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Newsletter

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Can a breeding program save the Common Midwife Toad in Flanders, Belgium?

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Loss and degradation of its habitat have led the Common Midwife Toad (*Alytes obstetricans*) in Flanders, Belgium, into a very precarious state. A Species Protection Program was launched in 2017. This is an official policy document with binding commitments. It proposes all kinds of measures, including the establishment of a breeding program as a last measure to strengthen existing populations and to establish new ones.

Midwife Toads are small amphibians (approximately 5 cm) that only occur in western Europe and north-western Africa. The Common Midwife Toad is the most widespread species of this genus. It is native to parts of Portugal and Spain, France, Switzerland, Luxemburg, Germany, Belgium and the Netherlands. Its name refers to the parental care behaviour of the males who wrap the fertilised egg strands around their hind legs and take care of them until the time of hatching, when the tadpoles are deposited into the water. The downside of this elaborate care is that only a few eggs are laid. Egg strands seldom contain more than thirty eggs. Thus, failed reproduction

may quickly endanger small populations. The reproductive period extends over a long period, particularly during the warm summer months. Tadpoles that are deposited after the end of July will, however, mostly not leave the water in the same year, as they will spend the winter in the water. These animals will only metamorphose during the next (late) spring or summer.

The Common Midwife Toad poses specific demands to its terrestrial and aquatic habitats. In the northern parts of its range (including Belgium), the terrestrial habitat has to be warm, has to



Male Common Midwife Toad (*Alytes obstetricans*) carrying egg strands around the hind legs. Photo: Jeroen Speybroeck.

offer sufficient shelter, and has to be close to permanent, fish-free water.

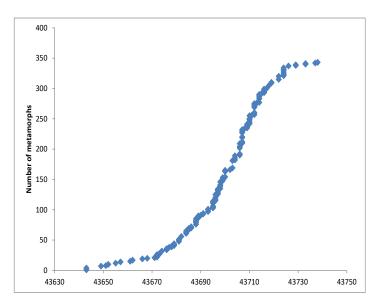
Until the second half of the last century, rural areas were unintentionally used and managed to the benefit of the species. For instance, cattle drinking ponds were available in close proximity to farm buildings, with worn-out joints and other crevices offering daytime, and winter shelter to adult animals. Active sand or stone quarries feature many open, warm patches, and the dug pits will fill up with fish-free water after a while. In recent times, however, cattle ponds have become less abundant and are not as well-managed. Joints in old buildings are neatly sealed, and old quarries are overgrown, with suitable, warm terrestrial habitat disappearing.

According to the most recent IUCN Red Data List of amphibians and reptiles in Flanders, the Common Midwife Toad is Threatened. If notable action is not taken soon, the species is likely heading towards the category Critically Endangered. At the European level, the species is included in Appendix II of the Bern Convention (1979) and Appendix IV of the Habitats Directive (1992).

The Species Protection Programme, developed by the Flemish Agency for Nature and Forests in 2017, aims to restore the number of midwife toads to a safe level in the remaining populations as soon as possible. This program will run for five years, and it is the first policy document containing precise, binding measures. In order to implement this plan, government agencies, scientific institutions, universities and non-profit organisations have joined forces. The remaining suitable terrestrial and aquatic habitats are examined, all known Flemish populations are monitored in a standardised way, and a breeding program will support the recovery of the populations.



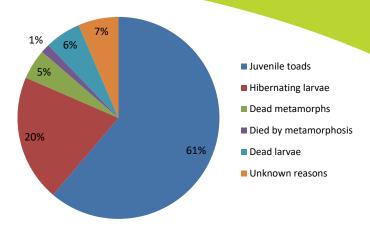
Midwife toad larvae eating blanched organic endive. Photo: Johan Auwerx.



Number of tadpoles that successfully made the transition to the terrestrial phase.

First results

In the summer of 2019, the Research Institute for Nature and Forest (INBO) started a breeding program, funded by the European LIFE BNIP program (LIFE14 IPE BE 002 BNIP). Midwife toad tadpoles were collected from the remaining five Flemish populations. As these populations are very small and likely to have undergone genetic erosion, larvae from Wallonia (southern Belgium) and the Netherlands were included as well. In total, 557 tadpoles from thirteen different populations were collected from the end of June to the beginning of July 2019, ranging from 1 to 171 individuals per site.



Fate of 557 collected tadpoles of the Common Midwife Toad at the end of the first rearing season.

To be able to ensure genetically balanced breeding groups, DNA samples were taken from all populations where tadpoles were collected. After their release, populations founded from or supplemented with captive-bred animals will be monitored. Information on the genetic profile will form the basis to evaluate the success of the breeding program, as well as that of site management measures.

Collected larvae were first placed in quarantine in the INBO breeding facility in Linkebeek. There, following a biosecurity protocol developed by Prof. An Martel and Prof. Frank Pasmans from the Wildlife Health lab of Ghent University in Belgium, they were

A fully developed juvenile Midwife Toad. Photo: Johan Auwerx.



treated to prevent amphibian diseases, such as chytridiomycosis, from entering the breeding group.

We aimed to have a minimum of 50% survival to metamorphosis. Larvae were given a varied diet, composed of blanched (organic!) endive and zucchini, frozen mosquito larvae, and algae flakes. They also appeared to nibble on dead conspecifics. With a water temperature of 25-30°C, offering a lot of food and regular water renewal, the larvae grew quickly, with only 6% mortality. As soon as the larvae developed four firm limbs, and before their tails began to reduce, they were transferred to smaller containers with lockable lids. During the period of metamorphosis (three to eight days), the animals may easily drown, therefore, they were put in containers with very shallow water and moist moss, stones and a limited number of small prey items (springtails, *Collembola*).

The first animals to leave the water were the larger tadpoles, and their advanced growth indicated that they hatched in 2018, hibernated as tadpoles, and were subsequently collected in 2019. Towards the end of July, the young of the year also started to metamorphose. By autumn, 341 of the 557 (61%) collected tadpoles successfully made the transition to the terrestrial phase, while 113 tadpoles (20%) were still in the water and would hibernate as larvae.

Mortality was limited, occurring at different stages. A limited number of larvae died during the rearing season (6%), some died during metamorphosis (1%), some as juveniles (5%), and some died or disappeared due to unknown causes (7%). To allow examination of cause of death at a later stage, all dead animals were stocked at -20° C.

At the end of the first rearing season, much better results were obtained than anticipated. A total of 341 juvenile toads and 113 tadpoles had survived. Thus, we actually had a surplus on what we had set as the required number to ensure genetically balanced breeding groups. Therefore, a number of animals were ready to be released in order to strengthen existing populations. Prior to their release, these surplus larvae (n = 99) and juvenile toads (n = 78) were screened for diseases (Rana-viruses and Bd). As the results were negative, they were subsequently released at three different locations.

Prospects

The young toads that remained in the breeding program are currently being raised until they become sexually active and until they are able to produce offspring for several successive years. Maturity is expected to be reached at the age of two or three years. The offspring will be released to strengthen existing populations but will also be used to establish new populations in locations optimally suited and managed for this purpose. To the best of our knowledge, large-scale breeding of this species has not been done before. INBO will draw up an optimal breeding protocol that can serve as a basis for similar tasks in neighbouring countries.



Small containers to support the critical period during which the animals exit the water. Photo: Johan Auwerx.

Monitoring

In 2019, standardised monitoring of all existing and recently lost populations of the Common Midwife Toad in Flanders was started by the NGO Natuurpunt. Together with a group of enthusiastic volunteers, calling male counts and larval surveys will be carried out over a period of three years, in order to obtain a detailed overview of the current state of conservation. As a first surprising result, the whistling call of seven Common Midwife Toads was heard again in a location where no observations have been made for ten years. Suddenly, it became the second most active population recorded in 2019. Hopefully, the Species Protection Program will bend the trend for this rare and intriguing amphibian.

