

amphibian ark



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A frog first: The reintroduction of the Endangered Pickersgill's Reed Frog back into the wild

Jeanne Tarrant, Threatened Amphibian Program Manager, Endangered Wildlife Trust, South Africa, and Ian du Plessis, Curator, Johannesburg Zoo, South Africa

September 2018 marked an exciting leap for amphibian conservation in South Africa. Through the collaborative efforts of the Johannesburg Zoo, Ezemvelo KZN (KwaZulu-Natal) Wildlife, The Endangered Wildlife Trust (EWT) and the South African National Biodiversity Institute (SANBI), the first reintroduction of an Endangered South African frog species back into the wild took place. This marked the culmination of a decade of work and is a first major step in a long-term project to be able to reintroduce the Pickersgill's Reed Frog (*Hyperolius pickersgilli*) (Raw, 1982) to new or recreated sites *in situ*.



The tiny Pickersgill's Tree Frog (*Hyperolius pickersgilli*) occurs only within the narrow strip along the KwaZulu-Natal coast in South Africa, and is restricted to approximately twenty-five sites of highly fragmented wetland habitat. Photo: Ian du Plessis.

This tiny frog species occurs only within the narrow strip along the KwaZulu-Natal coast and is restricted to approximately twenty-five sites of highly fragmented wetland habitat. Its total area of occupancy is just 12 km² (IUCN, 2016), however, the known sites have increased since the previous IUCN assessment in 2010, which totalled 9 km² (SA-FRoG, 2010), meaning the species was down-listed from Critically Endangered to Endangered in 2016. The species is threatened primarily by loss and degradation of its coastal wetland habitat caused by rapid urbanization, mining, agriculture, and industrialization as well as pollution, and the drying of its habitats caused by invasive plants (Tarrant & Armstrong, 2013).

Given its precarious state in the wild, the Pickersgill's Reed Frog was a high priority species for conservation action and recommended for *ex situ* rescue at a workshop held by Amphibian Ark in 2008 at the Johannesburg Zoo. At the time, the species was known only from eight sites. As of 2018, this has increased to twenty-five sites, but only two of these are protected (at iSimangaliso and Umlalazi Nature Reserve). The Johannesburg Zoo wanted to become more involved with amphibian conservation and entered into an agreement with Ezemvelo KZN Wildlife to breed Pickersgill's Reed Frog in captivity to establish an insurance population, in the event that this species might become extinct in the wild. This insurance population would also be able to provide specimens for reintroduction back to the wild at secure and well-managed sites. The first twenty adults for the breeding program were collected in Durban in 2012 and taken to the Johannesburg Zoo and National Zoological Gardens in Pretoria. Little breeding success was had at this time, however the Zoo continued to care for the frogs and maintain them in good health for a further five years. In 2017 a memorandum of agreement was signed between Ezemvelo KZN Wildlife and Johannesburg Zoo to formalise the process of establishing sustainable insurance populations of Pickersgill's

Reed Frogs and other endangered herpetological species that are found only in KwaZulu-Natal. A further twenty adult frogs were collected in September 2017, and very quickly the staff found themselves looking after hundreds of tadpoles, and subsequently juveniles. Much about the species' breeding biology and behaviour has been learnt through this project, including patterns of development, diet and husbandry – a manual on which has been documenting the process throughout.

The first batch of offspring (F1 generation), comprised about 400 specimens bred in captivity, of which 200 individuals were transported from Johannesburg to Mount Moreland's 'Froggy pond' in September 2018 for release back into the wild. The area of release is an 18 ha reedbed wetland from which the breeding stock was originally collected. Work done by the Endangered Wildlife Trust and the Mount Moreland Conservancy through support from Department of Environmental Affairs and the Airports Company of South Africa (ACSA) to remove alien vegetation has ensured that the site is well-managed and in an improved condition to what it was a few years ago.

The frogs to be released were allowed to acclimatize for a few hours on site (while the humans had a press conference), after which a 'ceremonial release' was held to allow VIPs including sev-



eral Department of Environmental Affairs representatives who enjoyed wading into the wetland and personally releasing a few of these minuscule 1 cm young frogs!

This project contributes to the Biodiversity Management Plan (BMP-S) for the Pickersgill's Reed Frog, which was gazetted by the Department of Environmental Affairs in June 2017. This plan, spearheaded by the Endangered Wildlife Trust, together with Ezemvelo KZN Wildlife, brings together many different organizations, including government, non-governmental, and civil society to affect the recovery of the species, with the ultimate aim that the species will one day be listed as Least Concern by the IUCN. The reward of this collaborative approach is evidenced by the release in September. In addition to the captive breeding program, the National Zoological Gardens (now SANBI) in Pretoria carried out genetic research which has shown that Pickersgill's Reed Frogs may be released to the wild throughout its native distribution range (Kotze et al, submitted), and also provided the testing to confirm that the frogs to be released on the day were free of the lethal chytrid fungus. The Endangered Wildlife Trust is working towards formally protecting sites for the species and has been rehabilitating wetlands for this frog species through funding provided by the Working for Water Natural Resources Management Programme through the Department of Environmental Affairs, in the process creating seventy-five jobs for local people across the greater Durban area.

It is indeed exciting to be part of a positive conservation story, especially one that not only involves one of the smallest frog species in the country, but one which has generated a lot of interest.

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Captive-bred Pickersgill's Reed frogs await their release. Photo: Jeanne Tarrant.



The Endangered Wildlife Trust Threatened Amphibian Program team. Photo: Jeanne Tarrant.

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Moving forward with the conservation program for Valcheta Frog in Argentina

Federico Kacolis, Coordinator, Wild Plateau Initiative La Plata Museum, Argentina

The Valcheta Frog (*Pleurodema somuncurense*) is one of the most endangered amphibians in Argentina. In 2012 we started a project aimed at improving the conservation status of this species by conducting a combination of *ex situ* and *in situ* management. After seven years of hard work, we can be assured that things are doing better for this frog. We successfully established an *ex situ* colony of this species at the La Plata Museum in Argentina and subsequently had three reproductive events. We fenced and restored some key reproductive habitats for frogs as a way to avoid habitat perturbation by cows that trample and feed on native vegetation. One of these restored habitats used to have frogs in the past but the local population living there had gone extinct due to this disturbance. After restoration and fencing of the habitat, we transported a lot of newborns from the *ex situ* facilities to this habitat in two separate reintroduction events. Two and a half years later, the reintroduced population is now showing reproductive events in the wild, confirming its establishment, and thus, the return of the species to this specific habitat.

However, not everything is that simple or so happy for the Valcheta Frog. The small distributional range and low population size, along with years of decline and local extinctions due to human-related disturbance are threatening the future of the frogs. Additional work is needed to bring the species to a point of self-viability. And this work should be based on a conservation strategy that includes management of the cows, habitat restoration, and reintroduction of new populations of this frog. However, it is also necessary to start working on trout management. The Rainbow Trout is an invasive predator that was introduced in the frogs' habitat some decades ago. Now, the trout is the top predator of the stream and predated not only on Valcheta Frogs but also on Naked Characins (*Gymnocharacinus bergii*), an endemic and endangered species of fish that shares this habitat with the frogs. Trout removal is of key relevance to allow movement of individuals between the frog populations as a way to reduce local inbreeding. Today, the local populations of the frogs are restricted to a few sites of the headwaters of the Valcheta stream, that are inaccessible for trout because of the existence of waterfalls. The removal of trout will not only enhance connectivity but also increase current suitable habitat for native frogs and fishes.

In this complex scenario, the *ex situ* colony of this species plays three key roles. Firstly it provides frogs which will be reintroduced in restored habitats. Secondly, it maintains a healthy population of this species to ensure its viability in the face of potential catastrophes in the habitat that might affect a significant percentage of the entire wild population (and this assumption is quite possible if we take into account the extremely low range of this species, that just reaches a total of 2 km²). And thirdly, the *ex situ* facilities allow us to continuously generate scientific knowledge related to the natural history of this species, that is of great help to improving management activities. In summary, the *ex situ* facilities are of key relevance in the conservation of the Valcheta Frog.

