amphibian ark Newsletter

Keeping threatened amphibian species afloat

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The European Green Toad rearing and reintroduction project at Nordens Ark, Sweden

Kristofer Försäter, Emma Nygren and Mats Niklasson, Nordens Ark Foundation, Sweden

The European Green Toad (*Bufotes variabilis*) is a continental species that requires warm summers and cold winters. They can be found in most of Europe with the south of Sweden being its northernmost range. In Sweden, shallow ponds in coastal areas with high solar radiation that heat up early in spring are their prime habitat. They can also be found in areas with ponds in rocky cliffs surrounded by sandy grass and shrub-dominated areas. Historically, the species could be found in more than 100 localities in southern Sweden but it is now restricted to a handful of small and fragmented localities.

The main threat to the species is the continuing loss of suitable land habitat and spawning water where overgrowth, drainage and lowering of groundwater levels are amongst the most significant problems, but also predation and road construction pose significant threats. Habitat restoration is highly prioritized and officially recommended in the 2010 Species Action Plan (www.naturvardsverket.se/Documents/publikationer6400/978-91-620-6406-8.pdf (in Swedish)).

Nordens Ark is a non-profit foundation in Sweden with over twenty-five years' experience of working with endangered species, both *ex situ* and *in situ*. Nordens Ark has been dedicated to amphibian conservation and actively involved in captive breeding and re-introduction of the European Green Toad since 1995. To develop rearing methods and improve the survival of as many released toads as possible has been a challenge since the very start of the project (http://nordensark.blob.core.windows.net/media/177323/annualreport-nordens-ark-2016-hemsida.pdf).

Advancing the husbandry

Breeding of Green Toads starts with eggs collected from nature. At present a former limestone quarry at Limhamn, Malmö is the main locality for egg collection. Short segmented egg strings, corresponding to about 100 eggs per string, are gathered and transported back to Nordens Ark. The eggs and toads are kept and bred in an isolated rearing facility in a biosecure manner.

Over the years much effort has been allocated to advancing and adjusting the husbandry and management in order to produce as many toads fit for release as possible. Large aquaria equipped with external filters enable us to keep large numbers of tadpoles in a stable aquatic environment. Once the toads metamorphose they are moved to a terrestrial setup. These are large plastic boxes with integrated lighting and heating including UVB, suited for the Green Toads.

The rearing facility for European Green Toads at Nordens Ark. Photo: Kristofer Försäter.

Historically, the European Green Toad (*Bufotes variabilis*) was found in more than 100 localities in southern Sweden but it is now restricted to a handful of small and fragmented localities. Photo: Claes Andrén.

We keep and breed all the live invertebrate food used for rearing in a large heated room. Continuous husbandry and welfare improvements have resulted in steadily growing numbers of toads being reared and in 2017 we managed to rear an all-time high of 6,000 toads in one season.

Reintroduction into the wild

This is a long-term project with toads being released to the wild every year. Prior to release, the toads undergo testing for both chytrid fungus and rana virus. Since 2009 the releases of Green Toads have focused on two localities on Öland: Ottenby in the south and the Natura 2000 sites at Högbyhamn in the north. Habitat restoration, such as new breeding ponds and hibernation areas has been done at both sites and the project is carried out in cooperation with the county administration in Kalmar. Between 2009 and 2015 a total of 1,760 toads were released. In 2017 we conducted the largest releases of toads to date, with a total of 4,695 toads. Annual monitoring shows that a small but vulnerable population has been established with successful reproduction confirmed by finding new metamorphs in the autumn.

Visions and challenges

With chytrid fungus (*Batrachochytrium dendrobatidis*) now present in all but one population of Green Toads in Sweden it would seem important that all new populations of Green Toads are from disease-free environments. And given the fact that there is only one small and fragile population (the old limestone quarry in Limhamn, Malmö) which is currently free from chytrid disease and is used to collect eggs for rearing, the plan is to now build a larger breeding facility at Nordens Ark and to collect and treat adults from other populations for chytrid before being introduced into the breeding population. In this way, we will increase genetic variation which could play a vital role in the establishment of new populations.

Development of a preliminary nutritional program for the maintenance of the Palm Rocket Frog and *Dendropsophus padreluna* in controlled conditions at the Santacruz Zoo Foundation, Colombia

Cindy Tatiana Gil Muñiz, Student of Zootechnics, La Salle University, Colombia; Susan Paola Castillo Vega, Project and Collection Coordinator, Santacruz Zoo Foundation, Colombia; and Haydy Monsalve Redwan, Executive Director, Santacruz Zoo Foundation, Colombia

The word amphibian derives from the Greek *amphi* "double" and *bios* "life" which refers to its larval life as tadpoles and to its adult life. Colombia is one of two countries with the greatest variety of amphibians in the world, with 733 species, especially for the anuran group (frogs), but this is affected by the different problems facing amphibians in our country. Frogs from the family Dendrobatidae are the most affected: studies report that between 1985-1995 large areas of forest (547,940 hectares) have been lost, therefore, conservation plans are of great importance, and this is where *ex situ* conservation institutions play an important role. The maintenance of species in controlled conditions is a challenge that involves several aspects, especially in amphibians since their requirements constantly change each phase of their lives.

Two species of high Andean amphibians; the Palm Rocket Frog (*Rheobates palmatus*, above) and *Dendropsophus padreluna* (below) were used to compare the consumption of three different food species at the Santacruz Zoo Foundation, Colombia. Photos: Santacruz Zoo Foundation.

Applied studies with these species have helped us to obtain information about their ecology, guiding us to understand and identify in more depth their needs and threats. This has led to the creation of conservation programs for a range of different species, where captive breeding institutions (zoos and rescue centers among others) fulfil an important role focused on this goal. For the success of these types of programs, research in the area of nutritional requirements is necessary as this information is often scarce.

This leads us to consider how we will develop and implement guidelines for feeding, and improving breeding and maintenance conditions of these amphibian species under controlled conditions. To answer this question we decided to compare three types of food for two species of high Andean amphibians; *Dendropsophus padreluna* and the Palm Rocket Frog (*Rheobates palmatus*) under controlled conditions, for conservation purposes.

Methodology

The Palm Rocket Frog, from the family Aromobatidae, measures between 1-5 cm and has skin covered by small granules. It is dark brown in color with irregular patches of light brown, its belly is white, with yellow color in the groin area, and it has sturdy legs. It has diurnal habits, feeds on insects and is listed as Least Concern in the IUCN Red List of Threatened Species. It is endemic to Colombia, associated with slopes of the mountain ranges and low hills, and it is present in departments such as Antioquia, Boyacá, Cundinamarca, Santander and Tolima. It can be found in areas between 200-2,520 meters above sea level.

Dendropsophus padreluna is a frog from the family Hylidae, which is distributed in sub Andean and Andean forests of the central region on the western slope of the eastern mountain range with records in the department of Cundinamarca

specifically in the municipality of Alban (farms of Padre Luna) and Pedro Palo lagoon. It can be found between 1,882-2,328 meters above sea level, in temporary pastures and lagoons, and as there are no known threats to this species, it is also listed as Least Concern in the IUCN Red List.