Natterjack Toad conservation in Denmark – a project for toads and humans

Lene Vestergren Rasmussen, Zookeeper; and Signe Ellegaard, Research Assistant, Copenhagen Zoo, Denmark

Three years ago, the Natterjack Toad (*Epidalea calamita*) conservation project at Copenhagen Zoo was granted almost 1,000,000 DKK (≈ 130,000 EUR) by the Danish Nature Fund. That funding period has now ended and we are providing an overview of the project. This article does not go into how we breed, feed, and care for the tadpoles and toads, as we are trying to present one of the other important aspects of a conservation project - nature conservation is about humans – that is one of my mottos and it is something that I have learned during my many years in the zoo field.

The Natterjack Toad is a small toad that is easily recognized by the yellow stripe running down the back of the brown-coloured toad. Once, it was an abundant species throughout Denmark, but the conditions for survival have been reduced in habitat which over the past decades have been exposed to modern agriculture, where small coastal ponds and wetlands have disappeared.

A Natterjack Toad population within fifteen kilometres of the coast might sound like something that belongs in the past, and that might actually be the case for the Natterjack Toads near Refsvindinge on Funen, Denmark. Once, the coast reached Refsvindinge, a small town which is now located at what we would define as inland. There are stories about how Queen Dagmar of Denmark in the 13th Century and seafaring sailors sailed to Refsvindinge from the Great Belt through winding channels. The same route was utilized by the Natterjack Toads of that time. From the flooded beach meadows south of Nyborg, the toads took the trip in the wake of the trade traffic of that time. The descendants from that population of Natterjack Toads have been able to survive in the temporary ponds that emerged before drains radically changed the Danish landscape. We are talking about a Natterjack

Toad population with ancestry that goes all the way back to the 13th Century. If any species has a justified living space in this area, it must be these toads. And with our help, they are currently recapturing their lost land (or water, we might say).

In 2011, Copenhagen Zoo, together with local landowners, dug the first of the new ponds in Refsvindinge, with a total of fourteen ponds already established and there are more to come. Previously, the last known breeding pond of the Natterjack Toads was drained when a new residential area was built. In fear of children drowning in the pond, the area was fenced and the water supply was reduced so pumps and drains could lead off the water. Ole Nikolajsen, a local schoolteacher, had followed the sad development of the Natterjack Toad population and made several unsuccessful attempts to increase the water supply. The toads still visited the disappearing pond where they laid eggs, even though it would dry out before the tadpoles could complete metamorphosis.

Ole Nikolajsen is a good example that is sometimes only takes one passionate person to make a significant difference, and we owe him a great deal of thanks. In 2008, he was so visionary that he contacted Amphi Consult, a consultancy company working on projects such as habitat restoration. They contacted the Copenhagen Zoo and the day after, a newly laid Natterjack Toad egg string from Refsvindinge arrived at the Zoo. I am sure that if Ole had not acted at that time, the Natterjack Toads would now be extinct in

A pair of Natterjack Toads (*Epidalea calamita*) in amplexus. Once, it was an abundant species throughout Denmark, but the conditions for survival have been reduced in a habitat where small coastal ponds and wetlands have disappeared. Photo: Henrik Egede-Lassen.





the area. That egg string was the beginning of our engagement in the area. The year after, the first pond, which was paid for by the Zoo, was established on the grounds of a local landowner who was willing to sacrifice some of his riding course for the toads. The critics thought that a single pond was not enough, but we had to start somewhere, and one pond has now turned into fifteen. In total, more than 35,000 tadpoles and 1,100 toads have now been released in these ponds.

Of course, there has also been challenges. Even though we have had a high level of professionalism by partnering with the leading amphibian expert in Denmark, Kåre Fog, for inspection and assessment of the ponds, challenges have arisen. Part of Refsvindinge is an old gravel pit area which makes it difficult to guess what is underground. An example is the local football club which wanted to donate an area of the farthest corner of the football field. Here, water was present all year round so it looked easy to work with. But after digging and making a big surface run-up, it did not seem to hold water. We have worked on improving this and it now looks as if it should be ready for breeding in the Spring of 2020. That waterhole is located close to Refsvindinge Nature and Culture Centre, which plays a large role in the project.

The Nature Centre was opened by a local group of passionate people in 2015 and since then it has developed into a great driving force in the project. The Nature Centre aims to develop outdoor nature and cultural projects and activities for schools, residents, day-cares, institutions, families, and tourists in order to promote understanding and awareness of nature in general and of the conservation of Danish amphibians, with special attention to the Natterjack Toads. Everyone who uses and visits the centre, knows about the toads' history in the area. Behind the Nature Centre building, breeding ponds have been established and indoors there is a Natterjack Toad terrarium, educational posters and the staff proudly wear T-shirts with a picture of the toad and the text "We are helping the Natterjack Toad".

Engaging the local community is an extremely important part of the project. Here, local landowners are being updated on the project by Lene Rasmussen and Eddie Back from Copenhagen Zoo before releasing tadpoles together. Photo: Copenhagen Zoo.

The centre is also responsible for contacting new landowners who wish to participate in the project by allowing the Zoo to create ponds on their land. There is public access to several of the ponds where information about the project and the amphibians is available. It is also possible to spend the night in shelters at the Nature Centre and if you are lucky, you might hear the calling males from the nearby ponds. It is hoped that these nature experiences can create a fascination for this often overlooked animal group – a fascination that in the long run can help securing its survival.

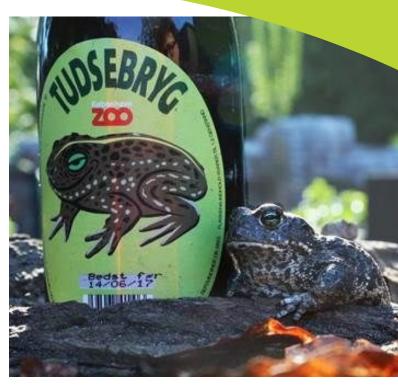
Recently, a field station was established at the centre which most recently was used by a biologist from Copenhagen Zoo when monitoring the Natterjack Toad population in the breeding season. In Spring of 2020, more research activities are to be conducted in the area, including radio-tracking of released adult toads and a study investigating the metamorphosis success of the captive-bred and released tadpoles.

Collaboration with and engagement in the local community has shown to be a very important and rewarding part of the project. This is expressed both through our collaboration with the Nature Centre, but also with the local brewery. Refsvindinge is a small town with only 600 citizens, but their brewery and its prizewinning beer 'Ale no. 16' is well-known all over Denmark. After releasing tadpoles in the area, we have often visited the brewery to buy a few beers to bring back home. Such a visit once led to a talk with the owners about the possibility of selling a beer that could raise money for the project. A month later the first pallet with "Toad Brew" arrived at the Zoo. Since then, lots of

pallets have arrived and have been sold at the Zoo's restaurants and shop and in 2018, a pond on the island of Ærø was created, solely from the money raised from the beer sales.

With new ponds and releases of tadpoles bred at Copenhagen Zoo, along with the commitment of local schools and locals who take part in helping the Natterjack Toads, it is possible to restore the area so that the Natterjack Toad can roam securely again. Securing local ownership as well as the Zoo's expertise with breeding goes hand in hand and has made the noticeable difference for the toads.

Our effort has already made a difference and singing males have been heard in the area. However, the population is very vulnerable and more needs to be done before we reach our final goal. It takes a lot of effort when going from almost no breeding ponds to re-establishing a sustainable population, and thus of course we do not stop here. A new partnership agreement has just been made between Copenhagen Zoo, Nyborg Municipality and Refsvindinge Nature and Culture Centre. The funds will be used for further development of the already existing work. This means establishing new ponds and caring for the already existing ones as well as conducting research and creating education and hosting activities for children, young people and families.