The maintenance of the *ex situ* facilities for the Valcheta Frog is a full-time task. With a focus on the wellbeing of the frogs, every day we are facing a new challenge. We have been supported by the Amphibian Ark since the beginning of this project, and the AArk has provided us with funds to build the facilities and to continue our work, and also has trained us in *ex situ* amphibian management. AArk staff have always been available to give us suggestions and information to solve the different problems we have faced during the past six years.



The new lab-container which was co-funded by a private donor (via the Amphibian Ark) and Fundación Azara, finally arrives at University of La Plata. This new lab is being setup specifically for the survival colony of the Valcheta Frog (*Pleurodema somuncurense*) and will help us enhance the *ex situ* habitat for this species. Photo: Federico Kacolis.



Two large terrariums were recently constructed for the Valcheta Frogs and will soon be placed in the new dedicated lab-container. Photo: Federico Kacolis.



Two and a half years after captive-bred frogs were released into protected habitat, the reintroduced population is now showing reproductive events in the wild, confirming its establishment, and thus the return of the species to this specific habitat.
Photo: Federico Kacolis.

In 2018, Amphibian Ark (via a private donor) and other donors (like the Fundación Azara) allowed us to purchase a new lab-container that has recently been established in the campus of the Faculty of Natural Sciences at the University of La Plata. They also helped us in supporting the construction of two large terrariums which will be placed in this new lab-container. We are confident that both the lab-container specifically designed for these frogs, and the new terrariums will help enhance the *ex situ* habitat for the survival colony of the Valcheta Frog, allowing us to further improve their wellbeing. We hope that this management promotes a higher level of fitness in the frogs to help us continue with the reintroduction program in coming years.

We are very happy with these achievements and very thankful to the Amphibian Ark and the other donors who helped us to carry them out. Good things are happening for the Valcheta Frogs and a lot of people are committed to them. By following this path we expect to achieve our final goal of ensuring a long-lasting and self-viable wild population of this species living in the headwaters of the Valcheta stream.

Abilene Zoo – Quarters for Conservation

Luis Carrillo, Training Officer, Amphibian Ark

Abilene Zoo is located west of Fort Worth, in Texas, USA. With more than 1,000 animals from around the world, the mission of this AZA accredited zoo is: *A place of learning and adventure, where families make memories, share the joy of discovery and become inspired to preserve wildlife.* Although it is small, there is no other zoo for about two and a half to three hours around it, so it attracts many visitors from the surrounding areas, and especially from local schools.

Quarters for Conservation is an exciting program that raises money to support wildlife conservation all over the world! Quarters for Conservation at Abilene Zoo started in the Fall of 2015, by donating twenty-five cents from every zoo admission to species conservation projects. In turn, each guest at the gate receives a token that symbolizes the donation, and they can vote for one of three animal projects by dropping the token in a vortex wishing well at a conservation kiosk.

Amphibian Ark was one of the winners of the 2018 Quarters for Conservation of Abilene Zoo. The presentation event started at 6:00 pm with more than sixty people, including the board of directors, staff, volunteers and special invitees in attendance. We had the opportunity to deliver a twenty-minute presentation about how the AArk has contributed to amphibian conservation worldwide, followed by a question and answer session. At the end the Conservation Committee had a ceremony prepared to hand over a check for US\$ 2,500 which was raised by the donations from zoo visitors.

The next morning, we had the opportunity to interact with the visiting public entering the Zoo's reptile house, explaining about the AArk, how people can support saving amphibians, and interacting with children. We gave copies of AArk's ten-year report to interested visitors, provided amphibian coloring sheets for kids to paint and gave away some frog figurines as free souvenirs. The Zoo lent us some live frogs and newts to further encourage people to approach our kiosk to appreciate and ask about them.

Quarters for Conservation is an exciting program run by Abilene Zoo, Texas, USA, that raises money to support wildlife conservation all over the world.



Amphibian Ark's Luis Carrillo gave a presentation about how Amphibian Ark has contributed to amphibian conservation worldwide, to Abilene Zoo board of directors, staff, volunteers and special invitees. Photo: Amphibian Ark.

We are very thankful to the Abilene Zoo staff to select Amphibian Ark as one of the 2018 projects/institutions to fund and to Abilene Zoo visitors for their donations.

We can all leap together and help amphibian conservation!

Luis Carrillo receives a check for US\$ 2,500 which was raised by the donations from zoo visitors and donated to the AArk to support our work. Photo: Luis Carrillo.



Amphibian Advocates

In this edition of the Newsletter, we are pleased to introduce two amphibian advocates: Teresa Camacho Badani, Manager at K'ayra Center for Research and Conservation of Threatened Bolivian Amphibians, and Luke Jones, Mountain Chicken Recovery Program Research Assistant from Durrell Conservation Trust in Jersey. Teresa's work is currently focused on threatened frogs from the genus *Telmatobius*, with additional programs for other threatened species being implemented in the future. Luke's studies and work on Mauritius, the UK and Jersey have led him to now work with The Durrell Wildlife Conservation Trust on the Mountain Chicken Recovery Program on Montserrat. We hope you enjoy reading about their amphibian conservation activities.

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!

Teresa Camacho Badani, Manager K'ayra Center for Research and Conservation of Threatened Amphibians of Bolivia at the Museo de Historia Natural Alcide d'Orbigny, Bolivia

I have always liked animals, nature, and of course, amphibians. Luckily my family shared this interest in nature, especially my father, with whom we would go to the highest peaks of Cochabamba or to the lowlands in the Bolivian Amazon. I think these trips encouraged me to become a biologist.

When I began my studies at the Universidad Mayor de San Simon, a great opportunity appeared - the Center for Biodiversity and Genetics (CBG) at the University needed research assistants to work in the field. I had no experience, and I was only in the third semester of my studies, and although I was one of the youngest students, I was able to be part of this project. At this point I found my way, I was fascinated by the wonders I saw outdoors, my country, and the diversity that we have. I became a "field biologist", by performing hard but enjoyable activities like walking a day and a half to get to the far away communities at Isiboro Secure National Park, staying twenty days camping without any communication, and getting home with my body full of ticks. But for me, being in nature was wonderful.

After finishing this project, I was hired as a research assistant at CBG, participating in several projects, initially with High Andean herpetofauna. At the end of my studies I was looking for a thesis subject, which I was hoping would be related to amphibians. Thanks to the project Conservation of Amphibians of Bosques yungueños of Bolivia with emphasis on Endangered Species at this same institution, I was able to document the population status, breeding phenology and activity patterns of two threatened amphibian species found only in cloud forests of Bolivia - the Quechua Toad (*Rhinella quechua*) - the species with the first record of chytrid fungus in Bolivia - and the Small Whistling Toad (*Microkayla iatamasi*). While looking for these species, I saw other unique amphibians, most of them in danger of extinction. At that time, I did not imagine that I would not see them again in the wild, as is the case with the first aquatic frog that I found in the field, the Sehuencas Water Frog (*Telmatobius yuracare*).

After finishing my degree, I was an intern at the Amaru Zoo in the city of Cuenca, Ecuador. The internship was funded through the Disney Wildlife Conservation Fund and the Amphibian Conservation Program at the Philadelphia Zoo. During that work, I focused on a conservation program for Critically Endangered harlequin toads, marsupial frogs and poison dart frogs. I also helped to collect data on the natural history of these and other species, as well as monitoring the last remaining populations of the Mazán Green Jambato (*Atelopus exiguus*). This trip to Ecuador changed my way of looking at conservation, and when I obtained a scholarship from the Organization of American States (OAS), I knew I wanted



to study in Ecuador. However, the university where I wanted to study was not part of the consortium of the OAS. In the end, my enthusiasm managed to convince them that it was the best option for me, and so I went to Ecuador. I got my Master's degree in Conservation Biology at the Pontifical University of Ecuador in Quito. The Herpetology Division of the QCAZ Zoology Museum allowed me to do my research and travel much of Ecuador looking for frogs, where we saw several threatened species such as Andersson's Jambato (*Atelopus palmatus*) and the Pebas Stubfoot Toad (*Atelopus spumarius*). I made great friends and colleagues at this institution.