Food items

The food items offered were:

Common cricket (*Acheta domestica*): An insect from the order Orthoptera, family Gryllidae. It is brown, has antennae and wings, females have a structure called ovopositor with which they lay their eggs on the ground and both sexes have segmented tails called "cerci". The adult is between 1.5-2.5 cm. They have an incomplete life cycle (egg, nymph and adult), they are omnivores, the female is able to deposit 30-150 eggs, and they require temperatures between 25-35°C.

Fruit fly (*Drosophila melanogaster*): This is an arthropod from the order Diptera, family Drosophilidae, melanogaster subgroup. It has complete metamorphosis (egg, larva, pupa and adult).

Woodlouse (*Oniscidea* sp.): Commonly known as moisture pill bugs or ground woodlouse, these are from the order Isopoda and sub order Oniscidea. They are flat and oval-shaped and are characterized by having a rigid and segmented exoskeleton. They feed on vegetable remains and stems with accumulation of water, living from mountain ranges to underground caverns.

The experiment was carried out in the facilities of the amphibian laboratory of the Santacruz Zoo Foundation in Colombia. Forty-two animals of the two species were collected (thirty Palm Rocket Frogs and twelve *Dendropsophus padreluna*).

Species	Group	Food offered	No. of individuals
Palm Rocket Frog	1	Crickets	5 per species and 5 duplicate
Palm Rocket Frog	2	Woodlice	5 per species and 5 duplicate
Palm Rocket Frog	3	Fruit flies	5 per species and 5 duplicate
Dendropsophus padreluna	1	Crickets	4 per species
Dendropsophus padreluna	2	Woodlice	4 per species
Dendropsophus padreluna	3	Fruit flies	4 per species

Table 1. Foods offered

The animals were divided into groups so we could provide the groups with the three food types (crickets, woodlice and flies). Six groups of Palm Rocket Frogs were formed, with five individuals in each group, and three groups of *Dendropsophus padreluna* were created, with four individuals in each group. The variable that was taken into account was the weight variation. The data obtained were subjected to a parametric statistical analysis using (simple ANOVA) with multiple range tests or (LSD) Fisher.

Results

Table 2 shows the analysis of the data obtained during the experiment for the species of Palm Rocket Frogs; since for *Dendropsophus padreluna* the number of individuals were not statistically significant to perform an analysis.

Group	Media	Homogeneous groups
1 Cricket	2,535	X
2 Woodlouse	3,021	X
3 Fruit fly	2,942	XX

Table 2. Evaluation of variable weight with respect to groups 1, 2 and 3.

The statistical analysis shows that there are significant differences between groups.

Table 3 shows the percentage of consumption for each one of the food items offered, and it is evident that the preferred food species was the cricket, followed by the woodlouse.

Food offered	Consumption %
Cricket	83
Woodlouse	72
Fruit fly	65

Table 3. Consumption percentage per food species.

Conclusion

The nutritional content of each one of the food species is different, with each one contains differing levels of minerals and nutrients. Therefore the provision of multiple food species for captive feeding is recommended because each of them provides essential nutrients for the optimal development of individuals.

Two new Amphibian Ark seed grants awarded

In August we called for applications for a second round of Amphibian Ark Seed grants. These competitive $5,000 grants fund start-up *ex situ* rescue projects for species that cannot currently be saved in the wild. Successful proposals reflect AArk values and should follow these guidelines:

- focus on species whose threats cannot be mitigated in nature in time to prevent their extinction and who therefore require *ex situ* intervention to persist
- work with species within their native range country
- involve range-country biologists
- adhere to recommended biosecurity standards for *ex situ* programs
- link *ex situ* programs to *in situ* conservation
- involve partnerships to maximize the likelihood of the program's long-term sustainability

We're very pleased to announce that two grants were recently awarded to new amphibian conservation programs, both in Latin America:

- **An *ex situ* conservation program for the Zippel's Frog, *Aromobates zippeli***, Enrique La Marca, Laboratory of Biogeography of the University of Los Andes at Merida, Venezuela.

  Aromobates zippeli, a Venezuelan Andean high montane frog species, is one of the most endangered *Aromobates*. There is an urgent need to establish an *ex situ* program, given the huge amount of habitat destruction and agrochemical pollution facing the remaining populations. This project is addressed to rescue populations of this endangered species through captive husbandry and breeding, as well as reintroduction back into the wild. The *ex situ* program for Zippel's Frog will be carried out in the Laboratory of Biogeography of the University of the Andes in Mérida, Venezuela, following recommended biosecurity standards and captive husbandry protocols developed by us with previous *ex situ* seed grants from AArk. This species has a close relationship to the AArk since it was named after a previous Program Director, Kevin C. Zippel.

  The project proposal can be viewed at www.amphibianark.org/seed_grants/Enrique-La-Marca-AArk-Seed-Grant-Proposal.pdf.

Zippel's Frog, *Aromobates zippeli*, is one of the most endangered *Aromobates*, and will soon be part of an *ex situ* conservation breeding program in Venezuela. Photo: Fernando J.M. Rojas-Runjaic.

- **Saving the giant frogs of Peru, *Telmatobius macrostomus***, Lizette Bermúdez Larrazábal, Parque Zoológico Huachipa, Peru.

  The Lake Junin Frog (*Telmatobius macrostomus*) is a high Andean species of lacustrine frog categorized as Endangered by the IUCN. This species is in danger of disappearing since in recent years their populations have been diminished on a large scale. Among the priority threats are over-exploitation for consumption and use in the preparation of curative extracts and the high degree of contamination of the bodies of water where they live. Despite the state's efforts to create protected natural areas to mitigate its threats, these have not been effective, and other measures are needed to support the population. We intend to manage a viable population of this species in captivity and achieve reproductive success so that they can be reintroduced in the future. Huachipa Zoological Park, has successfully maintained and reproduced the Titicaca Water Frog (*Telmatobius culeus*) in captivity, so we can manage a viable captive population, also there is an action plan which is being developed for the conservation of this species. Our experience will hopefully allow us to be successful with management of *T. macrostomus* which requires immediate management.

  The project proposal can be viewed at www.amphibianark.org/seed_grants/Lizette-Bermudez-Saving-the-giant-frogs-of-Peru.pdf.

Populations of the Lake Junin Frog (*Telmatobius macrostomus*) have diminished on a large scale in recent years but a captive program at Parque Zoológico Huachipa in Peru aims to help recover this species. Photo: Lizette Bermúdez Larrazábal.

The addition of these two seed grants brings the total number of AArk seed grants awarded since 2009 to twenty-six, in sixteen countries, with a total of US$127,109 being awarded. The complete list of seed grant recipients can bee seen at www.amphibianark.org/seed-grant-winners/.

Amphibian Advocates

We are pleased to share stories about another two dedicated amphibian advocates in this newsletter - Jonathan Kolby from the Honduras Amphibian Rescue and Conservation Center, who has been researching chytrid fungus in wild amphibian populations, as well as helping to protect endangered species in Honduras; and Olivier Marquis from Paris Zoo in France, who focuses his efforts on amphibian conservation and research both in the wild and in captivity. We hope you enjoy reading their stories.