"Toad Brew" – a special brew from Refsvindinge Brewery is being sold to raise money for new ponds. Photo: Copenhagen Zoo.

In 2011, Copenhagen Zoo, together with local landowners, dug the first of the new ponds for Natterjack Toads in Refsvindinge. Photo: Copenhagen Zoo.



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Amphibian Ark Conservation Grants

AArk has offered grants since 2009, and in the past eleven years, we have provided funding totaling just over US\$194,000 to forty-one projects in twenty-one countries. We offer a range of grants, including start-up grants, which provide initial funding to help newly-launched projects get started at the very beginning of their life; start-up grant extensions, which are additional funds, available to provide continued support for AArk seed or start-up grant projects that have met their stated objectives over the previous year; workshop attendance grants providing partial funding to assist attendance at ex situ amphibian conservation-related workshops; and mentorship grants, which support organizations that 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. Details of all of our grants, including guidelines and deadlines for lodging applications, can be found on the Conservation Grants page of the AArk web site, www.amphibianark.org/conservation-grants/.

Last year, we changed the application process a little, requiring all potential applicants to lodge a 200-word Project Outline, prior to submitting their full application. The outline is required to summarize the proposed outcomes of the project, provide brief details on the species, its conservation status, and other funding sources. All project outlines are

reviewed by our Grant Review Committee, with the principal investigators of approved outlines then invited to submit a full application. Feedback received from the Review Committee is provided to the applicants, which can then be considered when full applications are being written. We received seventeen project outlines this year, with thirteen of them resulting in full applications. Overall, the quality of the applications received this year was much higher than in previous years – no doubt the result of initial feedback and suggestions being factored into the applications.

Of the thirteen applications received, we are excited to announce that we are able to fund three start-up projects (US\$5,000 each, based in Ghana, India and Venezuela), US\$4,000 for a start-up extension grant based in Argentina, and US\$1,500 for a mentorship grant for capacity-building in Uganda. The successful projects this year are:

- Ex situ conservation of a critically endangered frog (Conraua derooi) in Ghana. Caleb Ofori-Boateng and Michael Gyapong Akrasi, Herp Conservation Ghana (Herp-Ghana); and Hamdia Mahama Wumbeidow, Forestry Research Institute of Ghana (FO-RIG)
- Developing ex situ facilities for the conservation of the Indian caecilian Gegeneophis tejaswini. Dr Ramachandran Kotharambath, Central University of Kerala, India
- Merida's harlequin frog is back after three decades! A proposal to rescue the species *Atelopus oxyrhynchus*. Enrique La Marca, Rescue of Endangered Venezuelan Amphibians (REVA) program of the BIOGEOS Foundation, Venezuela
- Rescuing the southernmost marsupial frog species (*Gastrotheca gracilis*) in Argentina. Dr. Mauricio Sebastián Akmentins, INECOA, UNJu-CONICET, Argentina
- Capacity Building of Uganda's in-country ex situ husbandry and captive amphibian breeding expertise. James Watuwa and Dr. James Musinguzi, Uganda Wildlife Conservation Education Centre, Uganda

Ex situ conservation of the critically endangered Togo Slippery Frog (Conraua derooi) in Ghana

Caleb Ofori-Boateng and Michael Gyapong Akrasi, Herp Conservation Ghana (Herp-Ghana); and Hamdia Mahama Wumbeidow, Forestry Research Institute of Ghana (FORIG)

The Critically Endangered Togo Slippery Frog (*Conraua derooi*) is an Evolutionarily Distinct and Globally Endangered (EDGE) amphibian (www. edgeofexistence.org/species/togo-slippery-frog/). The species is known from two localities in Ghana: the Togo-Volta highlands, which is along Ghana's border with the Republic of Togo and the Atewa Hills, one of the last upland evergreen forest in Ghana. The Atewa population is however recognised as a distinct species and a paper is currently being completed to name it (Blackburn et. al.,in review). It is feared that this distinct frog will become extinct even before it is named due to a planned bauxite mine in the Atewa Hills to repay loans secured from China. In late 2019, the bulldozers moved in to reopen roads and national security operatives secured the main entrances to the reserve. A recent map of the proposed mine coincides directly with the slippery frog's habitat and it is highly unlikely that this Atewa endemic frog has any chance of survival should the government proceed with its plans to mine the reserve in December.

The goal of this project is to save the distinct subpopulation of the Togo Slippery Frog in the Atewa Hills. We propose to establish an *ex situ* conservation program in collaboration with the Forestry Research Institute of Ghana (FORIG) and Fisheries and Aquaculture Department of the Kwame Nkrumah University of Science and Technology (KNUST).

We have been studying this frog in the wild for the past ten years. Therefore, we already have a fair amount of understanding of its habitat requirements. Notwithstanding, we plan to conduct additional field visits with one of our collabora-



tors from the London Zoo to gather additional habitat parameters. We believe this will help us to construct the breeding facility to mimic their natural habitat.

Developing ex situ facilities for the conservation of the Indian caecilian Gegeneophis tejaswini

Dr Ramachandran Kotharambath, Assistant Professor, Central University of Kerala, India

The indotyphlid caecilian *Gegeneophis tejaswini* (discovered and described by the applicant in 2015) has a small, fragmented distribution within human-inhabited areas in the foothills of the Western Ghats, within 20 km of the applicant's institution. The major threats to the species are a lack of knowledge of the species' basic biology and widespread urbanization and intensification of agriculture. The planned captive breeding program will (1) generate valuable new knowledge on the species' biology, and (2) establish and develop capacity and expertise for captive maintenance and breeding of Indian caecilians in India.

The applicant has maintained live caecilians since 2016 in a rudimentary facility, and approximately forty individuals are currently present including larvae, juveniles and adults from all three genera occurring in the region. The new *ex situ* facility will be built as per international husbandry standards and specific caecilian requirements, by vastly improving the current rudimentary setup. The project will build new knowledge on the husbandry of Indian caecilians in general and *G. tejaswini* in particular. It will generate novel data on the be-



havior, ecology, reproduction and physiological requirements of *G. tejaswini*, which otherwise are very difficult to obtain due to its highly subterranean life.

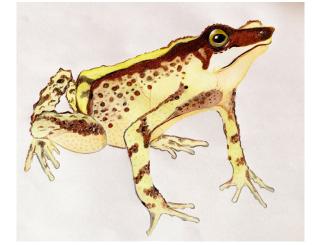
The project will be the first focused, dedicated captive breeding attempt for any Indian caecilian species (none of which are maintained in zoos in the Western Ghats region), and is expected to generate data also on other sympatric caecilian species. New data will help the applicant to write improved conservation status assessments and *in situ* and *ex situ* conservation management plans. The project will build and/or cement collaborative links with ZSL London Zoo and the Natural History Museum, London. The new facility will foster the husbandry component of the applicant's planned caecilian conservation work in the coming decade.

Merida's harlequin frog is back after three decades! A proposal to rescue the species Atelopus oxyrhynchus Enrique La Marca, Rescue of Endangered Venezuelan Amphibians (REVA) Conservation Center, Venezuela

Atelopus oxyrhynchus has been "lost" for more than three decades. It was not only the first Venezuelan harlequin frog described, but also the first in disappearing from the record by the late 1980's. Our recent finding of many tadpoles in a pristine mountain stream in the Merida Andes rises hope to rescue the species. We rescued the larvae from water pools that in the same day desiccated due to intense dry regional weather conditions. Tadpoles present signs of chytridiomicosis which, added to the climate risk, suggest that a captive husbandry program is mandatory for this Critically Endangered species. Additionally, the locality lies adjacent to a National Park, and close to the city of Mérida, posing an interesting case where the local community, official institutions, researchers and other key actors can interact to get a better conservation scenario for the emblematic frog that can be a flag or umbrella species to protect other amphibians and their environments.