After finishing my Master's degree, I returned to Bolivia, and started working at the Alcide d'Orbigny Natural History Museum in the city of Cochabamba and I was soon designated as Chief of the herpetology department. The amphibian captive breeding center of the museum at that time was working only as a project, but now it is a research center called Centro K'ayra, which is the only one in Bolivia authorized by the Ministry of the Environment for the *ex situ* management of amphibians, with a license to operate as a wildlife custody center.

In the K'ayra Center we currently have several species of the genus *Telmatobius* and will soon incorporate other species from other groups that are threatened and need our help. I have a great multidisciplinary team that includes biologists, veterinarians, environmental engineers and several volunteers from different careers and universities. Together we will continue working for the benefit of amphibian conservation in Bolivia.

Luke Jones, Mountain Chicken Recovery Program Research Assistant, Durrell Wildlife Conservation Trust, Jersey, and Mountain Chicken Recovery Program

Unlike many of the previous amphibian advocates I am very much a new face on the scene! I currently have the privilege of advocating for one of the world's most unusual amphibians, an apex predator in its own right, and all-in-all a general oddity, not the least because of its name! The Mountain Chicken (*Leptodactylus fallax*) has been one of those species that many have looked towards as a case study for the devastating impact that introduced invasive species can have on an ecosystem. This species has suffered one of the most well-documented and rapid declines of any vertebrate in recent times, as a direct result of the introduction of the deadly amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) to its native habitat. In the past I had studied the species, yet I hadn't dared to dream I would have the opportunity to work with them, let alone to be a part of their reintroduction back to the island of Montserrat, in the Caribbean.



Like many of you reading this, I was that child drawn to everything weird, wild and wonderful - obsessed with zoos, wildlife documentaries and the beautifully diverse myriad of creatures they contained. It wasn't until I was introduced to the books of Gerald Durrell that my passion and excitement found a channel. Up until this point I hadn't really been exposed to wildlife conservation as a concept that an individual could have a significant impact upon, let alone a career that one could pursue. Reading his books was like a call to action! They focused my passions and gave me the courage I needed to pursue something that I believed to be truly valuable. They gave me hope for the impact that a single individual could have!

The inspiration I garnered from Durrell's books led me to pursue a BSc in Animal Science, at the end of which I was fortunate enough to be offered a position working and studying in Mauritius on my Post Graduate Diploma (PGDip) in Endangered Species Recovery, with The Durrell Wildlife Conservation Trust and the Mauritian Wildlife Foundation, both of which had been founded by the man that had inspired me all those years ago.

My PGDip was my first true taste of fieldwork, living and working *in situ*. Flying out to Mauritius I spent eight months working within the Mauritian Wildlife Foundation on a wide array of wildlife conservation projects and initiatives. These varied hugely in their approach and utilized a variety of tools in the conservationist's arsenal, from the more traditional methods such as captive breeding

programs, habitat manipulations and management and invasive species control through to the pioneering use of analog species and their role in whole ecosystem restoration projects. All of this fieldwork was supplemented with Durrell-led lectures, from inspirational and world-renowned conservation biologists such as Carl Jones, Nik Cole and John Ewen. It was whilst studying in Mauritius that I fully realized a passion for creatures of the herpetological variety. How could you not, with the beautifully vivid Mauritius Ornate Day Gecko (*Phelsuma ornata*) tucked into every nook and cranny of the field stations, or after having had the privilege to work with one of the world's rarest and most elusive snakes the Keel-scaled Boa (*Casarea dussumieri*)?

I left Mauritius filled with inspiration and aspirations. The PGDip had opened my eyes to the more human side of wildlife conservation projects, the needs of local people, employees and the impacts that exceptional leaders can have in recruiting others to their vision. With this in mind I accepted a lecturing position in Animal Management at West Midlands Safari Park in the United Kingdom, during which time I developed several modules including wildlife conservation and team management, and hopefully pushed a few more individuals towards pursuing a career in conservation. The role also helped me to develop skills in community outreach and public engagement, experiences that have proven to be priceless when it comes to the level of community engagement required of a re-wilding project such as the Mountain Chicken Recovery Program.

Which brings me to where I am now! Working for The Durrell Wildlife Conservation Trust on the Mountain Chicken Recovery Program, a multi-institutional partnership of zoos and government organizations, we are working to combine the knowledge gained from *ex situ* conservation work and research to inform and develop an *in situ* re-wilding strategy for the species. This has involved the development and adaptive management of environmental solar heating initiatives, establishing a native insect breeding facility on the island, as well as a whole array of surveys and public outreach initiatives. All of which is gearing towards the creation of a "SAFE haven" (SAFE = Saving Amphibians From Extinction) for the Mountain Chicken within its native environment. A SAFE haven that doesn't isolate the Mountain Chicken from the Bd fungus but rather aims to facilitate its adaptation to the presence of Bd within the environment.

Our collective ambition is to not only have self-sustaining populations of Mountain Chickens returned to their former range, surviving alongside the disease that almost caused their extinction, but also to develop a new tool for the conservationists' arsenal, one that will allow us to manage amphibian populations *in situ* at risk of extinction due to Bd.

You can find out more about our project and follow our journey on our website (www.mountainchicken.org) or across our social media platforms:

Facebook: Mountain Chicken Project

Twitter: Mountain Chicken Recovery Programme @ReWildCaribbean

Instagram: mountainchickenproject

Experiment: www.experiment.com/mountainchicken

Collaboration for Fire Salamander conservation

Annemarieke Spitzen and Tariq Stark, Reptile, Amphibian and Fish Conservation the Netherlands (RAVON), Nijmegen, the Netherlands; Anna Rauhaus and Thomas Ziegler, Zoologischer Garten Köln, Cologne, Germany; Kathleen Preißler, Technische Universität Braunschweig, Germany; Sergé Bogaerts, Salamandervereniging, the Netherlands and DGHT/AG Urodela, Germany; Sebastian Steinfartz, Technische Universität Braunschweig, Germany; and Stefan Lötters, Universität Trier, Germany

Until recently, the Fire Salamander (*Salamandra salamandra*) was a species of low conservation concern in Europe and listed in the IUCN Red Data Book as Least Concern. Due to the invasive chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*), populations have collapsed, and some are now threatened with extinction. *Bsal* is thought to have originated in South-East Asia and may cause lethal chytridiomycosis in urodelans (newts and salamanders) (Martel et al., 2014), whilst some anurans (frogs and toads) may act as vectors (Nguyen et al., 2017). Although *Bsal* is deadly to nearly all European salamanders and newts and poses a massive threat to European urodelan diversity, the genus *Salamandra* is specifically vulnerable. In 2013, *Bsal* was first described after records of massive die-offs in Fire Salamander populations in the Netherlands. In the following years, *Bsal* was found to be present in populations in Belgium and Germany as well (Spitzen et al., 2016; Dalbeck et al., 2018), with the affected populations showing no signs of recovery and the pathogen remaining present.

Upon the discovery of *Bsal* and the subsequent population declines, the Netherlands set up a captive assurance colony with a subset of the remaining Dutch Fire Salamanders. Due to the continuous spread of *Bsal* into new areas within Belgium and Germany, both countries aim to develop a captive breeding program for Fire Salamanders. Thus, to ensure best possible international collaboration and an effective sharing of knowledge and resources, the 'Ex situ Salamandra Group' (ESG) was initiated. The ESG had its initial meeting in September 2018 at Cologne Zoo (Germany). See the list below for the founder members and participants.