The profiles of all of our Amphibian Advocates can be found on the AArk web site at www.amphibianark.org/amphibian-advocates. If you would like to nominate an Amphibian Advocate to be featured in a future edition of the AArk Newsletter, please send us an email at newsletter@amphibianark.org and we'll add your suggestion to our list!

Jonathan Kolby, Honduras Amphibian Rescue and Conservation Center

As long as I can remember, I've been fascinated by reptiles and amphibians. Most of my earliest childhood memories involve wandering the woodlands and wetlands of New Jersey and returning home with a muddy complexion and a pet frog in my pocket. I never imagined that twenty years later, I would find myself racing around the world searching for outbreaks of emerging amphibian diseases and building a frog rescue center in Honduras to save frogs from extinction.

My involvement in herpetological conservation began in 1997, when I volunteered to join a reptile and amphibian biodiversity survey in Hong Kong and mainland China with The Conservation Agency. After spending a month bushwhacking through tropical river valleys, exploring small uninhabited islands, and catching my first bamboo viper, I quickly learned that fieldwork was in my blood. During this trip, I became good friends with Dr. Skip Lazell, President of The Conservation Agency, who soon became my mentor. He helped me write my first publication and invited me to join him on three more expeditions to Hong Kong as well as to Kenya, New Caledonia, and the US Virgin Islands.

Among other conservation activities, Jonathan Kolby is building a frog rescue center in Honduras to save frogs from extinction.

In 2006, I stumbled across an opportunity to join a survey team in Honduras with Operation Wallacea, working to document reptile and amphibian biodiversity in Cusuco National Park, a cloud forest threatened by illegal logging and poaching. I worked with a small team of herpetologists spread across this forest and in just six weeks, we discovered nearly thirty species not previously documented in this region.

While preparing to return to Cusuco in 2007, I read several IUCN Red List assessments of frogs in Honduras that were disappearing for unknown reasons. A couple weeks later, I attended a course on amphibian disease monitoring provided by the US Fish and Wildlife Service, and one of the instructors, Katy Richards-Hrdlicka, taught us how to sample frogs and tadpoles for chytrid fungus. I previously knew very little about amphibian declines and the global emergence of amphibian chytrid fungus, and after this class, I decided to immerse myself into this alarming conservation issue and investigate whether chytrid fungus might be driving the decline of endangered frogs in Honduras.

After this class, Katy became my mentor in all things chytrid and taught me how to prepare my first grant proposal to conduct fieldwork. I was incredibly excited to be awarded funding by the National Geographic Young Explorers program, and together with support from several additional institutions, my research ideas were suddenly catapulted into reality. In 2007, I returned to Cusuco and sampled every amphibian I could find for chytrid. I was alarmed to discover that frogs throughout this forest were positive for chytrid, and the highest rates of infection were detected among the endangered species which had been reported to be experiencing dramatic population declines for unknown reasons. My research showed it was time to take action, and I decided to do everything I could to ensure these frogs would not silently disappear due to chytrid. With help from Amphibian Ark, I was introduced to Jessi Krebs at Omaha's Henry Doorly Zoo and Aquarium in the USA. Jessi expressed a strong interest in helping me develop a conservation program. With his support, the Honduras Amphibian Rescue and Conservation Center (HARCC) was quickly becoming a reality.

I continued returning to Cusuco to study long-term patterns of chytrid infection in wild amphibian populations and measure environmental conditions in order to help develop captive husbandry protocols. Based on five additional years of field data, I decided to focus the HARCC frog rescue operation on three endangered species which appeared most likely to face extinction in the near future: the Cusuco Spike-thumb Frog (*Plectrohyla dasypus*), Exquisite Spike-thumb Frog (*Plectrohyla exquisita*), and Mossy Red-eyed Frog (*Duellmanohyla soralia*). In consultation with Jessi Krebs and Brandon Greaves at the Henry Doorly Zoo, we developed the strategic framework for HARCC: a head-start and reintroduction effort to supplement the vulnerable populations of adult frogs breeding in the wild in Cusuco, with a simultaneous captive breeding effort to establish the first captive assurance populations in case these species suddenly crashed. In 2013, I formed a partnership with Lancetilla Botanical Garden and Research Institute in Tela, Honduras, where I was granted permission to establish HARCC Headquarters. In 2014, HARCC construction officially began with the arrival of two ocean shipping containers that we would transform into frog rescue laboratories. After numerous trips to Honduras accompanied by highly skilled staff from the Henry Doorly Zoo, Phoenix Zoo, USA and Atlanta Botanical Garden, USA, I'm so excited to announce that HARCC is almost ready to open!

When fully operational, HARCC will protect three amphibian species from extinction, offer opportunities for Honduran university students to gain hands-on experience in conservation, and establish a model conservation framework that can support other amphibian rescue programs in Latin America and worldwide. With every great conservation challenge comes an opportunity for innovation and capacity building, and I couldn't be more excited to watch HARCC develop and seek new ways to help protect the future of amphibians.

Working in conservation has been incredibly fulfilling and equally challenging. I dedicate a great deal of my "free time" to sharing my work with new audiences in hopes of inspiring others to help protect nature. While working on my PhD to understand the global spread of chytrid fungus, I also learned about the importance of using social media and science outreach to better communicate with the public about my research, why I do it, and why they should also care. To share information about HARCC, I helped create a HARCC YouTube channel with video updates, a frog rescue website, and social media accounts on Facebook, Instagram, and Twitter. In 2016, I started working with Katie Garrett, an amazing science videographer from London, and we created a video about HARCC for National Geographic. I've been incredibly impressed with the public's level of engagement and interest in HARCC, and it continues to encourage and inspire me.

Two of the species that will be managed at the Honduras Amphibian Rescue and Conservation Center are the Mossy Red-eyed Frog (*Duellmanohyla soralia*, above) and the Exquisite Spike-thumb Frog (*Plectrohyla exquisita*, below). Photos: Jonathan Kolby.

I recently completed my PhD at James Cook University, Australia, where I studied the global spread of chytrid fungus. During this work, I discovered the first record of chytrid in Madagascar, evidence of chytrid spreading in rainwater, and exceptionally high amounts of chytrid being spread by the international wildlife trade. For my day job, I'm currently working for the US Fish and Wildlife Service as a CITES Policy Specialist where I help ensure that the global trade in animals and plants does not cause their extinction.

It's easy to become overwhelmed by the increasing challenges to protect biodiversity and wild places from habitat loss, diseases, pollution, and climate change, but I also feel that there has never been such an important time to work in conservation. The decisions we make over the next few years will impact generations to come, and I feel lucky to be able to contribute my efforts during this critical time to protect biodiversity and global health. In the future, I hope to combine my skills in science and policy to develop international systems that will reduce the spread of chytrid and other emerging pathogens through the wildlife trade, giving the world's wildlife a better chance of survival.

Olivier Marquis, Curator of Reptiles, Amphibians and Invertebrates, Paris Zoo, France

I was born in 1978 in Paris, and grew up in a small village in a natural landscape in the Paris suburbs. As far back as I can remember I've always been interested in animals, primarily under-appreciated species like amphibians, reptiles, and invertebrates. I spent all of my time in the forest and in ponds looking for frogs, newts, snakes, and spiders.