Goals for the project:

- To assess the ecological requirements and current threats of Atelopus oxyrhynchus, addressed to develop appropriate captive husbandry conditions to guarantee survival of the species.
- 2. Establish a long-term captive husbandry conservation program, aimed to maintain an *ex situ* genetic stock to preserve this amphibian.



Atelopus oxyrhynchus. Drawing by Luis Saavedra.

- 3. Set an *in situ* educational environmental program and start considering all variables for a reintroduction program with mitigation of threats.
- 4. Implement a community conservation program involving local leaders, national park rangers and other key people, highlighting the project, the conservation efforts of funding organizations, and the species' importance in nature. The main goal here is to have these actors participate in joint conservation efforts for the species and the habitats where this harlequin frog live in.

Rescuing the southernmost marsupial frog species (Gastrotheca gracilis) in Argentina (Start-up grant extension)

Dr. Mauricio Sebastián Akmentins, Instituto de Ecorregiones Andinas, Unidad Ejecutora del Consejo Nacional de

Investigaciones Científicas y Técnicas, Argentina

The Banderita Marsupial Frog (*Gastrotheca gracilis*) is the southernmost species of marsupial frogs. This species was missing for twenty years, but the rediscovered populations are facing new threats. Despite the success in the implementation of the population supplementation program for the Banderita Marsupial Frog, the conservation status of this endangered species is still a matter of concern. Therefore, we propose making an upgrade of the existing facilities in the Reserva Experimental Horco Molle for the development of an *ex situ* research program to reach the entire cycle of captive breeding of this threatened

marsupial frog species. Also, we will continue the supplementation program of *G. gracilis*' population in the Reserva Provincial Los Sosa and we will establish an intensive search program focused in detecting the presence of this endangered species in the northern distribution range where it is missing for nearly thirty years.

During the second reproductive season of 2019/2020, we continued with the supplementation program of Banderita Marsupial Frogs in the Reserva Provincial Los Sosa with a successful release of a second cohort of forty-six froglets into the wild. In this opportunity, the release of the froglets was made with the support of the environmental authorities of Tucuman province government. As a result of these two consecutive experiences of rearing tadpoles and juveniles in captivity, we compiled the obtained information in the Husbandry Guidelines for this threatened species.

During the coming year we propose to upgrade the existing facilities of the Reserva Experimental Horco Molle to develop the *ex situ* research program. The improvements of the facilities will be destined to reinforce the biosecurity levels and to condition space for the raise of live food supply for the frogs. In addition, we will install a permanent glass window for providing the frogs a natural light cycle. Also, the breeding facility will be included in daily guided trail of Reserva Experimental Horco Molle, so that visitors can learn about this project.



Gastrotheca gracilis facility. Photo: Sergio Omar Saguir.

Capacity-building of Uganda's in-country ex situ husbandry and captive amphibian breeding expertise. (Preparations for establishment of the first ex situ supporting program for Uganda's frog species) (Mentorship grant)

James Watuwa and Dr. James Musinguzi, Uganda Wildlife Conservation Education Centre

In general, there is a lack of training, knowledge, expertise, and comparative information for most basic aspects of captive amphibian breeding in Uganda using standardized approaches. The goal of the proposed project is to build capacity of Uganda's in-country ex situ husbandry and captive amphibian breeding expertise. This will enhance preliminary preparations for establishment of the first ex situ amphibian breeding program for Uganda's endangered frog species.

The project will install equipment needed to setup basic amphibian breeding facilities at Uganda Wildlife Conservation Education Centre (UWEC ZOO), identify one or two common, local amphibian species (most likely *Leptopelis kivuensis*) that zoo keepers can work with as surrogate species for *Leptopelis karissimbensis* which is threatened due its declining populations in the wild.

Ian du Plessis, Curator: Reptiles, Fish and Amphibians from Johannesburg City Parks and Zoo in South Africa, will be our mentor and he will guide our staff in the process of selecting appropriate species, determining which equipment will be needed to house the species, and consequently to provide hands-on-training for amphibian keepers at the Uganda Wildlife Conservation Education Centre and other Ugandan amphibian scientists as shall be identified.



Leptopelis kivuensis. Foto: Bernard Dupont

The overall outcomes of this project are:

- 1. Basic amphibian breeding facilities established at Uganda wildlife Conservation Education Centre (UWEC ZOO).
- 2. Founder population of Leptopelis kivuensis a surrogate species for Leptopelis karissimbensis established.
- 3. Staff at UWEC ZOO will be trained in aspects of amphibian husbandry, live food production, biosecurity and captive breeding.
- 4. Captive husbandry and breeding protocols developed which will eventually be applied to Leptopelis karissimbensis.

A giant leap for amphibian conservation: South Africa's "Frog Lady" wins 2020 Whitley Award

Reprinted from www.whitleyaward.org, with thanks to the Whitley Fund for Nature

A conservation biologist from South Africa has won a prestigious Whitley Award worth £40,000 to support her quest to save threatened amphibians. Jeanne Tarrant, known locally as the "Frog Lady", works for the Endangered Wildlife Trust (EWT), where she manages the Threatened Amphibian Programme. EWT is the only NGO in South Africa to include frogs as a conservation focus.

The Whitley Awards, often referred to as 'Green Oscars', are awarded annually to individuals from the Global South by UK-based conservation charity the Whitley Fund for Nature. Jeanne is one of six conservationists to be recognised this year for their achievements in nature conservation.

Amphibians are the most threatened group of animals on the planet with 41% of all species at risk of extinction. Almost two-thirds of the country's 135 frog species are found nowhere else, making South Africa a priority for amphibian conservation. Despite this, a combination of threats from habitat loss due to mining, agriculture and pollution are putting the country's frogs at risk.

In some South African cultures, frogs can be associated with witchcraft, making them often feared by locals. Jeanne's educational work aims to dispel such myths and raise awareness and appreciation of the important role frogs play in the health of the environment and ecosystem. The EWT's national awareness Leap Day for Frogs has attracted some 15,000 participants over the past five years. Jeanne has inspired school children with her "Frogs in the Classroom" learning program, gaining young fans and earning her the title of the "Frog Lady".

Growing up in the southern Drakensberg mountains of KwaZulu-Natal, Jeanne was surrounded by nature. Following her undergraduate studies, she worked in the UK for five years before

One of the species Jeanne works with is the Endangered Pickersgill's Reed Frog (*Hyperolius pickersgilli*), with the number of known localities of this tiny 2 cm amphibian on the rise thanks to her efforts. Photo: Courtesy of Jeanne Tarrant/EWT.



Jeanne Tarrant, a conservation biologist from South Africa has won a prestigious Whitley Award worth £40,000 to support her quest to save threatened amphibians.

Photo: Courtesy of Jeanne Tarrant/EWT.

returning to her homeland of South Africa to specialise in the research of threatened South African frogs.

Some of the species that Jeanne and her team conserve include

the Critically Endangered Amathole Toad (*Vandijkophrynus amatolicus*), which had not been seen for over thirteen years until Jeanne and her colleagues re-discovered it in 2011. Jeanne also works with the Endangered Pickersgill's Reed Frog (*Hyperolius pickersgilli*), with the number of known localities of this tiny 2 cm amphibian on the rise thanks to her efforts.

In addition to education and field work, Jeanne works with government to ensure enhanced protection for frogs on a policy level. Supported by WFN, her team will produce a ten-year conservation and research strategy for South African frogs and protect 20,000 ha of amphibian habitat conserving eight species.