During the meeting, presentations were given on the current state of knowledge and ongoing research on Fire Salamanders and *Bsal* in the three countries and how these research lines can help *ex situ* conservation efforts. Fruitful discussions took place about subjects including the genetic aspects of how to prioritize popula-

During the meeting in Cologne, presentations were given on the current state of knowledge and ongoing research on Fire Salamanders and *Bsal* and how these research lines can help *ex situ* conservation efforts. Photo: Thomas Ziegler.

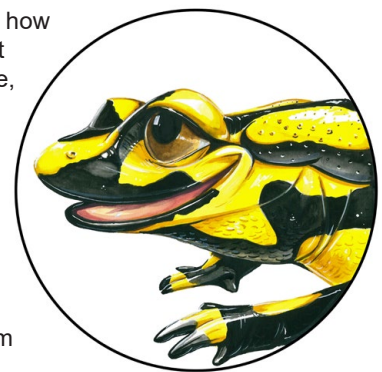


Attendants of the first meeting of the 'Ex situ Salamandra Group' held at Cologne Zoo, Germany, in September 2018. Photo: W. Kujawski.

tions (e.g. management units) and how to determine the founder sizes that should be considered. Furthermore, the development of a scientifically based husbandry protocol was initiated, and a Fire Salamander logo developed by Christian Niggemann from Cologne Zoo.

As a concrete output and start of the project, we checked the Zoological Information Management System (ZIMS) and the Zootierliste (www.zootierliste.de) for European zoos and institutions that currently hold individuals of *S. s. terrestris* (Tables 1-2), and *S. salamandra* but without assignment of subspecies status according to these databases (Tables 3-4), as there might be further *S. s. terrestris* among them. Certainly, this information may not be complete, but it provides at least a rough overview about the numbers of holding institutions and individuals held therein. The overview is also intended to raise awareness in institutions to check their collections, the particular (sub)species they hold, and the origin of individuals.

In total, 110 individuals of the subspecies *S. s. terrestris* are kept in seven institutions according to ZIMS (34 males, 46 females, 30 undetermined sex), and in additional four institu-



The new Fire Salamander logo designed by Christian Niggemann from Cologne Zoo.





Due to the invasive chytrid fungus *Batrachochytrium salamandrivorans*, populations of Fire Salamanders have collapsed in Europe, and some are now threatened with extinction. Photo: J. Herder.

tions according to Zootierliste. According to ZIMS, 121 *S. salamandra* (without defined subspecies status) individuals are kept in 29 institutions (7 males, 6 females, 199 undetermined sex), and in an additional forty-one institutions according to Zootierliste (see tables). Currently, populations within the subspecies *S. s. terrestris* sensu lato are in our focus, but future projects will likely include other subspecies / genetic lineages. As a follow-up step, a questionnaire will be sent to the zoos and institutions that were found to keep Fire Salamanders, so we can obtain a more detailed overview about provenance, subspecies status and genetic purity of breeding stock. We cordially invite institutions with or without captive collections of Fire Salamanders that are interested in participating in the ESG or in respective breeding programs to contact us.

Close collaboration between all institutions with *ex situ* Fire Salamander colonies was agreed upon and the next meeting will be held in March 2019 in GaiaZOO (Kerkrade, the Netherlands), where a studbook for the Dutch fire salamanders was established

Country	Number of institutions	Total number of individuals (males.females.undetermined)
Germany	2	19 (1.1.17)
Lithuania	1	5 (0.0.5)
Netherlands	1	74 (30.44)
Poland	1	4 (0.0.4)
Slovenia	1	4 (3.1)
United Kingdom	1	4 (0.0.4)

Table 1: Overview of institutions keeping Fire Salamanders (*Salamandra salamandra terrestris*) according to ZIMS (accessed 21 November 2018).

Country	Number of Institutions
Czech Republic	1
Germany	3

Table 2: Overview of additional institutions keeping *Salamandra salamandra terrestris* (compared to Table 1) according to Zootierliste (accessed 21 November 2018).

recently. During this second meeting the next steps towards an effective international standard for *ex situ* breeding colonies for salamanders and newts will be taken and further tasks will be allocated to the participants to reach this goal.

Acknowledgements

We are grateful to Theo B. Pagel (director, Cologne Zoo) for housing the founder meeting at the Cologne Zoo, Germany.

For further information, please contact the authors.

Country	Number of institutions	Total number of individuals (males.females.undetermined)
Austria	1	4 (2.2)
Czech Republic	1	4 (0.0.4)
Denmark	2	15 (1.0.14)
France	1	2 (0.0.2)
Germany	5	31 (2.0.29)
Hungary	3	16 (0.2.14)
Italy	2	25 (0.0.25)
Netherlands	1	43 (0.0.43)
Poland	2	22 (0.0.22)
Portugal	2	5 (0.0.5)
Spain	1	1 (0.0.1)
Switzerland	2	16 (2.2.12)
Ukraine	2	3 (0.0.3)
United Kingdom	4	25 (0.0.25)

Table 3: Overview of institutions keeping *Salamandra salamandra* according to ZIMS, for which subspecies information was not provided or lacking (accessed 21 November 2018).

Country	Number of Institutions
Austria	2
Belgium	1
Czech Republic	2
Germany	19
Hungary	1
Italy	1
Lithuania	1
Netherlands	4
Romania	1
Serbia	1
Sweden	1
Switzerland	3
Ukraine	1
United Kingdom	3

Table 4: Overview of institutions keeping additional *Salamandra salamandra* (compared to Table 3) according to Zootierliste, for which subspecies information was not provided or lacking (accessed 21 November 2018).

Participants of the first workshop

Zjef Pereboom (Antwerp Zoo/KDMA, Belgium)
Timm Reinhardt (Bundesamt für Naturschutz, Germany)
Emile Prins (GaiaZOO, the Netherlands)
Tjerk ter Meulen (GaiaZOO, the Netherlands)
Annemarieke Spitzen (RAVON, the Netherlands)
Tariq Stark (RAVON, the Netherlands)
Sergé Bogaerts (Salamandervereniging the Netherlands and DGHT/AG Urodela, Germany)
Kathleen Preißler (Technische Universität Braunschweig, Germany)
Pia Oswald (Universität Bielefeld, Germany)
Stefan Lötters (Universität Trier, Germany)
Joana Kuchenbecker (Zoologischer Garten Köln, Germany)
Christian Niggemann (Zoologischer Garten Köln, Germany)
Anna Rauhaus (Zoologischer Garten Köln, Germany)
Thomas Ziegler (Zoologischer Garten Köln, Germany)

Absent founder members

Philippe Jouk (Antwerp Zoo/KDMA, Belgium)
Barbara Caspers (Bielefeld Universität, Germany)
An Martel (Ghent University, Wildlife Health, Belgium)
Frank Pasmans (Ghent University, Wildlife Health, Belgium)
Maarten Gilbert (RAVON, the Netherlands)
Sebastian Steinfartz (Technische Universität Braunschweig, Germany)
Miguel Vences (Technische Universität Braunschweig, Germany)
Norman Wagner (Trier Universität, Germany)

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KÖLNER ZOO



An update on the *in situ* conservation of the Lemur Leaf Frog through habitat improvement and forest management practices in Costa Rica

Brian Kubicki, Costa Rica Amphibian Research Center, Costa Rica

Over the last year progress with the forest management project within Costa Rican Amphibian Research Center's Guayacán Rainforest Reserve has progressed very well. With the support of a donation from the Amphibian Ark, approximately seven hectares of forest targeted for forest management within the private reserve has had the majority of its selected undesirable plant species removed, allowing for the reestablishment and advancement of a dominance of an understory flora that is characteristic of old growth forests in the region. Unquestionably, the shift of an understory floral dominance from fern back to natural broadleaf vegetation will greatly increase the overall productivity for a variety of species of both flora and fauna for years to come.