Early on, I used captivity as a way to observe and contemplate these shy and secretive species. I constructed my first terrarium at nine years old and have continued until today. Early in my schooling it was clear that my personal and future professional life would be dedicated in one way or another to gaining knowledge about, and working towards the conservation of reptiles and amphibians.

At thirteen years old I decided to ask a reptile and amphibian lab director, Professor Alain Dubois, if I could gain work experience in his lab. He was very surprised to see this young boy asking to join his lab, but he eventually accepted. This was my first step in the world of amphibian research. In the following years I continued to be involved with the lab during my studies and had the wonderful opportunity to participate in the description of two new species of anurans in Vietnam. (Ohler, A., Marquis, O., Swan, S., & Grosjean, S. (2000). *Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species*.- Herpetozoa, Wien; 13 (1/2): 71 - 87.

All of the choices I made during my high school and university studies were directed towards subjects of animal biology and ecology, and when possible, to reptiles and amphibians. I eventually received my PhD with Prof. Claude Miaud (University of Montpellier), focused on studying local adaptations to UVb radiation in high altitude frog populations. After one post-doc appointment on population dynamics of lizards and two years of teaching at university, I decided to leave the university. In 2008, I had the opportunity to create and direct the first French training centre in herpetology and herpetoculture for private and professional keepers, scientists, vets, firemen, and all people

Early in his schooling, it was clear to Olivier Marquis that his personal and future professional life would be dedicated in one way or another to gaining knowledge about, and working towards the conservation of reptiles and amphibians.

who were interested in, or had to work with reptiles and amphibians and needed theoretical and practical training.

In 2012 I heard about the reconstruction of the Paris Zoo, France and a project to develop a totally new reptile and amphibian collection plan. Since the Paris Zoo belongs to the National Museum of Natural History, they were looking for a new curator with zootechnical and research/conservation experience to manage the species collection and develop *in situ* and *ex situ* conservation and research projects. I applied for the position, eventually got the job, and officially started my role in August 2013. Because the Paris Zoo had never had any amphibians or reptiles in its collection plan, I assisted in the construction of completely new vivariums, and the off-exhibit part of the vivarium until April 2014.

Since then, I've been able to find time to discover how zoos work in research and conservation and have become involved. In 2014, Gerardo Garcia from Chester Zoo, UK, Chair of the Amphibian Taxon Advisory Group (ATAG) of the European Association of Zoos and Aquariums (EAZA), and Warren Spencer from Artis Zoo in the Netherlands, Vice-Chair of the ATAG, offered me an opportunity to join the team as second Vice-Chair of the ATAG.

I am not alone in my work in research and conservation at the zoo. To me, research and conservation requires a necessity for team work and collaboration with other zoos, scientists, local or national associations, and sometimes private keepers too. As I've been a private reptile and amphibian keeper for more than twenty-five years, and I'm now working in a zoo myself, my goal is to make captivity a tool for gaining support for research and conservation.

Some of my past contributions to amphibian conservation were the financial support of the national *Bd* (chytrid) survey in Madagascar and also financial support for the survey of the invasive species *Duttaphrynus melanostictus* in Madagascar via the Amphibian Survival Alliance.

Prof. Claude Miaud, Dr. Norin Chai (from La Menagerie du Jardin des Plantes, Museum of Natural History of Paris) and I also initiated a national screening of *Bd* occurrence in amphibian collections in French zoos.

An example of one of my conservation projects that's still in progress is the conservation of *Ichthyosaura alpestris reiseri*, a subspecies of alpine newt endemic of one lake in Bosnia and Herzegovina, and suspected to be extinct in the wild. It's a really nice project that involves the Bosnian Herpetological Society (Emina Sunje from ATRA association) for the field components, and Paris Zoo and some private keepers (Jean Raffaelli from the French Urodela Group) for the *ex situ* part of the project.

My future goals are to continue to initiate, support, and/or collaborate on studies about European, Malagasy, and South American amphibian species with the support of Paris Zoo and the ATAG. I do not consider myself an expert on amphibian research and conservation, and my personal goal is to improve my own knowledge and skills to be able to continue to contribute to the world of amphibian knowledge and conservation.

Reintroduction of the Northern Pool Frog to the UK - Interim progress report, November 2017

Yvette Martin, Amphibian Conservation Officer; Karen Haysom, Species Programmes Manager; and Jim Foster, Conservation Director, Amphibian and Reptile Conservation Trust, UK

Introduction

The Northern Pool Frog (*Pelophylax lessonae*) became nationally extinct in the UK in 1995, largely because of outright habitat loss and the degradation and fragmentation of its remaining habitats. The species had often been considered an introduced species until the late 20th century, unfortunately therefore escaping conservation attention. A reintroduction program initiated by the Amphibian and Reptile Conservation (ARC) Trust and partners in 2005 has restored one population to a specially prepared UK site using wild-to-wild translocation of Swedish founders. Whilst that intervention appears to have been successful to date, the result is that the UK has had only a single population of Northern Pool Frogs in recent years. This is clearly a perilous situation: should any harm come to that population, the species would again risk being extirpated from the UK.

The ARC Trust is now working to establish a second population in order to increase the resilience of the species in the UK, countering the risks inherent in having only a single population. The current project plan (Baker & Foster, 2015), aims to establish this second population using a head-starting approach, taking spawn from the first population and then releasing well-grown larvae and metamorphs at the second reintroduction site.

The Amphibian and Reptile Conservation Trust in the UK is now working to establish a second population of Northern Pool Frogs (*Pelophylax lessonae*) in order to increase the resilience of the species in the UK. Photo: Jim Foster.

An Amphibian Ark Seed Grant, awarded in May 2017 is supporting ARC in working towards two primary outcomes: 1) the UK conservation status of the Northern Pool Frog is considerably improved, via the establishment of a second viable population; and 2) head-starting methods for Northern Pool Frogs are deployed and evaluated, with the results documented in the literature in order to assist with reintroduction efforts for this species and other amphibians with a similar life history.

This interim progress report summarises work undertaken between July and November 2017.

Design, construction and equipping of *ex situ* facility

Work began in June 2017 to investigate established amphibian head-starting facilities across the world, to inform the design of a facility for pool frogs. The Amphibian Pods page on the AArk web site (www.amphibianark.org/amphibian-pods/) provided a basis for the research. Following this desk-study we had discussions with Chelsea Tomas of the Atlanta Botanical Gardens, USA and Chris Michaels of London Zoo, UK. A design specification for an appropriately equipped 20 ft shipping container was drafted and used as the basis for soliciting quotes from three sources, two of which responded.

In addition to scoping the equipment needed to set up a biosecure unit e.g. air conditioning, lighting etc., we also investigated the cost of equipment needed for amphibian housing and husbandry. A fully-costed plan was developed, covering both the capital costs of establishing the unit and its subsequent running costs, including the salary of a dedicated ecologist to run the facility.