Jeanne said: "While South Africa has excellent environmental legislation,



illegal developments continue to destroy frog habitats. Our aim is to not only improve appreciation of frogs through research and education but use our slippery friends as flagships for the wider conservation of vital freshwater and terrestrial areas that are under the increasing threat of humans.

"The fact that almost half of amphibians are experiencing declines should be a massive wake-up call to humanity that all is not right with our planet – most people however are unaware that amphibians are even in trouble."

Edward Whitley, Founder of the Whitley Fund for Nature, said: "Jeanne is an inspiring leader who tirelessly advocates for amphibians – an often overlooked group. We hope that this Whitley Award will allow her to spread her important message far and wide and bring about real change for amphibians and their habitat through science, policy, and community education."

Jeanne's educational work aims to raise awareness and appreciation of the important role frogs play in the health of the environment and ecosystem.

Photo: Courtesy of Jeanne Tarrant/EWT.

While normally presented to winners by charity Patron HRH The Princess Royal at an annual Ceremony in London, the 2020 Whitley Awards Ceremony was postponed due to the COVID-19 pandemic. Whilst the winners will receive their funding now, they will be invited to attend a ceremony and related events in London later this year to celebrate their achievements, should circumstances allow.

Note from the Amphibian Specialist Group

We would thus like to introduce you to the new ASG Southern Africa Chair, Jeanne Tarrant. Jeanne is well known to many, if not most of you: she has been extremely active in amphibian and habitat conservation in South Africa and is one of this year's recipients of the Whitley Awards (congratulations, Jeanne!). Jeanne is also co-chair of the ASG Habitat Protection & Management Working Group, https://www.iucn-amphibians.org/working-groups/thematic/habitat-protection-2/.





Saving the Giant Lake Junin Frog in Peru

Lizette Bermúdez, Huachipa Zological Park, Peru; Luis Roque and Roberto Elías, Denver Zoological Foundation, USA; José Flores, Michael Tello and Luis Añes, Huachipa Zological Park, Peru; and Mauro Tambella, Mundo Marino, Argentina

The Lake Junin Frog (*Telmatobius macrostomus*) is an endemic High-Andean amphibian from Junin and Pasco in central Peru. Currently, it is categorized as an Endangered species by the IUCN Red List and Peruvian Legislation, however, population density data is still insufficient, the last scientific studies and the surveys from local people reveal that the frog population is decreasing. The main threats are the overharvesting for human consumption, contamination of the lake and the presence of an invasive exotic species of trout.

In 2017, we were awarded an Amphibian Ark Conservation Grant to create a captive breeding center to maintain a viable population of Lake Junin Frogs, which will possibly be released back to the wild to increase its natural population.

In 2018, the implementation of the breeding center was completed, which has two independent areas. The quarantine area with three glass tanks and the breeding area with another three glass tanks and a concrete tank. Both areas include life support and water-cooled by chiller systems. Protocols for the management of life support systems, biosecurity and water monitoring were established, and personnel have been trained to handle the systems and animals.

Protocols for live food management were developed, such as tubifex worms, fish and earthworms, and feed tests were performed with pellet food, taking as a model previous work with the Lake Titicaca Frog (*Telmatobius culeus*).

In 2019, through R.M. 026-2019-MINAGRI-SERFOR-

The Lake Junin Frog (*Telmatobius macrostomus*) is an endemic High-Andean amphibian from Junin and Pasco in central Peru, and it is categorized as an Endangered species by the IUCN Red List. Photo: Roberto Elías.

DGGSPFFS, we received permission to collect five adult males, five adult females and forty tadpoles (or metamorphs) for a period of 51 months. On June 17, 2019, the first collection was made in Capilla, Carhuamayo district of Junín department, located 4,216

The four individual frogs have completed their metamorphosis and they are in good health, and apparently well-adapted to captivity.

Photo: Marcial Sumari.



meters above sea level. Two metamorphs and two tadpoles were collected and were subsequently brought to the center and placed in two aquariums in the quarantine area. All the specimens were tested for chytrid fungus (*Bd*) during their capture.

Since that time, we have been carrying out an evaluation of the development and adaptation of the specimens, as well as standardization of the management protocols. To date, the four individuals have completed their metamorphosis and they are in good health, and apparently well-adapted to captivity.

On June 17, 2019 two metamorphs and two tadpoles were collected and were brought to the captive breeding center and placed in two aquariums in the quarantine area.

Photo: Luis Castillo Roque.



AArk Husbandry Document library

The Husbandry Document library on the AArk web site (www.amphibianark.org/husbandry-documents) currently has over 160 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.

Five new documents have been added recently:

Husbandry Guidelines for La Banderita Marsupial Frog, Gastrotheca gracilis (English)

Author: Mauricio Sebastián Akmentins, Martín Boullhesen and

Elena Correa

Publication: May 2020

www.amphibianark.org/wp-content/uploads/2020/05/Husbandry-Guidelines-Gastrotheca-gracilis.pdf

Husbandry Guidelines for La Banderita Marsupial Frog Gastrotheca gracilis (Spanish)

Author: Mauricio Sebastián Akmentins, Martín Boullhesen and

Elena Correa

Publication: May 2020

www.amphibianark.org/wp-content/uploads/2020/05/Pautas-de-Manejo-Gastrotheca-gracilis.pdf

Species Action Plan for La Banderita Marsupial Frog Gastrotheca gracilis (English)

Author: Mauricio Sebastián Akmentins and Martín Boullhesen

Publication: April 2020

www.amphibianark.org/wp-content/uploads/2020/05/Species-Action-Plan-Gastrotheca-gracilis.pdf

Species Action Plan for La Banderita Marsupial Frog Gastrotheca gracilis (Spanish)

Author: Mauricio Sebastián Akmentins and Martín Boullhesen

Publication: April 2020

www.amphibianark.org/wp-content/uploads/2020/06/Gastrothecagracilis-Plan-de-Acción.pdf

Species Action Plan for *Atelopus oxyrhynchus* (English) **Author:** Enrique La Marca, REVA (Rescue of Venezuelan Endangered Species), Venezuela.

Publication: May 2020

www.amphibianark.org/wp-content/up-loads/2020/06/Species-Action-Plan-Atelopus-oxyrhynchus.pdf

Amphibian Translocation Symposium Videos

Luis Carrillo, Training Officer, Amphibian Ark

Many amphibian populations have been decimated in the wild and many others are severely fragmented, meaning that colonizing suitable habitat is almost impossible. Translocation is one of the tools in the conservation toolbox, and often requires the help of captive breeding programs as a source of animals. It has become more relevant to amphibians due to the continued population declines for many species. However, we still need to better understand the different factors that affect the success of amphibian reintroduction programs.

Reintroduction in conjunction with threat mitigation and habitat protection should be one of the major goals of almost any comprehensive amphibian conservation program. To be successful, program managers should properly plan reintroduction programs. The IUCN (www.iucn.org) has produced Guidelines for Reintroductions and Other Conservation Translocations (www.iucn.org/content/guidelines-reintroductions-and-other-conservation-translocations), and the Reintroduction Working Group of the Amphibian Survival Alliance has drafted specific guidelines for amphibians.

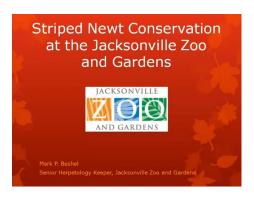
In late 2019, the Amphibian Ark hosted an online translocation symposium, which was divided into themes, with each of the themes being covered over three days each week, during two-hour presentations. Experienced program managers with previous experience in amphibian reintroductions, amphibian disease risk assessment, habitat management and restoration, threat management, and post-release monitoring shared their successes and failures. Other speakers included small-population managers, amphibian conservation-ists and general reintroduction specialists.