We have already begun to notice a definite increase in the abundance of amphibian species, both terrestrial and epiphyllous, observed within the forest management areas. Due to the removal of the fern that formed a solid mat covering of the forest floor, an abundant coverage of thick open leaf litter has taken its place, greatly benefiting not only amphibians, but other taxa of fauna that are associated with leaf litter communities. Additionally, now that these forest management areas within the Guayacán Rainforest Reserve have an understory that is dominated by broad leaf plant species instead of fern, the localized populations of epiphyllous amphibian species have also increased noticeably.

One of the key amphibian species that was targeted to benefit from this forest management project was the Critically Endangered Lemur Leaf Frog (*Agalychnis lemur*). The Guayacán Rainforest Reserve is known to have one of the most robust remaining populations of this beautiful frog species, and the forest management project is located within the core area of the reserve where several metapopulations are known to be actively breeding. The dominance of a natural understory flora within this section of the



The Critically Endangered Lemur Leaf Frog (*Agalychnis lemur*) from the forest management project within the Guayacán Rainforest Reserve in Costa Rica, managed by the Costa Rican Amphibian Research Center. Photo: Brian Kubicki.

private reserve will greatly increase the migration potential between the different metapopulations, resulting in stronger genetic flow and population viability.

It has been impressive to see how fast the recolonization rate of the native understory floral community has taken place with the forest management areas, even with more delicate and slower growing taxa such as palms (family Arecaceae). Despite the excellent progress that has been achieved thus far with the forest management progress within the Guayacán Rainforest Reserve, we realize that we will need to continue to manage the selected

forest areas for several years to come in order to ensure that the delicate balance of the dominant floral community does not fall back into that of the ferns and other undesirable species.

Future plans include expanding our forest management practices to additional sections of secondary forest within the Guayacán Rainforest Reserve that have an abundance of fern and other undesirable species. We hope that our efforts and successful results may inspire others to undertake similar methods of forest management within other regions of tropical forest to aid in the *in situ* conservation of amphibians and other taxa of both flora and fauna.

A section of forest in the Forest Management Project. This section is in the third phase of management, when creeping terrestrial ferns and their rhizomes are being removed. Photo: Brian Kubicki.



REVA: to the rescue of threatened Venezuelan amphibian species

Enrique La Marca, Luis Saavedra and María Evelyn Escalona, REVA and Universidad de Los Andes, Mérida, Venezuela; and Michelle Castellanos, REVA and Universidad Central de Venezuela, Caracas, Venezuela

We are motivated by a passion: amphibians. But it is a passion against the tide. People ask us, why do we raise frogs? Under the current situation? Why? What for? And we have to explain repeatedly the details that are like lights that illuminate the darkness of the moment. Venezuela is one of the eight countries with the greatest amphibian diversity in the world, but at the same time it also has a high number of endangered species; but it is also the country that occupies the first place in a hyperinflationary spiral that seems to sink the expectations of progress of many.

Full of enthusiasm, on May 11, 2018 we inaugurated an initiative called REVA (from the abbreviations that identify the program of Rescue of Endangered Venezuelan Amphibians). It was created as a program within the BIOGEOS Foundation, a local NGO founded in 2004 and dedicated to the study and divulgation of biological diversity. REVA is made up of a small team: a director, two technical assistants and a research assistant (in the same order as they appear in the authorship of this article). It also has an assistant for invertebrates and a GIS specialist in charge of digital cartography.

REVA is not the first initiative for breeding amphibian species in the country. There were a few previously that dealt with individual species, although only a couple of them had conservation as their primary purposes. However, REVA is the only one of the latter that remains active. It focuses on frog species that inhabit cold ecosystems (especially cloud forests and paramo), particularly those in the genera *Aromobates*, *Atelopus* and *Pristimantis*, without detriment to any others that merit protection. Some of these species, such as Mucuchíes' Frog (*Aromobates zippeli*), have lost much of their habitat and are found in isolated populations with major threats that go against their genetic integrity and survival. Many of the species studied are not even within the national system of protected areas (national parks and similar), so their situation is even more precarious. All this makes it necessary to establish initiatives for *ex situ* captive breeding (that is, within captive breeding centers far from their original places of origin).

The REVA (Rescue of Endangered Venezuelan Amphibians) team in a sub-paramo locality in the Venezuelan Andes. In the background, from left to right: Mauricio Ramírez, M. Evelyn Escalona and Luis Saavedra. In front, Dr. Enrique La Marca. Photo: REVA archive.



The first achievements of REVA are promising. We are generating novel information about the natural history, biogeography and systematics of the species that we raise, and we are developing recommendations for their inclusion (or modification of status) in the IUCN Red List of Threatened Species. Also very important is the support we are giving to the new generations so that they can learn and train in *ex situ* conservation tasks and that they themselves can be duplicators of those experiences. In an environment where many students and university professors have left the country, it is still a compensatory alternative to continue learning. Also, as some comment, we are a source of inspiration for those who observe our achievements despite the current crisis.

We initiated conservationist campaigns with inhabitants in rural areas but also other outreach campaigns for the general public through short videos and press releases. The ultimate goal is to bring information about the global amphibian crisis to a wider audience through awareness programs. These actions are accompanied by work such as cleaning and restoring the habitat of threatened populations, like those that we have started in the localities of Mucuchíes' Frog and *Aromobates duranti*.



Partial view of *ex situ* breeding facilities of *Aromobates* frogs in the cold room of REVA. A donation via Amphibian Ark in 2017 allowed the cool room at the REVA facilities to be installed. Photo: REVA archive.

In the future, expectations are to continue with conservation efforts and not give up in the face of adversity. The teaching of conservation values will always be present among our goals. The preservation of species not only satisfies scientific purposes, but also gives the possibility to future generations to know and admire these creatures that play an important role in ecosystems and that amaze with their diversity of life strategies.

REVA has received international support through *ex situ* projects such as those of Amphibian Ark (for example, with Mucuchíes' Frog and *A. duranti*) and with an *in situ* project under the sponsorship of the Mohamed bin Zayed Species Conservation Fund (with *Pristimantis telefericus*). We hope that similar alliances with other international and national conservation organizations will allow us to achieve many more goals.

Neotropical amphibian management and conservation training course, Guatemala City, Guatemala

Luis Carrillo, Training Officer, Amphibian Ark

Amphibians are the most endangered group of organisms on the planet with over one-third of the species threatened with extinction, due to threats ranging from water quality to infectious diseases. Currently Guatemala has more than 145 amphibian species with almost eighty of them threatened, mainly due to habitat loss for timber and habitat modification for crops. In 2010 an Amphibian Ark Conservation Needs Assessment (www.conservaionneeds.org) was completed for Guatemalan species, where thirty-four species were recommended for Rescue (*ex situ* conservation). But to date, no *ex situ* assurance colonies of endangered amphibians have been set up in the country.

To plan and set up a successful amphibian conservation program there should be trained personnel that can initiate and manage *ex situ* conservation programs and so, during August this year, Amphibian Ark and the Universidad del Valle de Guatemala (UVG) organized a training course on the *ex situ* management and conservation of neotropical amphibians. The course was financially supported by grants from Columbus Zoo and Aquarium (USA), Manchester Metropolitan University (UK) and Amphibian Ark.

The goal of the training course was to build capacity among biologists and other professionals within the Mesoamerican region to provide technical skills necessary for the long-term management of *ex situ* assurance populations of endangered amphibians. Twenty-three professionals representing twelve different institutions from Guatemala, Costa Rica, El Salvador, Panama, and Mexico attended this training course.

This five-day, intensive course consisted of a mix of lectures, group activities, practical workshops, and a field trip designed to deliver the most relevant information about neotropical amphibian husbandry and also to put into practice what the students had learned during the lectures/group work sessions.

Twenty-three professionals representing twelve different institutions from Guatemala, Costa Rica, El Salvador, Panama, and Mexico attended this training course. Photo: Luis Carrillo.



Some of the participants at the recent neotropical amphibian management and conservation course in Guatemala building terrariums which will be used to house their animals. Photo: Luis Carrillo.

The course content was designed to:

- deliver up-dated neotropical amphibian 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 neotropical amphibians.