Advice on habitat management

ARC instigated and chaired a meeting of the Pool Frog Working Group (an advisory panel of experts set up prior to the initial reintroduction project in 2005) in September 2017. The group visited the first reintroduction site with Jonathan Preston (Reserve Manager at Thompson Common) to discuss priorities for habitat management over the coming winter season. A revised three-year management plan is being produced for the first reintroduction site. This will take into account recommendations from the expert group, as well as professional advice from Professor Phil Bishop of the University of Otago, New Zealand, who visited the site and undertook studies of water quality during the summer this year. The management plan will focus on works designed to open up the site for the pool frogs and encourage their spread across the site. A draft will be produced by March 2018.

Clinical examination of Northern Pool Frogs in the wild. Photo: Yvette Martin.

Project management

Project management is an ongoing task. Fundraising will remain the major priority until enough money has been secured to set up the head-starting facility.

Species surveillance data (recording counts of adults, juveniles, spawning events and metamorphs) is being collated for the 2017 field season. A draft report has been produced detailing productivity on the site and the final version will be available by March 2018.

Disease risk management advice

Consultation with the Institute of Zoology, UK is ongoing. A Disease Risk Management protocol was produced in May 2017 and a management protocol for collecting, transporting and releasing spawn was produced as a collaboration between ARC and London Zoo.

Captive management advice

A note on the management of captive pool frogs was produced by Chris Michaels at London Zoo in collaboration with ARC. The note provides advice on housing, environmental management, feeding, monitoring development, dealing with ill health and metamorphosing tadpoles. Consultation with London Zoo is ongoing. London Zoo have offered staff time 'in kind' to advise on amphibian husbandry prior to and once the unit is established.

Partnerships for the Titicaca Water Frog

Arturo Munoz, Bolivian Amphibian Initiative, Bolivia; Jeff Dawson, Durrell Wildlife Conservation Trust, Jersey; Robert Hill, Zoo Atlanta, USA; and Tim Steinmetz, Kansas City Zoo, USA

The Titicaca Water Frog (*Telmatobius culeus*) is a Critically Endangered aquatic frog which is endemic to the high altitude Lake Titicaca that straddles the borders of Bolivia and Peru. It is one of the largest frog species in the world and their unique, warty fold of skin helps it absorb oxygen in the cold waters of Lake Titicaca. Brought to global attention by Jacques Cousteau in the 1960s and 70s, today the species faces a number of threats to its survival including pollution, over-harvesting for human consumption and potentially the amphibian chytrid fungus, Chytridiomycosis. In Bolivia the frog has disappeared from many areas of the lake including almost all of the area known as Lago Menor. This situation was a major factor that led to Arturo Muñoz establishing the Bolivian Amphibian Initiative (BAI) to help save this iconic species as well as other threatened Bolivian amphibians.

In 2010, using grants from US Fish and Wildlife Service, The Rufford Foundation, Amphibian Ark, Stiftung Artenschutz, Durrell Wildlife Conservation Trust and Idea Wild among others, the BAI was able to establish a captive breeding facility for the water frog and other *Telmatobius* species at the Museo de Historia Natural Alcide d'Orbigny in Cochabamba and to improve its work carrying out *in situ* research, capacity building and educational activities.

In 2015 massive mortalities of Titicaca Water Frogs in the wild were reported and there was an urgent need for action, so in 2016, with the support of SOS Save Our Species, Stiftung Artenschutz, Amphibian Ark and others, and with the assistance of herpetological staff from Kansas City Zoo and Zoo Atlanta in the US, and Durrell Wildlife Conservation Trust in Jersey, BAI was able to expand its breeding facilities in Cochabamba to hold two populations of this species.

Durrell Wildlife Conservation Trust based at Jersey Zoo has a long association with Arturo and the BAI. Indeed it was Arturo's visit to Jersey in 2006 to attend Durrell's Amphibian Biodiversity and Conservation course that was part of the catalyst for him to establish the BAI. Since then BAI and Durrell have helped deliver amphibian conservation and husbandry courses in Bolivia, and in conjunction with AArk, in Argentina, with two further BAI staff coming to Jersey to undertake training.

In 2014 Durrell launched three dedicated amphibian conservation programs - Saving Amphibians From Extinction and the program's first collaboration in the Tropical Andes region was with BAI.

One of the major threats facing the species on the Bolivian side of the lake is accidental by-catch from fisherman and over-harvesting for human consumption - primarily in restaurants or as 'frog

Tim Steinmetz, from the Kansas City Zoo, USA, helping to construct the second facility at the Bolivian Amphibian Initiative for Titicaca Water Frogs. Photo: Arturo Muñoz.

Working with Titicaca Water Frogs in the new facility at the Bolivian Amphibian Initiative. Photo: Arturo Muñoz.

juice' drinks. To address such issues effectively requires changing people's attitudes, which in itself requires a basic understanding of local people's knowledge of and views towards the species. The BAI and Durrell collaborated to undertake a Knowledge, Attitudes and Perceptions survey of lake communities in Bolivia which was conducted through an Imperial College London MSc research project in 2016. These findings are being used to help guide and inform the next steps of BAI's community work.

In January 2016 staff from Zoo Atlanta and the Kansas City Zoo travelled to Cochabamba to assist with the construction of the second facility to hold additional frogs from two populations. Since then, staff from both facilities have given talks at their own facilities and at professional venues to bring light to the work being conducted by BAI. Kansas City Zoo staff have been back to Bolivia and Peru to take the next step in collaboration with BAI and other Bolivian institutions such as the Bolivian Government and the La Paz Zoo, Vesty Pakos, where a new captive breeding facility for the species will be created with the support of the BAI. The team has also been to the lake to meet local community members and to coordinate the next steps that Kansas City Zoo will be supporting with BAI's team.

Given the nature of the threats facing the Titicaca Water Frog and that its range spans two countries, effective conservation requires multiple partners working together to implement conservation measures and actions. Efforts by AArk are bringing these multiple partners from Peru and Bolivia together to look at the issues affecting the species across the lake and to decide how to work together and holistically to address them. This partnership can help not only the Titicaca Water Frog to survive but also its ecosystem.

Some of the international collaborators during a field trip to Lake Titicaca in Bolivia, to rescue additional frogs. Photo: Dirl Ercken.

2017 North American Salamander Biology, Management and Conservation Training Course

Luis Carrillo, Training Officer, Amphibian Ark

Last October, AArk and Zoo Atlanta organized and ran the second North American Salamander Biology, Management and Conservation Training Course, and we're very thankful for the generosity of Zoo Atlanta and Wharton Center in hosting this course. This year we had ten participants representing zoos, NGOs, universities, and research institutions, giving the group a great variety of backgrounds and experience but with all of them interested in salamander conservation.

Fourteen globally-recognized amphibian biologists, veterinarians, and conservationists comprised the course's faculty, who were ready to share their knowledge and experience with the students, and we are really thankful to them.

Objectives of the training course were:

- to provide technical skills necessary for long-term management of *ex situ* assurance populations of salamanders, from species selection to reintroductions, with a focus on husbandry, health, biosecurity and population management,
- to promote the establishment of assurance colonies for imperiled prioritized salamander species,
- to aid in ensuring the sound care, welfare and management of captive threatened North American salamander species.

Course content and learning design

The five days consisted of a mix of lectures, hands-on practical exercises, and fieldwork. Topics covered during the course included: salamander biology and management, enclosure design and construction, breeding techniques, biosecurity and disease control, monitoring and surveys of wild populations, habitat management and restoration.

