets to better reflect the nutritional content of the wild diet. Nutritional analysis of captive and wild diets is recommended for other species in conservation breeding programs to improve captive husbandry and ultimately fitness.

Authors: Jayson, S., Ferguson, A., Goetz, M., Routh, A., Tapley, B., Harding, L., Michaels, C., and Dawson, J.

Publication: Zoo Biology. 37. 332-346. 10.1002/zoo.21442. 2018

www.researchgate.net/publication/327698881_Comparison_of_the_nutritional_content_of_the_captive_and_wild_diets_of_the_critically_endangered_mountain_chicken_frog_Leptodactylus_fallax_to_improve_its_captive_husbandry

The importance of natural history and species-specific approaches in amphibian *ex situ* conservation (English)

Due to the importance of *ex situ* components of the response to the on-going amphibian extinction crisis, the numbers of captive amphibian species and populations is growing. However, *ex situ* projects are currently often poorly supported by knowledge of the captive husbandry requirements of individual amphibian species, many of which are being taken into captivity for the first time. Natural history data and measurements of wild environmental parameters are critical in designing appropriate captive environments, but are absent for the majority of species held in captivity. This has resulted in the failure of some *ex situ* projects and is likely to affect many future initiatives. Publication biases away from natural history and amphibian-specific research, the inaccessibility of data in academic literature for conservation institutions and lack of time for preparative surveys before 'rescue' attempts are largely responsible for this data deficit. In many cases, conservation groups must collect their own data where existing information is insufficient. We suggest important parameters to record in the field and discuss the importance of considering the microclimates in which wild amphibians live when determining the methodology of recording parameters. Furthermore, we highlight the important role that public databases should fulfil to store and disseminate data. All in all, this perspective piece demonstrates the need for natural history data and outlines a road map for their efficient collection and for their practical integration into conservation programmes.

Authors: Michaels, Christopher J., Gini, Beatrice F. and Preziosi, Richard F.

Publication: Herpetol. J. 24:135–145. (2014)

www.academia.edu/66088406/The_importance_of_natural_history_and_species_specific_approaches_in_amphibian_ex_situ_conservation

Recent Studies in Reptile and Amphibian Welfare: Some Relevant Publications for the Zoo Herpetologist (English)

Animal welfare has become a major driving force behind the operations and management of accredited zoos and aquariums, with institutions across the globe committed to structured approaches to assessing and managing the wellbeing of animals in their care (Mellor et al. 2015). Although much

of the focus on animal welfare in zoological parks historically has centered on just a handful of taxa, particularly high-profile mammalian species such as elephants, primates, large carnivores and cetaceans (e.g., Goulart et al. 2009; Melfi 2009; Maple and Perdue 2013), other taxonomic groups including reptiles and amphibians have received increased attention and resources aligned with their welfare in recent years. Today, animal welfare programs factor heavily into the captive management of reptiles and amphibians at many zoological parks, incorporating enrichment and behavioral husbandry initiatives and periodic welfare assessments that seek to optimize the wellbeing of captives and promote more evidence-based approaches to their care. Our collective understanding of reptile and amphibian welfare has advanced considerably over the past three decades, and now encompasses many different fields of inquiry including, but not limited to ecology, ethology, cognition and learning, physiology, captive husbandry, and veterinary medicine. Laying the essential groundwork for the body of research that has focused on reptile and amphibian welfare to date, Warwick, Frye, and Murphy's *Health and Welfare of Captive Reptiles* (Warwick et al. 1995) was the first major work to tackle the subject of welfare in captive reptiles. Covering many important topics including physiology and functional anatomy (Lillywhite and Gatten).

Author: Mendyk, Robert

Publication: *Herpetological Review*. 53. 176-180. 2022

www.researchgate.net/publication/359646218_Recent_Studies_in_Reptile_and_Amphibian_Welfare_Some_Relevant_Publications_for_the_Zoo_Herpetologist

Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior (English)

Good nutritional husbandry is crucial to maintain high welfare standards in captive animals. Both direct effects of diet on growth, development, and maintenance and indirect effects of feeding regimes on behavior may be important. Despite this, many questions remain as to how we should best feed many of the species that are commonly kept in captivity. In this study, we investigate the impact of mixed versus invariant diets on growth and behavior in the axolotl (*Ambystoma mexicanum*), an aquatic amphibian of severe conservation concern that is frequently maintained in captive collections. We then use our results to provide advice on feeding management in the context of improved welfare. These

data suggest that providing a mixed diet is not necessarily beneficial to either growth or welfare of captive animals. In the case of axolotls, an invariant diet of bloodworm should increase growth rates, but the diet (mixed vs. invariant) does not influence behavior. Overall, our results suggest that mixed diets in themselves may not be beneficial to the growth or welfare of axolotls as compared with a high-quality invariant diet.