The course was also designed to encourage the participation and sharing of knowledge and expertise among instructors and



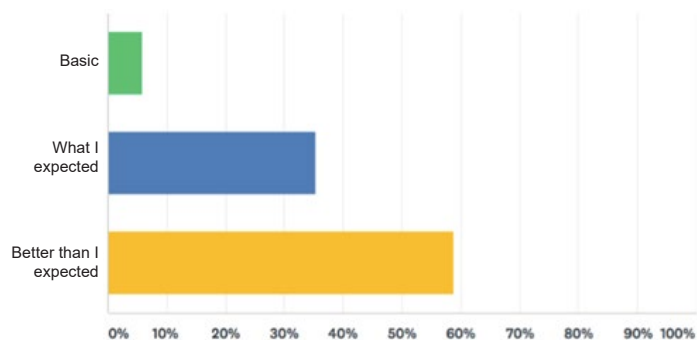
students, providing spaces to do so in a comfortable environment of camaraderie.

To evaluate the effectiveness of the course a post-workshop evaluation was delivered to all participants. A summary of their feedback is presented in the accompanying graphs.

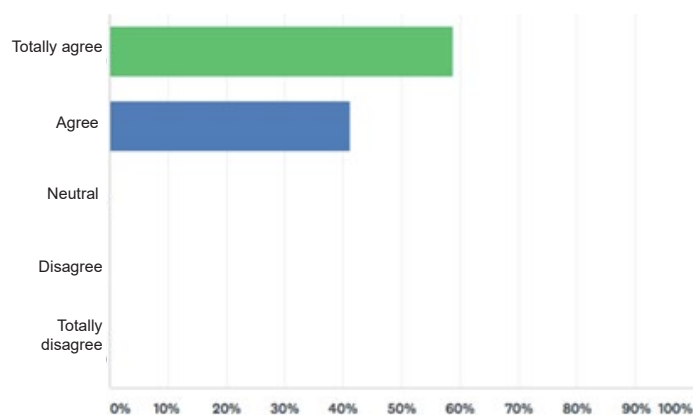
As a side product of the course, a report of the amphibian species found during the field trip and their relative abundance was prepared and delivered to the president of the ecological house development and protected area, Cumbres de Vista Hermosa, where one of the field trips was carried out.

The UVG already has a lab for amphibian biological and behavioral research. During the course students and instructors built tanks to properly keep amphibians that will be used by UVG to keep their animals and to use as a model to build additional tanks. UVG has committed to implement an *ex situ* conservation program and is currently in the process of planning it.

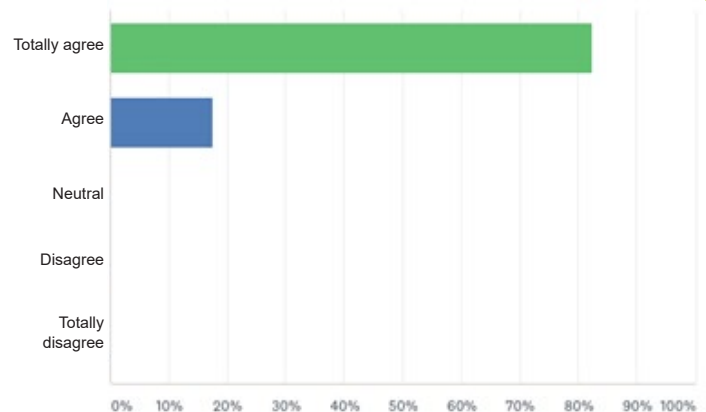
We are very thankful for the support from Columbus Zoo and Aquarium and Manchester Metropolitan University since without their support this training course couldn't be possible.



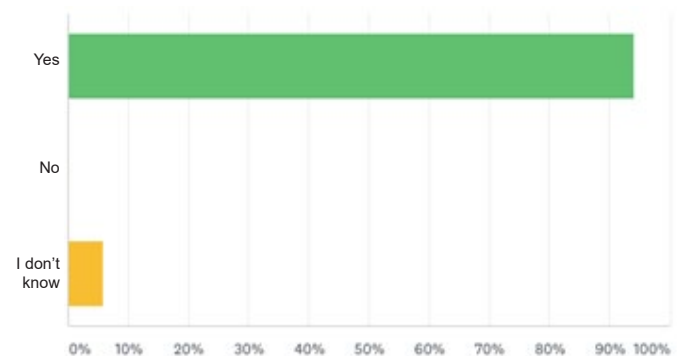
93% of the participants said that information and knowledge acquired were very good to excellent.



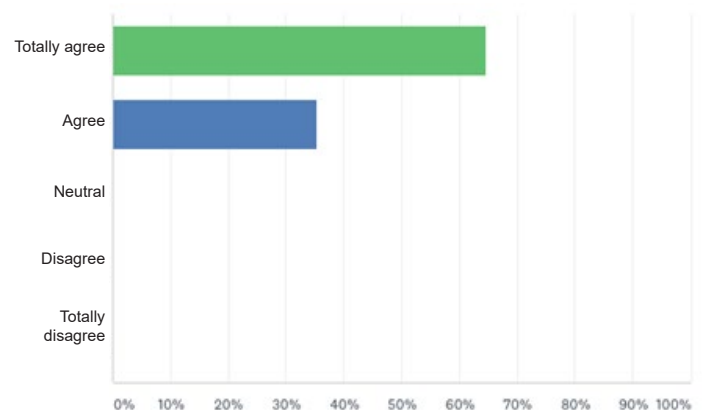
100% of the participants said the course met their expectations.



100% of the participants said the course was good for their personal and professional development.



95% of the participants said people met during the course would be a good support for their amphibian conservation programs.



100% said that they would recommend this course to their colleagues.

Focused Future

Matthew O'Donnell, Manchester Museum, United Kingdom

Over many years, Manchester Museum in the UK has developed a large and world-renowned collection of tropical amphibians and has established captive breeding programs for a range of rare and endangered Central American frog species. However, their revised collection plan for 2019 reflects a new program of research, learning, and social responsibility-led activities that will focus on a highly reduced number of species - but one which will increase their capacity for making an even greater difference to the conservation of each species being kept.

Each Endangered or Critically Endangered species maintained at Manchester will be associated with a multi-disciplined collaborative project to include high profile research, population monitoring, in-country environmental education, and an opportunity for all to be actively involved in supporting the individual species concerned. Species in the 2019 collection plan include all frogs of the genus *Cruziohyla*, the Endangered Blue-sided Treefrog (*Agalychnis annae*) and Critically Endangered Lemur Leaf Frog (*Agalychnis lemur*), as well as two Critically Endangered bufonids from Central America.

Due to our more focused efforts on specific groups of frogs, and our recent discoveries, our work has caught the attention of amphibian film-maker Katie Garrett. She has a particular interest in neotropical tree frogs and has been fascinated in the work being conducted. Her new film, *Sylvia's Leaf Frog*, captures the essence of the amphibian collection at Manchester Museum and some of the associated work being carried out, and can be seen at www.vimeo.com/301586310.

Katie Garrett combines her experience as a trained biologist and her passion for reptiles and amphibians with her skills as a professional film-maker to produce some incredible documentary work. She has previously worked at the Natural History Museum in London and over the past six years has had a real focus on the amphibians of Central America. Her work here has included documenting conservation volunteers, wildlife diseases, and

Katie Garrett holding one of the frogs at the Manchester Museum, United Kingdom.



Blue-sided Treefrog (*Agalychnis annae*). Photo: Katie Garrett.



Sylvia's Tree Frog (*Cruziohyla sylviae*). Photo: Katie Garrett.

the important amphibian conservation work being conducted in Honduras by Dr. Jonathan Kolby. She has covered his rescue efforts in Honduras for several films but has also put in her fair share of hours helping to build the new amphibian rescue facility there (www.jonathankolby.wordpress.com/honduras-amphibian-rescue/).