The course content was designed to:

- inform students about the critical situation of many salamanders species in the US,
- deliver updated salamander husbandry and breeding methods and techniques,
- inform students about infectious diseases and biosecurity in captive assurance colonies,
- provide the students with information and skills to successfully breed and maintain different species of North American salamanders.

The course was designed to encourage the participation and sharing of knowledge and expertise among instructors and students, providing spaces to do so in a comfortable environment of camaraderie.

Evaluation

Students received a pre- and post-training evaluation to measure their increase in knowledge related to the captive management of salamanders from course lectures, exercises, and fieldwork. A post-workshop survey was also sent to all the students to assess the course itself and as a way to evaluate the effectiveness of the course in an indirect way. The summary of the results are:

- 100% of participants said the course content was as good or better than they expected
- 100% of participants said information and knowledge acquired during the course were excellent

Spring Salamander (*Gyrinophilus porphyriticus*) seen during the field trip to Wharton Center. Photo: Mark Beshel.

- 100% of participants said the level of presentations, questions and discussions around them were excellent
- 100% of particpants said there were enough opportunities for networking during the course
- 100% of participants said they met people during the course who will be valuable for their future work in salamander and other *ex situ* amphibian conservation and who will be a source of information if they have salamander management questions
- 100% of participants said the level of the faculty was very good
- 100% of participants agree or strongly agree that the course was useful for their professional development and met their expectations.

Some quotes from this year's participants:

"*The course was very in-depth and well planned. The instructors, leaders, and fellow classmates were amazing and I loved networking with everyone! Great class!!! Well worth it!!!!*"

"*The course went above and beyond my expectations. Hearing about first-hand experiences from the true experts in the field was extremely helpful and informative, and restored my hope in salamander conservation for the future. The course was well organized although laid back, and all of the instructors were knowledgeable and approachable. I would recommend this course for anybody working with any or all Caudata species in any way, shape, or form!*"

Ten participants representing zoos, NGOs, universities, and research institutions participated in AArk's second North American Salamander Biology, Management and Conservation Training Course in Atlanta, USA earlier this year. Photo: Luis Carrillo.

"*I came to this course with some background knowledge of salamanders and walked away with more information than I could imagine. Not only was this course useful but I also gained new friends who I can count on to help further my knowledge of salamander husbandry, behavior, and conservation.*"

During the field trip to the Wharton Center, a Black-bellied Salamander (*Desmognathus quadramaculatus*) was also seen. Photo: Mark Beshel.

Managing *ex situ* amphibian programs during adverse conditions

Enrique La Marca, Venezuelan Andean Reptile and Amphibian Conservation Center and Universidad de Los Andes at Merida, Venezuela; and Michelle Castellanos, Universidad Central de Venezuela, Venezuela

Venezuela is a country which is currently facing unusual and critical political situations. During the first semester of 2017 the hostile confrontations and adverse conditions compromised normal activities at our conservation center for endangered frogs in Merida city. The unrest conditions prevailing then, especially in the largest cities, also affected the relatively small mountain town where the conservation center is located. Protesters, police and military forces took the streets in confrontations and riots that impeded the normal personal and vehicular transit at cold points, sometimes for periods up to a week or more. For this reason, facilities remained unattended for long periods of time, compromising our ongoing *ex situ* projects with the amphibian species *Mannophryne collaris*, *Aromobates meridensis* and *Leptodactylus* sp. Three main problems arose, namely, energy shortages, regular cleaning and feeding of the captive animals that affected, respectively, automated illumination and sanitary conditions of containers, and daily provision of food for the animals.

Recent hostile confrontations and adverse political conditions compromised normal activities at the conservation center for endangered frogs in Merida city in Venezuela, requiring special management procedures to be put in place for frogs within the center. Photo: Danny Canro.

To deal with this abnormal situation, we temporarily set up new captive husbandry facilities. Adult frogs were moved to "vivaria", large plastic containers that were provided with a gravel substrate, bromeliads and a small receptacle with water and aquatic plants, all previously treated for biosecurity considerations. Each vivarium held one or two pairs of frogs. Water was added to the substrate and to the "tank" plants to provide adequate humid conditions for the relatively long unattended periods of time, and containers were moved to receive natural light, avoiding overheating. In each container, live food was provided through two small containers, one containing a small colony of mealworms (*Tenebrio molitor*), and a small glass jar with a colony of wingless fruit flies (*Drosophila melanogaster*).

During the unrest, adult frogs were moved to large plastic containers with each containing a supply of live food in two small containers, and sufficient water to ensure appropriate levels of humidity. Photo: Danny Canro.

For the tadpoles, up to four larvae were put together in "larvaria", large plastic containers that already bore algae grown on the base and sides. Some decaying leaves were added to the water in each larvarium. To feed the tadpoles, we replaced the regular tadpole or fish food with a specially developed formula consisting of a mix of spinach leaves and oak flakes, that remained unspoiled for up to a week.

Once the critical situation reverted to "normal", some of the experimental enclosures were left under the new conditions for several months. What we found was that the fruit fly colonies (each lasting up to three weeks) were best to provide food supply, although some of the meal worms also added to the frogs' menu in the vivaria. Algae in the larvaria, once the tadpole's food was completely eaten, were relevant, not only as an extra food supply, but also by providing oxygen to the water. That, along with the low pH provided by the decaying leaves most probably contributes to getting rid of unwanted bacteria. The natural lighting conditions were fine as a substitute for the automated lighting system.

Our experience suggests that similar kind of settings may be adequate to deal with situations where *ex situ* husbandry facilities need to be left unattended for several continuous days, providing that the frogs have natural light and temperature conditions similar or close to the ones they experience in their natural environments, and that their size and food habits are appropriate for their dietary regime.

Studying the barriers to amphibian captive breeding programs in Latin America, Africa and Asia

Berglind Karlsdóttir, Master of Science (MSc), Imperial College, London, UK

Urgent captive breeding programs are essential for the survival of 9% of the 2,300 species assessed by the Conservation Needs Assessments to date (Baker et al. 2017). But what are the main barriers to these programs in achieving their long-term conservation goals, and what can the Amphibian Ark and other partners do to help them get there? I set out to answer this question for my Master's thesis, with my partners; the Amphibian Ark and Durrell Wildlife Conservation Trust in Jersey.

As part of this research, I interviewed twenty-five managers of captive amphibian breeding programs in the relevant regions. I am now working on a publication of the results, and the development of guidelines on what to expect when running a program. If you have any questions, or would like to read my thesis, please email me at Berglind.karlsdottir@durrell.org. I greatly appreciate any feedback on my work, or recommendations on how to make the guidelines even more useful.