Learning from each other's successes and failures allows amphibian conservation program managers to gather new and unpublished information and project experiences that could be helpful to better design their reintroduction protocols; avoid unsuccessful practices or strategies; and connect with amphibian management experts and other program managers from different regions of the world.

The twenty-seven presentations which were delivered over the course of the symposium were recorded, and are available on the AArk web site (www.amphibianark.org/translocation-symposium/). The complete set of videos is also available as a playlist on YouTube (www.youtube.com/playlist?list=PLVjpGsWSiXYsClh7TG4K5qAsB_I9CNcXz).







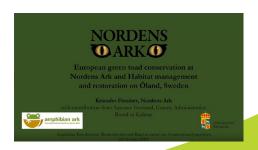












Amphibian Ark George and Mary Rabb Research Fellowship

This fellowship supports early or mid-career scientists and conservationists from all countries in research-based professional development that furthers amphibian conservation. 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. Applicants must be formally associated with a mentor or lead scientist at a relevant university, NGO, zoo/aquarium, or other established institution. The fellowship carries a \$5,000 stipend and is open to applicant from all countries.

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.

Application materials and the letter of support from the mentor should be e-mailed to: Joseph Mendelson, AArk Scientific Advisor, jmendelson@zooatlanta.org. Submission deadline: 1 September 2020. 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 essentials 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 (AArk) 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.





Check out our Amphibian Ark t-shirts, hoodies and sweatshirts!

We're continuing to help support amphibian conservation programs for threatened species by raising awareness and resources, with the sale of AArk clothing. Please join us and check out our new T-shirt designs featuring some of your favourite frog species, or show your support by proudly wearing our new AArk Rescue Team t-shirts

We've recently added many different designs and colors, in men's women's and children's sizes.

Some of the items feature species from our partners' breeding programs, and all profits from these shirts will go directly to supporting amphibian conservation programs.

Head to the AArk clothing store at www.amphibianark.org/AArk-products.htm and check out our clothing items!

Your continued support is helping to save the most threatened amphibians!



More than twenty-one partners celebrate first-ever **World Water Frog Day**

Teresa Camacho-Badani, Sophia Barrón Lavayen and Ricardo Zurita, Museo de Historia Natural Alcide d'Orbigny, Centro K'ayra de Investigación y Conservación de anfibios amenazados de Bolivia

Water frogs and semi-aquatic frogs of the genus *Telmatobius* (Anura: Telmatobiidae), represent a characteristic component of anuran communities in the Andean and extra-Andean mountainous regions of western South America, from the Loja Basin in Ecuador to the province of San Juan in Argentina, through Peru, Bolivia and northern Chile. Usually, the distribution range of water frog species is usually restricted. The Giant Water Frog (Telmatobius gigas) for instance, has only been observed in some rivers and streams in the Huayllamarca basin in Bolivia. The critically endangered Salta Water Frog (Telmatobius atacamensis), its turn, is known only from two localities at 3,800 m elevation in Argentina.

According to the International Union for Conservation of Nature (IUCN), out of sixty-three water frog species described, 86% are currently threatened with extinction. Even worse, eight species may be already extinct in nature, such as the Vellard's Water Frog (Telmatobius vellardi) in Ecuador, which has not been seen since 1968.

Even though bringing us hope, conservation efforts related to

water frogs have been increased over the past few years. Worth mentioning are: the work of Amphibian Survival Alliance (ASA) partner Grupo RANA and the Denver Zoo on the Lake Junin Water Frog (Telmatobius macrostomus) in Peru; the rescue of the last individuals of the Critically Endangered Loa Water Frogs (Telmatobius dankoi) from their dried-up habitat in Chile. conducted in partnership with national and international wildlife organizations such as National Zoo of Chile, the Amphibian Survival Alliance, Amphibian Ark, the IUCN SSC Amphibian Specialist Group and ASA partner Global Wildlife Conservation: and the development of the Conservation Action Plan for the worldwide famous Sehuencas Water Frog (Telmatobius yuracare), which has become a symbol of the amphibian conservation movement. The K'ayra Center of the Museo d'Orbigny in Bolivia conducts the largest ex situ conservation program for water frogs in the world, currently hosting more than 600 individuals belonging to five Telmatobius species.

Why create a special day for Telmatobius water frogs?

It all started when more than ten zoos in Europe received Titicaca Water Frogs for the first time in 2019 and showed interest in supporting their in situ conservation in South America. The idea came from BCA Zoo (UK) and ASA partner Chester Zoo (UK), which were interested in creating a day to highlight and encourage the conservation of Titicaca Water Frogs. Based on this first proposal, we decided to expand the idea to include all Telmatobius species, since these are one of the most threatened amphibians in the Neotropics.

Even though there are already several initiatives aiming for the conservation of water frogs, we believe research and joint efforts should be increased. That is why the Alcide d'Orbigny Natural

History Museum in Bolivia, Global Wildlife Conservation and the Amphibian Survival Alliance decided to create the "World Water Frog Day". The initiative was celebrated and ioined by several institutions all around the world, such

as the BCA Zoo (UK), Amphibian Ark, Universidad Cayetano Heredia (Peru), Denver

Zoo (USA), Natural Way Peru, Balsa de los Sapos (Ecuador), Pro Fauna Ayacucho (Peru), IUCN Amphibian Specialist Group (ASG), Asociación Boliviana de Herpetología, Zoológico Nacional de Chile, Chester Zoo (UK), Kansas City Zoo (USA), Grupo RANA (Peru), Universidad Andrés Bello (Chile), Museo de Calama (Chile), Centro Jambatu (Ecuador), Instituto de Ecorregiones Andinas (INECOA, Argentina), Asociación Red Chilena de Herpetología, and Joel Sartore Photo Ark of National Geographic among others.



The Giant Water Frog (Telmatobius gigas). Photo: D. Alarcón/ D. Grunbaum.

Why April 1st?

We were looking for a representative date for the entire genus and found out that this was the day the first water frog individual was recorded in history. On April 1st 1831, the Prussian naturalist F.J.F. Meyen was camping in a cave in Palca (Peru) when he heard the call of the first water frog collected in history. These firsts individuals were later used by A.F.A. Wiegmann to describe the genus Telmatobius and the species Peru Water Frog (Telmatobius peruvianus) in 1834 and 1835.



Romeo, the Sehuencas Water Frog (*Telmatobius yuranare*) at the Centro K´ayra del Museo de Historia Natural Alcide d´Orbigny. Photo: D. Alarcón/ D. Grunbaum.

Why are water frogs so threatened?

Diseases

One cause of extinction of these species are diseases such as chytridiomycosis, especially in cloud forest where some species of water frogs have not been seen in a long time, such as the *Telmatobius edaphonastes* water frog, which is endemic to Bolivian cloud forests, considered to be one of the least aquatic water frogs with historical records of seeing it perching in the forest. It has not been seen for more than twenty years and the latest record of these species in scientific collections is from 1998 with a prevalence of 100% chytrid fungus.

Pollution

Water frogs spend a large part of their lives in the water - some of them never come to the surface - which is why contamination can be deadly to them. For example, in 2015 more than 10,000 Titicaca Water Frogs (*Telmatobius culeus*) were found dead in the Bolivian side of Lake Titicaca, and later in 2016, the same number

of dead frogs were found floating on the Peruvian side. The reasons for these deaths point to water pollution such as heavy metals and other pollutants from mining, pesticides, urban waste and sewage that are expelled directly into the lake by populations on the shore.

Traffic

One of the most trafficked species of water frogs is the Titicaca Water Frog. In 2019 more than 2,500 frogs were confiscated by the National Forest and Wildlife Service of Peru (SERFOR). The frogs were in the road from Lake Titicaca to markets in Lima, the capital of Peru, for consumption in markets as juices, as people considered them to be a cure for several diseases. In 2020, the Bolivian Forest Police and Environmental Preservation (POFOMA) also confiscated Titicaca Water Frogs and the *Telmatobius marmoratus* water frog which were being commercialized for rituals

How did we celebrate the first Water Frogs Day in 2020?