Katie has produced pieces for National Geographic, Geographical Magazine (Royal Geographical Society), and bioGraphic (California Academy of Sciences). Her work has toured international wildlife film festivals and one of her films was recently nominated as a finalist at the Jackson Hole Science Media Awards. In the future she hopes to make more impact driven documentaries, particularly to highlight the on-going chytrid crisis and with a focused aim of bringing as much attention to the plight of amphibians as she possibly can. For more information on Katie's work, check her web site, www.katiegarrett.co.uk.



The largest Titicaca Water Frog (*Telmatobius culeus*) in the K'ayra Center is known as "The Pirate" and measures 124 mm in length from cloaca to face, and weighs 235 grams!

Photo: D. Alarcón / D. Grunbaum.

The K'ayra Center for Research and Conservation of Threatened Amphibians in Bolivia has made many advances over the past year. Within its *ex situ* program, we have five species of water frogs from the genus *Telmatobius* and others which are possibly new species, awaiting their description.

One of the species with the largest number of individuals in our center is the Titicaca Water Frog (*Telmatobius culeus*) – we have more than 150 individuals and many of them are from the second F1 captive-bred group last year. With the increase of individuals, there is more work for our technicians, but thanks to them, and to the daily care they give the frogs, our new juvenile Titicaca Water Frogs are growing very well. Periodically the technicians change the aquarium water, the frogs are given UV dose baths and we constantly monitor their health and development. We also raise our own food, including cockroaches, small fish, isopods and earthworms, the latter being the frogs' favorite food. We also give them a vitamin supplement in the form of pellets.

Gerardo Garcia from the Chester Zoo, UK, presenting the lecture "The Critically Endangered Titicaca Water Frog: Bolivian and Peruvian conservation efforts". Photo: Gerardo Garcia.

The K'ayra Center's advances in *ex situ* management and conservation of the Titicaca Water Frog from Lake Titicaca in Bolivia

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

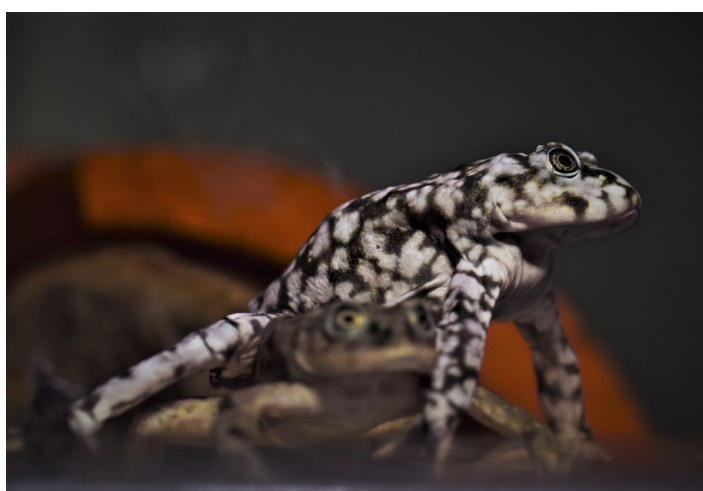


One of the Titicaca Water Frogs at the K'ayra Center in Bolivia.
Photo: D. Alarcón / D. Grunbaum.





Participants of the prioritization workshop for the implementation of the Binational Action Plan for the Conservation of the Titicaca Water Frog and the Titicaca Grebe. Photo: Yuri Beraún.



Juvenile frogs born in Centro K'ayra in 2017.
Photo: Teresa Camacho Badani.

Our job is to care for the Titicaca Water Frogs, as well as undertaking research, and we also collaborate with other national and international institutions to share and strengthen the knowledge

A prioritization workshop for the implementation of the Binational Action Plan for the Conservation of the Titicaca Water Frog and the Titicaca Grebe (*Rollandia microptera*), held in Copacabana, Bolivia in late 2018. Photo: Yuri Beraún.

we have about breeding these species in captivity. This year, during the First Bolivian Congress of Herpetology, there have been advances in the *in situ* and *ex situ* work made by the staff at the Center and researchers of the Natural History Museum Alcide d'Orbigny on the Titicaca Water Frogs, which included ecology and successful cases of veterinary care for this species.

Because of interest in this species from both countries where it occurs – Bolivia and Peru – we recently attended the annual meeting of the European Association of Zoos and Aquariums (EAZA) held in Athens, Greece, where along with Roberto Elías and staff from the Denver Zoo in the USA and the Peruvian University Cayetano Heredia, Teresa Camacho Badani from the K'ayra Center presented the efforts of Bolivia and Peru for the conservation of this Critically Endangered frog. The "*Prioritization for the implementation of the Binational Action Plan for the Conservation of the Giant Frog and the Diver of the Titicaca Bolivia - Peru*" was also discussed at length during a workshop held in Copacabana, Bolivia, where researchers and strategic actors from Bolivia and Peru developed a strategy to conserve this species.

There have been some great advances in the conservation of the Titicaca Water Frog, but because of its threats, there is still much work to be done. We hope that Centro K'ayra will continue to participate in these conservation efforts and we are grateful for the support of institutions such as the Kansas City Zoo in the USA, Amphibian Ark and other donors who make it possible for us to continue our efforts.



The Amphibian Foundation expands captive breeding program for rare frog

Lacey Avery, Communications Lead, and Mark Mandica, Executive Director, Amphibian Foundation, Atlanta, USA

A rare frog resides in south-eastern US. In fact, the Gopher Frog (*Lithobates capito*) is the state of Georgia's rarest frog, making it an Amphibian Foundation priority species. Despite evidence of successful introduction into protected habitat, the species continues to decline in some areas. In 2018, the Amphibian Foundation expanded its headstarting program to include captive propagation for the species in an effort to bring up numbers of releasable frogs each year. This captive breeding effort is being mirrored between the Foundation and conservation partners at Zoo Atlanta.

The expansion was made possible with the completion of the Amphibian Foundation's Amphibian Research and Conservation Center (ARCC—nicknamed 'Metamorphosis Meadow'), a private outdoor lab that holds twenty tri-phasic (wetland, upland, ecotone) mesocosms that mimic the natural environment under controlled conditions. The one-of-a-kind conservation resource was created initially to mimic ephemeral wetlands for one of our other priority species - the imperiled Frosted Flatwoods Salamander (*Ambystoma cingulatum*). Three of the mesocosms have been modified to support the captive propagation of the Gopher Frog; each with loose sandy soil, native vegetation, artificial burrows and an ephemeral wetland. The Amphibian Research and Conservation Center was funded by the generous donations of the Andrew Sabin Family Foundation, the Turner Foundation and the concerned public, through a YouCaring crowdfunding campaign.

Like Flatwoods Salamanders, Gopher Frogs are temporary wetland breeding amphibians, and breed in fish-less wetlands that dry out periodically throughout the year. Both amphibians are endemic to the Longleaf Pine ecosystem, which has been reduced to three percent of its original range in the south-eastern US coastal plain.

Gopher Frogs also need open canopy ponds with wiregrass, which unfortunately disappears when naturally occurring wildfires are suppressed. This is one of the reasons Gopher Frogs are disappearing from protected lands. The mesocosms will assist the frogs with reproduction, by providing the proper hydrology

and other environmental cues necessary. The offspring, once produced, will be reared through metamorphosis through a head-start program provided by our partners at US Fish and Wildlife Service - Warm Springs, Georgia.

A glimpse of establishment and hope

The Amphibian Foundation works with partners to save amphibians from extinction by raising awareness and leading one-of-a-kind conservation and research activities. Our captive propagation program allows us to raise these amphibians in captivity and produce offspring that can be released into protected habitat in the wild.

Since 2009, staff at the Amphibian Foundation have been head-starting Gopher Frogs - rearing eggs through the sensitive larval stage. The metamorphs and late-stage larvae were experimentally released into protected land owned by The Nature Conservancy. Over the past several years, there has been some evidence that the experimentally released population has begun to establish at the release site. We hope to detect further signs of positive establishment in the upcoming field seasons.