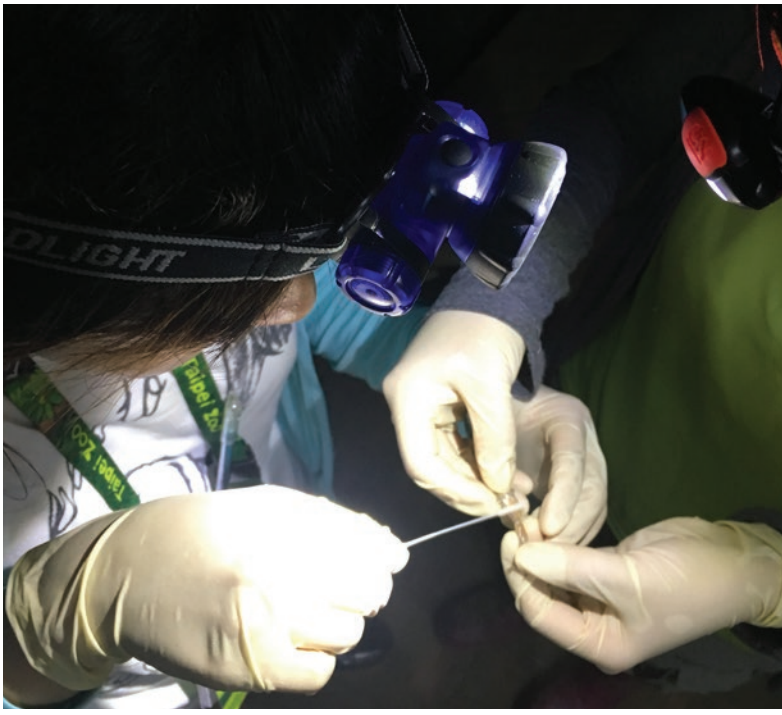
Main conclusions from the study

- Good planning from the beginning of a program is essential. It is important to select the right species, with the highest need and likelihood of success. It is also important to plan where captive-bred animals will be reintroduced, or how the habitat will be improved. An exit strategy is important for program managers to agree, at the start of the program, on how and when to end it.
- Resource needs vary over time and between projects. The resources needed when a program is being set up are likely to be very different from the resources needed during later stages of the program. The most difficult transition for many programs occurs when they prepare for reintroductions, as they need to spend a lot of time in the field, and require money for tracking equipment and/or genetic analyses. External partnerships and support is often needed for bridging the gap between *ex situ* and *in situ* activities, especially at this stage.
- Funding is always needed, but some problems relate to the way in which funding is provided rather than the lack of funding itself. For example, programs might apply for funding for one aspect of a program, but then have to spend money on other unexpected essential aspects such as broken equipment, or in some cases, programs struggle with the lack of funding options for staff salaries. Fluctuations in income can cause managers to pause or drop aspects of a program that are critical to their long-term success. Secure long-term funding frees up a lot of management time from fundraising, and allows managers to plan for the future. If you are helping to fund a program, ask the manager where the funding is most needed.
- The biological and financial barriers to captive breeding programs are often discussed, but we found that the human dimensions of these programs, which are often forgotten, are equally important. Management and relationships can make or break a good program. Knowledge sharing is one of the most important resources, and external expertise is essential for long-term success as well as instant problem solving. Easily available access to information and expertise is key and is available from a range of resources on the Amphibian Ark web site (www.AmphibianArk.org).

The managers

The participants in this research project were from sixteen different countries spanning Latin America, Africa and Asia. The programs they manage had been set up in zoos, museums, research institutions, aquariums or vivariums. Each program was unique in their operational structure, such as size, number of species, staff, funding models, outreach activities, assets and barriers. However all programs had a passionate leader, ensuring that objectives were being met. To these managers, their programs are not just their jobs; they are life-projects, and they will go to great lengths to fulfil the responsibilities that they feel are resting on their shoulders.

Enrique la Marca, recording the release of captive-bred *Mannophryne collaris* and *Leptodactylus meridensis* into Merida's Botanical Gardens in Venezuela. Photo: Michelle Castellanos.



A research team lead by participant Ming Hsung from Taipei Zoo in Taiwan does potential pathogenic screening of Taipei Frogs.
Photo: Lin Yo-yo.

Yuki Taguchi, one of the participants in the study, with a Japanese Giant Salamander in the field. Photo: Aoko Taguchi.

Many managers expressed a desire to "spread the word" about their projects, the insights they have provided or the problems they face. My thesis will never do justice to the amount of valuable information they provided me. The quotes below are my best attempt at making up for this.

At the start of my research, a small donation towards one of the amphibian programs was proposed. Congratulations to Enrique La Marca from Venezuela on receiving this $200 donation, for his commitment to the study.

"I think that it's kind of a slippery slope to think that science has now saved frogs when it's people. These people in the local communities around these streams are going to be the ultimate people that will be saving the frogs at the end of it."

"So I have been dealing with different authorities for 10 years trying to convince them that this is a new kind of project that can help a lot of species that's not something they understand. Maybe they're going to get in trouble so they're not going to do anything for us. And they will try to make me turn around the idea."

"Because of the message you send with the frogs it's not the same that you say with a giant panda or with an elephant …. I think amphibians are very good ambassadors for close encounters."

"I definitely think it's important that we hopefully see a little bit more of a shift towards in situ action which I think tends to be a lot more long-term beneficial for taxa that are of special concern."

"I think the biggest failure was the initial planning. Why? Because I would have liked to start with another species, not just with what …. is most attractive."

Improving our knowledge and *ex situ* management of the Ecuadorian Tiger Frog

Diego Almeida Reinoso, SARgrillo, Farm Insect Rearing Program, Ecuador; and Cecilia Proaño Viteri, Unidad Educativa de Armed Forces Military College, Ecuador

Introduction

Ecuador is considered to be a mega-diverse country because it houses a large number of species of vertebrates and plants in a relatively small area of about 230,000 km^2. As for amphibians, it ranks third in the world with a total of 590 species described (Ron et al., 2017). However, the number of threats faced by amphibians is large and perhaps anthropogenic actions such as cutting down forests, advancing the agricultural frontier, using fungicides and pesticides are the ones that most affect amphibians, in addition to physical environmental factors such as emerging diseases and climate change. The synergy of all these factors has caused the decline of a large number of species not only in Ecuador but throughout the world. In this sense, actions aimed at the conservation of amphibians are urgent. This is the case for the Ecuadorian Tiger Frog (*Hyloscirtus tigrinus*), a species that was first reported in Ecuador in 2012 and is listed as Critically Endangered in the IUCN Red List of Threatened Species.

External terrariums created for the Ecuadorian Tiger Frog (*Hyloscirtus tigrinus*). Photo: Diego Almeida Reinoso.

We present the results of observations of an individual Ecuadorian Tiger Frog kept in a terrarium in an outdoor environment at the Amphibian Conservation Program of the Herpetológica Gustavo Orcés Foundation in Quito, Ecuador. It was brought into these facilities as a tadpole in August 2013, metamorphosed in July 2014 and was transferred to the facilities of the Insect Breeding Program at Granja SARgrillo in March 2015.

Juvenile development observations

Eight months after its metamorphosis, in March 2015 the individual was transferred from the facilities of the Amphibian Conservation Center to the facilities of the Insect Breeding Program at the SARgrillo farm and maintenance trials were started in an exterior enclosure.

Treatment for chytrid fungus, using Itraconazole, administered by drip. Photo: Diego Almeida Reinoso.