Originally, we had planned several in-person activities in different parts of the world, such as the opening of the Titicaca Water Frog Exhibitions at the Museo de Historia Natural Alcide d'Orbigny in Cochabamba, Bolivia, and at the BCA Zoo in UK. Unfortunately, because of the COVID-19 pandemic, we had to cancel these events. However, this did not stop us from filling the social networks of more than twenty-one institutions, newspapers and TV shows with photographs, information and videos of Telmatobius, to remind the general public of their importance. We have also recognized and highlighted the institutions and people around the world who are making great efforts to conserve water frogs.

We hope that each year this will be a bigger event, that will help to spread the word about the importance of conserving this group of amphibians. By doing so, we aim to encourage research and collaborations, to strengthen relationships between institutions, and to bring the people closer to this highly threatened group of animals.





Project planning for the implementation of the Pickersgill's Reed Frog program at the Amphibian Research Project of the Johannesburg City Parks and Zoo

lan du Plessis, Curator: Reptiles, Fish and Amphibians; Cassandra A. Becker, Nutritionist; and Piet Malepa, Manager Animal Welfare, Johannesburg City Parks and Zoo, South Africa

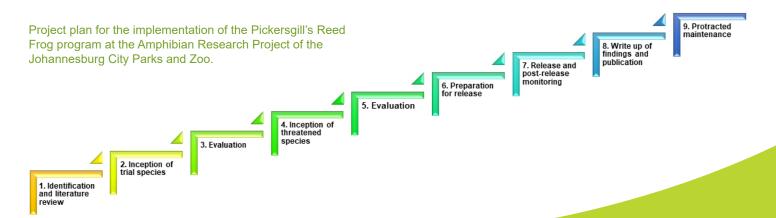


A project plan was designed and implemented as a guideline to ensure success with the *ex situ* breeding program for Pickersgill's Reed Frog (*Hyperollius pickersgilli*) at the Johannesburg City Parks and Zoo. Photo: Donovan Marais.

Background

In 2006 the Johannesburg City Parks and Zoo committed to assist with the conservation of endangered amphibians by concentrating on South African species under the Amphibian Research Project. After years of trials and research, the Amphibian Research Project was requested by Ezemvelo KwaZulu-Natal (KZN) Wildlife to combine resources in the conservation of South African amphibians. There was an approved Biodiversity Management Plan from the National Department of Environmental Affairs in South Africa. In 2017 a Memorandum of Understanding was signed between Johannesburg City Parks and Zoo and Ezemvelo KZN wildlife. The Amphibian Research Project was then ready to move over from common species that were used to set the platform for the project to the first endangered species, the Pickersgill's Reed Frog (Hyperolius pickersgilli). A project plan was designed and implemented as a guideline for the team to ensure success.

Step 1: Identification and literature review	 Literature review on husbandry, nutrition, veterinary, enrichment, standard operating procedures (SoPs), agreements, and memorandum of understanding (MoU) as well as ethical approval. Research done by utilizing various literature sources to establish a platform for initiating the project. Identification of candidate trial species, to mimic target species in order to design husbandry manuals, and to draft SoPs. Hyperolius marmoratus was identified as the trial species as they are potentially the closest analog to the Pickersgill's Reed Frog. Enclosure design and setup. Set goals step by step to achieve objective.
Step 2: Inception (trial species)	 Implementation of trial species breeding project (breeding cycle). Regular reviewing of protocols. Improvement of the guidelines of husbandry methods. Creation of a suitable and sustainable ex situ environment, housing and feeding system. NB. This step needs to be conducted over a period of two years.
Step 3: Evaluation	 Complete evaluation of steps 1 and 2. Prepare for introduction of endangered species, in this case Pickersgill's Reed Frog. Additional research and literature review in preparation for species-specific husbandry for the Pickersgill's Reed Frog.
Step 4: Inception (threatened species)	 Introduction of the Pickersgill's Reed Frog to the program. On-going evaluation and data collection.



Step 5: Evaluation	 Improve the guidelines of husbandry methods according to the requirements of the Pickersgill's Reed Frog. Regular reviewing of protocols for suitability regarding Pickersgill's Reed Frog. Continued breeding of Pickersgill's Reed Frog in order to produce a sustainable insurance population to F1 generation.
Step 6: Preparation for release	 Prepare administrative requirements for release and post-release monitoring (e.g. permits, transport). Identification of suitable release site. Preparation of specimens for release based on protocols (e.g. tagging of specimens, desensitization).
Step 7: Release, and post- release monitoring	Release of specimens into approved site. Post-release monitoring.
Step 8: Write up of findings, and publication	Preparation of literature publication – husbandry manuals and journal publication.
Step 9: Protracted maintenance	 Continual evaluation of protocols. Post-release monitoring. Insurance population maintenance.

Based on the project plan above for the endangered species, the Amphibian Research Project at the Johannesburg City Parks and Zoo have released, reinforced and reintroduced 371 captive-bred Pickersgill's Reed Frogs within KwaZulu-Natal, South Africa between 2018 and 2020. It is crucial that this model be followed step by step to ensure optimum flow even though continuous monitoring is required to evaluate success and eliminate any potential risk. Post-release monitoring proved that these specimens adapted successfully into their natural environment and constant post-release monitoring is still on-going to date. This proves that the model drafted and implemented is successful and it will be adapted to new species that will be introduced into the Amphibian Research Project at the Johannesburg City Parks and Zoo.



(Left- right) Piet Malepa, Ian du Plessis and Dr Adrian Armstrong during the 2020 release of Pickersgill's Reed Frogs within Kwa-Zulu-Natal. Photo: Timothy Netsianda.

Strengthening the amphibian conservation and education program at the Santacruz Zoo, Colombia

Susan Paola Castillo Vega, Project and Collection Coordinator and Biologist; and Ruth Viviana Parra, Legal Representative, Santacruz Zoo, Colombia

During the course of our investigations as part of the Amphibian Conservation and Education Program at the Santacruz Zoo, we have observed the behavior of *Pristimantis renjiforum*, an endemic species of our region and list in the IUCN Red List as Endangered. Inventories and population censuses have been developed in different areas of the region and at the *ex situ* conservation level, vocal activity, life cycle monitoring, nutrition, preventive medicine and both biological and ethological description of the species have been investigated. These processes have been developed with the participation of trained personnel, veterinarians, zoo technologists and biologists, as well as students from related careers, who through agreements established with different universities, have been able to develop research projects, practices and theses.

Pristimantis renjiforum is an endemic species in the Peñas Blancas Comprehensive Management District in Cundinamarca, Colombia, and is listed in the IUCN Red List as Endangered. Photo: Susan Paola Castillo Vega.

Pristimantis renjiforum is a species found in the Peñas Blancas Comprehensive Management District, which is located between the municipalities of San Antonio del Tequendama and Granada in Cundinamarca, Colombia, within a total area of 5,985 hectares. It has been found at elevations ranging from 1,500-2500 meters above sea level. The area is characterized by being an Andean cloud forest ecosystem, in which diverse species of native flora and fauna are found and is protected by the CAR (Regional Autonomous Corporation) and the mayoralties of the corresponding municipalities.

The developed studies have allowed us to determine that for some time this species has been displaced at the level of the habitat and the vegetative layer, this, together with the low population density recorded, guides us as an organization, with emphasis on conservation and education. In 2020 we are conducting a diagnosis of the species and its habitat, which will help us to document the current distribution, population census, causes of displacement and possible solutions to establish protection strategies for the species. Along with government entities and involving the local community from different areas, we hope to increase the biodiversity and conservation of amphibians and contribute to the protection of this reserve, which is currently affected by indiscriminate activities of deforestation, agriculture and illegal trafficking.