Authors: Dean J. Slight, Hazel Nichols, and Kevin Arbuckle

Publication: *J. Vet. Behav.* 10:185–190. 2015

www.sciencedirect.com/science/article/abs/pii/S1558787814001300?via%3Dihub

Amphibian Conservation Needs Assessments (English)

Conservation Needs Assessments (CNAs) use current knowledge of species in the wild to determine those with the most pressing conservation needs and provide a foundation for the development of holistic conservation action plans that combine *in situ* and *ex situ* actions as appropriate.

Authors: Johnson, K. and Carrillo, C.

Publication: *WAZA News* 1/22, 2022

www.amphibianark.org/pdf/CNA-WAZA-magazine-2022-01.pdf

Pautas de Manejo *Ex Situ*, Sapito de las Sierras, *Melanophryniscus nigriscans* (Spanish)

Husbandry guidelines for the Critically Endangered Darwin's blackish toad (*Melanophryniscus nigriscans*) in Argentina.

Authors: Igor Berkunsky and Manuela Santiago

Publication: March 2022

www.amphibianark.org/wp-content/uploads/2022/05/Pautas-de-Manejo-Ex-Situ-Melanophryniscus-nigriscans.pdf

Implementation and development of new processes for the *ex situ* reproduction of harlequin frogs at the Amphibian Conservation Center - Amaru

Jackeline Arpi, Fausto Siavichay and Nataly Aguilar,
Amphibian Conservation Center Amaru (CCA-Amaru),
Ecuador

Species of the genus *Atelopus* have been considered of great importance both for research and conservation, because they are included in the Red List of the International Union for the Conservation of Nature (IUCN, 2004) in the categories Endangered, Critically Endangered or Extinct. The destruction and degradation of their habitats, infectious diseases, invasive species and climate change are some of the pressures they face.

In Ecuador, twenty-four species of this genus have been registered distributed between the coast, Sierra and Amazonia (Ron, 2021) inhabiting natural regions such as montane forest, tropical humid Amazonian forest, montane humid forest and paramo (alpine tundra ecosystems). Around twenty-five species are protected at the Amphibian Conservation Center - Amaru (CCA-Amaru), including five harlequin frogs: the Rio Pescado stubfoot toad (*Atelopus balios*), *Atelopus* aff. *spumarius*, the Azuay stubfoot toad (*Atelopus bomolochos*), *Atelopus exiguus* and *Atelopus nanay*.

These last three species inhabit moorland areas where habitat conditions are extreme such as environmental and water temperatures that oscillate around 12°C and 10°C respectively, so the *ex situ* reproduction of these species has been a real challenge for the CCA-Amaru team. However, thanks to the support of the Amphibian Ark, some equipment was installed, which favors their reproduction, including specific UV lamps and heat emitters for amphibians and a chiller that lowers the temperature of the water, simulating the conditions in which they are found in the paramos of Azuay.

The equipment is now operational. UV lamps are used for a certain period of time in laboratories where there is not enough sunlight for the species to capture and assimilate vitamin D. Heat emitters are used with species that inhabit the eastern and western areas of Ecuador and the chiller is being used in reproduction terrariums for harlequin frogs. All processes are observed by CCA-Amaru technicians.

The CCA-Amaru work team hopes to be successful in the next stages of the project, such as the development of the larvae and the survival of the metamorphs. This phase is critical since these are the first reproduction trials with these *Atelopus* species from the paramos of southern Ecuador.

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Ron, S. R., Merino-Viteri, A. Ortiz, D. A. 2021. Amphibians of Ecuador. Version 2021.0. Zoology Museum, Pontifical Catholic University of Ecuador. <<https://bioweb.bio/faunaweb/amphibiaweb>>, accessed on January 3, 2021.



The authors – part of the team at the Amphibian Conservation Center - Amaru (CCA-Amaru), Ecuador. Photo: Fausto Siavichay.



An Azuay stubfoot toad (*Atelopus bomolochos*) tadpole.
Photo: Juan Carlos Sanchez.



Some of the new equipment in the amphibian laboratories which was purchased with the support on an Amphibian Ark grant.
Photo: Fausto Siavichay.

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