With this individual, the conventional treatment techniques against chytridiomycosis were modified, with the aim of reducing the stress caused by the treatment and handling the frog as little as possible. The treatment consisted of bathing the individual inside the terrarium, through a trickle of Itraconazole solution for five minutes and for a period of ten days. The terrarium was also sprayed with the solution during the time the frog treatment lasted. The excess was eliminated every day with water sprays. With this technique, the frog was not touched by us at any time.

In its natural habitat, this species is exposed to temperature variations, with minimal ranges of 3°C - 5°C and maximum temperatures of 18°C - 22°C. Based on this premise, our observations of the development of the individual kept in an external container, under natural shade that allows the passage of UVB rays from the sun and exposed to changing weather conditions began. With a terrarium set up in such a way that it presents the optimum conditions in terms of humidity, shelters, and adequate food it is possible to keep frogs in external enclosures without neglecting biosecurity parameters.

There have been several advantages of this type of husbandry:

1. Terrarium conditions - the basic principles are:

- a reservoir of water that helps maintain adequate humidity inside the terrarium, when evapotranspiration occurs (water is transferred from the land to the atmosphere by evaporation from the soil and other surfaces and by transpiration from plants),
- a false floor which protects the reservoir of water and on which sphagnum moss, rocks, sticks and plants are placed. These are necessary elements of enrichment of the terrarium,
- a mesh cover over the terrarium which allows the passage of UVB rays from the sun and internal ventilation.

A terrarium with these conditions ensures the welfare of these frogs when maintained in outdoor spaces.

2. Food - The provision of a varied diet of as many invertebrates as possible is the key to success in the *ex situ* management of amphibians. In this sense, we tried to provide the largest number of invertebrate species, among which we have two species of crickets, scale insects, moths, butterfly larvae, terrestrial molluscs (slugs), two species of earthworms, tenebrios and galleries. We routinely provided additional vitamin supplements (calcium, vitamin A and vitamin D3) once a week.

3. Irrigation - frequent watering, especially on the hottest days, has allowed us to maintain a fresh and tolerable environment inside the terrarium.

Under these conditions we observed a totally healthy individual with a progressive but slow growth, just as it would happen in their natural habitat. As of June 2017; almost four years since it metamorphosed, the individual maintained in these conditions continues to do well, with the coloration patterns of a sub adult frog, and with a cloacal-face length of 60 mm. This is an indicator of how large the species can be. Changes in coloration patterns are evident as seen in photographs.

In August 2016 we placed a device to measure the temperature inside the terrarium. With this data logger programmed to take the temperature every thirty minutes, we know exactly what the minimum and maximum temperature were and the hours at which the highest and lowest peaks have been recorded. With this information we can program, among other things, aspersions, times and hours of spraying and replicating the same actions with new individuals and with similar species of the same genus that are at risk in the country and that are brought into conservation programs.

The minimum temperature range recorded in the month of August 2016 was 8.5°C - 9°C between 04:30 and 07:00. The range of highest frequent temperature was 24°C to 26°C, although in some days of extreme heat peaks of up to 29°C were recorded, between 12:00 and 16:00 hours. However, the degree of tolerance to temperatures which are different to those recorded in their natural habitat is evident. Proof of this is the frog's state of health.

The chart on page 20 shows the fluctuation of temperature patterns over the 24 hours of several days.

Conclusions and discussion

As of June 2017, we maintain the only sub-adult individual Ecuadorian Tiger Frog known in Ecuador. So far we do not know of any wild adult individuals and only tadpoles have been found in a single location in the north-east of Ecuador.

(Above and below): Color changes in a juvenile Ecuadorian Tiger Frog over a two-year period. March 2015. Photos: Diego Almeida Reinoso.

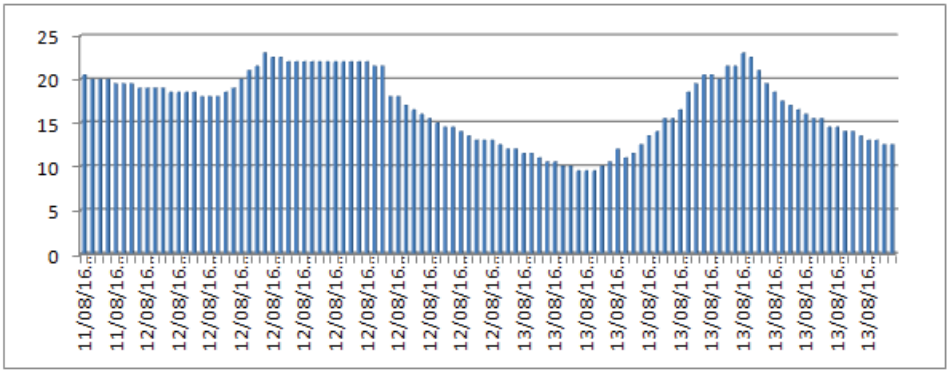
February 2016.

December 2016.

June 2017.

During all this time we have learned many aspects about the biology, behavior and management of the Ecuadorian Tiger Frog. With well set-up terrariums and adequate food it is possible to maintain and raise individuals in excellent condition and others with similar biological traits, not only of the Hylidae family, but of all the families whose species live over 3,000 meters above sea level, and at costs considerably lower than those required in other types of facilities. These species are in danger.

There are several advantages of amphibian management in outdoor terrariums. Perhaps one of the most important is the incidence of natural UVB light that allows the absorption of vitamin D and therefore the proper development of frogs. We have observed the individual resting, perched on a leaf or trunk just where a beam of light strikes, while on hot days we have found it resting on the base of the



The fluctuation of temperature patterns (°C) over the 24 hours of several days.

terrarium, on the sphagnum and protected under shade. Other advantages are the influence of the natural period photo, the incidence of rain and natural atmospheric changes.

This type of management has allowed us to demonstrate that species that live in altitudinal zones above 3,000 meters, can adapt very well to climate conditions in somewhat lower areas (1,600 meters above sea level). Perhaps one of the most frequent mistakes we have made in amphibian management centers is to try to standardize a certain temperature and maintain cold environments all the time, without considering that in the high zones, changes, especially in the temperature, are extreme, and the frogs are adapted to these changes. Variations in temperature ranges are essential for the normal development of these frogs.

With the temperature data obtained, the hours and the timing of daily spraying can be programmed and not done under the subjectivism of the handlers. The form of water supply in the terrarium also influences the frogs' welfare. This can be by subtle drip or micro spray. The general idea is to create the least amount of stress for the animals.

The technique used for the maintenance of this frog has worked extremely well, and the idea is that those who are working on amphibian conservation both within and outside of Ecuador can hopefully extract information that could help them in some way to improve their technique management.

Finally we must remember that many times it is the small details that allow us to achieve success.

Thanks

This contribution to our knowledge of the *ex situ* management of the Ecuadorian Tiger Frog would not have been possible without the support of Katty Garzón from the Herpetológica Gustavo Orcés Foundation who allowed the frog to leave the Herpetológica Gustavo Orcés Foundation to come to the premises of SARgrillo. We also thank Andrés Merino, director of the Balsa de los Sapos of the Pontifical Catholic University of Ecuador who kindly provided us with a data logger device and helped us by downloading the data for analysis.

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