It is important to highlight that the location of human population settlements in many cases are involved or in contact with the ecosystems of these animals, and are the main causes of population decline, however, through their contribution and actions it is possible to achieve protection results on a larger scale in a short period of time, especially if working with children and young people, who see and highlight the need to care for more easily.

To achieve our objectives, the Santacruz Zoo applied for and won a grant from CBOT 2019 (Chicago Zoological Society), receiving funds that will allow us to complete the proposed diagnosis, but also contributing to advances in *ex situ* conservation of two more species of amphibians from the Tequendama region (the Palm Rocket Frog (*Rheobates palmatus*) and *Dendropsophus padreluna*). We will also contribute through working hard with different communities to generate reasonable defenders who can make themselves heard in favor of this group of animals, strengthening the institution's Amphibian Conservation and Education Program and improving the amphibian protection programs of Colombia and the world.

In the Peñas Blancas Comprehensive Management District there are also other species of the genus *Pristimantis*, such as the Bogota Robber Frog (*Pristimantis bogotensis*), *Pristimantis susaguae* and the Two-colored Robber Frog (*Pristimantis bicolor*), among others. Our diagnosis will identify possible problems specific to each of these species, which will focus in the future on developing more research involving these amphibian species.



Santacruz Zoo in Colombia plans to work with government entities and local communities from different areas, to increase the biodiversity and conservation of amphibians such as *Pristimantis renjiforum*, in the Peñas Blancas Comprehensive Management District. Photo: Susan Paola Castillo Vega.

Ex situ conservation strategy for the Lake Pátzcuaro Axolotl at the Zacango Ecological Park

Mtro. Biol. Manuel Antonio Pérez Rodríguez, Research and Conservation Area, Zacango Ecological Park, Mexico; Dr. Omar Domínguez Domínguez, Laboratory of Aquatic Biology, Michoacana University of San Nicolás de Hidalgo, Mexico; Ojo de Agua Community, Lake Pátzcuaro Fishermen's Cooperative, Mexico; and Dr. José Antonio Ocampo Cervantes, Project Manager at the Cuemanco Biological and Aquaculture Research Center, UAM Xochimilco, Mexico

Introduction

Beginning in June 2019, we used funds from the Conservation Start-up Grant, awarded by the Amphibian Ark, to purchase materials for modification of the facilities for this project at the Zacango Ecological Park in Mexico, so we could collect Achoque (Ambystoma dumerilii) eggs that remain attached to fishermen's nets at Lake Pátzcuaro in Mexico. We bought special fish tanks to allow the separation of specimens by size, thermostats to help with temperature control, management networks, a reverse osmosis filter to equalize the physicochemical conditions of the water, containers for transportation of axolotl eggs collected at the lake and other equipment necessary for the operation of the project inside and outside the facilities.

In November 2019, the staff of the Zacango Ecological Park made four collection trips to Lake Pátzcuaro to obtain axolotl eggs. Our objective was to collect the eggs, which were by-catch from fishing nets, and which would otherwise become food for the crayfish and carp that inhabit the lake - the fishermen generally discard them as they have no economic value for them. These eggs were taken to the facilities within the Zacango Ecological Park, so we could begin to take care of them, during their development into axolotls.

For the second part of the project and after obtaining a total of 760 hatched fry, maintenance tasks were carried out, regular growth measurements were taken, mortality and survival records were made, the quantity of food offered was recorded, control of temperature, size classification, and medical treatment offered for these specimens, as part of the animal welfare program established within the Park for all the species that are housed here.

Ex situ management

From our *ex situ* management of the animals at the Park, we were able to obtain the following data:

- Of the 2,353 eggs collected, 41.3% were fertilized.
- The hatched fry were kept in fish tanks with water which was 50% reverse osmosis filtered water and 50% well water.
- Water changes were made daily to 50% of the total tank volume.



Live adult axolotls were found inside the fishing traps and the state of their health and body condition parameters were evaluated. Photo: Manuel Antonio Pérez Rodríguez.



Extraction of fishing traps in which adult axolotls and *Ambystoma*dumerilii eggs are trapped.

Photo: Manuel Antonio Pérez Rodríguez.



After the eggs which were rescued from fishing nets hatch, ex situ management includes carrying out growth and maintenance measures on the specimens until they reach a size suitable for release. Photo: Manuel Antonio Pérez Rodríguez and Hector Javier Castelán Ortiz.

- Once a week, physical and chemical tests were carried out on the water to control the parameters.
- The diet was changed according to the size and depending on the growth of the axolotls. During the first stage of development we fed water fleas (*Daphnia*); during the second stage we fed tubifex worms; and during the third stage of development, we fed live charales.
- With the support of the Michoacana University of San Nicolas de Hidalgo and the Cuemanco-Xochimilco Biological Research Center, specimen marking tests were carried out using a temporary tattoo technique.

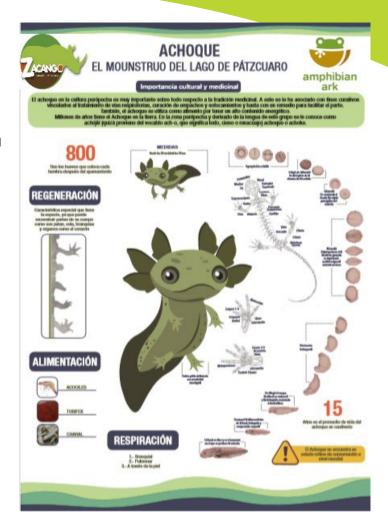
Conclusions

With the information obtained, the data were based on a growth matrix, and these were analyzed in order to be compared through the linear Von Bertalanffy equation. The scatter plots graph shows the growth in size of the fry with data taken from the first day of hatching and during development, until reaching 4 cm in length. This graph shows a sigmoid shape and a curve that does not fit the expected data was observed, with a decrease in the growth of the specimens maintained ex situ. This demonstrates the vulnerability of the species, prior to the ex situ conditions. These results mean we will use a more controlled approach in the future and should be further reviewed after the second period from 2020-2021. This will influence the ex situ work by counting erythrocytes and erythrocyte size, making estimates of biomass conversion and variants in the quality of water used.

Complementary actions

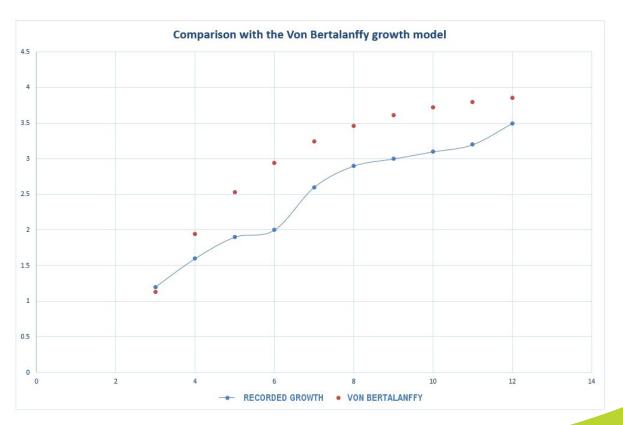
During this first year of obtaining the grant, in addition to working with the axolotls in the Park, various other actions were carried out, including:

- · Egg collection in Lake Pátzcuaro.
- Holding the first Ambystoma dumerilii ex situ management workshop.
- · Practice marking with the tattooing technique.
- · Dissemination of informative material.



Support material created at the Zacango Ecological Park to share the importance of conserving the Lake Pátzcuaro Axolotl.

Photo: Manuel Antonio Pérez Rodríguez.



Comparative graph of expected growth vs. growth obtained.

Amphibian Ark donors, 2019-2020

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