With support from local and national partners – the University of Georgia, Zoo Atlanta, the Georgia Department of Natural Resources, and the U.S. Fish and Wildlife Service - the ten-year head-start program expanded into the captive propagation program. We hope to continue to see success and improvement in the species population over time.

For updates on these projects and more, sign up for the Amphibian Foundation mailing list: [StayInformed.amphibianfoundation.org](https://stayinformed.amphibianfoundation.org).

In 2018, the Amphibian Foundation began a captive propagation program for the Gopher Frog (*Lithobates capito*) to bring up numbers of releasable frogs each year. The Gopher Frog is the rarest frog in Georgia, USA. Photo: Amphibian Foundation.



Experiences of the frog group in the conservation of two high Andean amphibians from the central Andes of Peru

Oscar J. Damián-Baldeón and Luis Castillo, RANA Group, Peru

The Junín Giant Frog (*Telmatobius macrostomus*) and the Wancha or Junín Riparian Frog (*T. brachydactylus*) were once abundant in the 1950s and constituted an economic resource for the local population. Unfortunately, their populations have declined drastically due to over-exploitation, invasive species and habitat degradation. Historically, these frogs inhabited lakes, streams and wetlands in the high Andean regions of central Peru, found in the Junín National Reserve, the Historic Sanctuary of Chacamarca and the National Sanctuary of Huayllay (Natural Protected Areas). Both species are of ecological importance not only because they are a source of proteins, but because of the variety of services they provide to ecosystems.

Recent explorations only found nine adult Junín Giant Frogs and one adult Junín Riparian Frog, in twenty dry and wet exploration zones, while monitoring zones that included the three Protected Natural Areas (Watson et al., 2017). This indicates the great difficulty of finding them and the risks they face.

Adaptation of environmental education manuals “The Disappearance of the Frogs” for teachers in Latin America and Peru

In this context, the RANA Group (Answers and Actions for Nature and its Threats) is implementing conservation projects for these endemic amphibians, with research components, environmental education and citizen science supported by The Rufford Foundation, Crown Funding campaigns, National Geographic Society, Denver Zoo, the Natural History Museum of the National University of San Marcos, ECOAN NGO, Junín Local Education Management Unit (UGEL Junín), and the National Service of Natural Protected Areas by the State (SERNANP) among other allies.

San Pedro de Pari educational center, where the RANA group and SERNANP developed the environmental education workshop using the *Salvando las Ranas* manual adapted to Latin America and Peru. Photo: Grupo RANA.



The Wancha or Riparian Frog (*Telmatobius brachydactylus*) was once abundant in the National Reserve in Junín, Peru in the 1950s, but unfortunately, their populations have declined drastically due to over-exploitation, invasive species and habitat degradation. Photo: Oscar Damián-Baldeón.

We must use tools that promote awareness and change of attitudes in local students, and so we have been implementing environmental education and citizen science programs. At the time we were implementing environmental education activities we identified the *Vanishing Frogs* manuals (www.amphibianark.org/education/links-to-curriculum-materials/) developed by Amphibian Ark, as a powerful tool that informs, sensitizes and encourages the change of habits and attitudes, and promoting youth leadership, using the context of the current amphibian crisis as a scenario.





Lake Junín or Chinchaycocha, within the National Reserve of Junín, historical habitat of the Junín Giant Frog (*Telmatobius macrostomus*). Photo: Oscar Damián-Baldeón.

The RANA Group saw the need to adapt and translate these manuals to the Peruvian and Latin American reality. In the Peruvian case, this lesson plan can be used to address the current “National Plan for the Study of Regular Basic Education”, approved in 2016 by the Ministry of Education. The Spanish versions are now available on the Curriculum Materials page on the AArk web site.

Next year the RANA group will implement environmental education and citizen science activities with the modified manuals in the Chinchaycocha Lake basin with the student populations of the local peasant communities. These projects will be financed and supported by our allies.

Historically, the Junín Giant Frog inhabited lakes, streams and wetlands in the high Andean regions of central Peru, found in the Junín National Reserve. Photo: Oscar Damián-Baldeón.



Bromeliad amphibian monitoring task force: Building capacity to use eDNA in the search for cryptic frogs

Alcina Santos and Geraldo Moura, Federal Rural University of Pernambuco, Brazil; Clara Ribeiro, Filipe Rego and Moacir Tinoco, Catholic University of Salvador, Brazil; and Ricardo Khouri, Oswaldo Cruz Foundation, Brazil

Bahian frogs at risk

The Glass Frog (*Vitreorana eurygnatha*), Leaf-litter Frog (*Chiasmocleis sapiranga*), Beaked Toad (*Dendrophryniscus proboscideus*) and Bromeliad Tree Toad (*Phyllodytes gyrinethes*) are just a few of the “lost” species from the north coast of Bahia and Pernambuco, in Brazil. A few of them, such as the Leaf-litter Frog was last recorded in our project in 2005. A few of them have not been recorded for ten or more years ago, such as the Glass Frog. Some of these species appear on the list of most wanted species of the world, for being such difficult taxa to find.

Our long term monitoring program has been able to monitor a large number (more than sixty species) of amphibian species (anurans and caecilians), and since 2001 our project has done research in Atlantic Forest ecosystems of the coast of Bahia, on the north-eastern region of Brazil, corresponding to less than 20,000 km² of natural sand dune habitats with a mixture of dunes, lagoons and forest. These ecosystems are the white sand dune habitats - locally known as Restinga - one of the most endangered ecosystems in Brazil.

The sand dune ecosystem and amphibian assessment

The Restinga habitats are dry, salty, windy and very little fresh water is available, and finding and monitoring amphibian species is not easy. It seems that traditional methods and techniques such as pitfall and funnel traps, visual and acoustic searches failed to detect and monitor species that are usually present, but don't seem so obvious in their natural habitat. It is not a matter of choice or combination of methods and their quality, but in such harsh environments amphibian species tend to reduce their activity pattern, therefore making it difficult to detect them in their natural habitats.

What do we do?

The Herpetofauna of the North Coast of Bahia project has been monitoring over 180 species of amphibians and reptiles and has recently trialled a new amphibian conservation management initiative. The project is building capacity to use eDNA in order to detect, monitor and manage endangered, rare and cryptic species. Our eDNA task force brings together professionals from three research institutions and using their expertise, experienced biologists, geneticists and biomedics work together to establish a highly efficient method to positively detect amphibian species, even when they are not there anymore. The Catholic University of Salvador (UCSAL), the Federal Rural University of Pernambuco (UFRPE) and the Oswaldo Cruz Foundation (FIOCRUZ) are the main institutions involved in the task force to date.

Our method consists of sampling bromeliad species in different biomes in Bahia and Pernambuco. We are looking at the Restinga and thus compare the use of eDNA, its persistence and efficiency to detect and monitor amphibian species. We compare with the Brazilian savannah (Cerrado) at Chapada Diamantina region in Bahia, and the Caatinga in Pernambuco. This last one is a dry land ecoregion with high temperature, like the Restinga, but showing a very harsh water shortage, therefore, we trust, will present different rates of eDNA persistence, thus printing different efficiency for the purpose of the project - detect, monitor and support public policies for the protection of endangered and rare species in an *in situ* conservation program.

By using DNA fragments released by frogs in their environment, such as phytotelm, soil, water bodies and even on other material such as leaf litter, researchers sample the environment and



A typical Restinga habitat, showing the high variety of landscape components including dunes, lagoons and forest. Frogs are found in a variety of habitats. Photo: Moacir Tinoco.

collect a portion of the intended material, and then send it to the laboratory to extract, amplify and sequence DNA fragments present in the material, and match them with selected primers during the sequencing process. So far, our genetic methods and protocol consists of extracting and amplifying DNA from phytotelm water samples, therefore polymerase chain reaction (PCR) methods are applied in order to prepare samples for DNA sequencing and thus determine which species are present in the samples.

Previous to that, we analyzed the sequences of the 16s region of the mitochondrial anuran DNA available at GenBank. Through

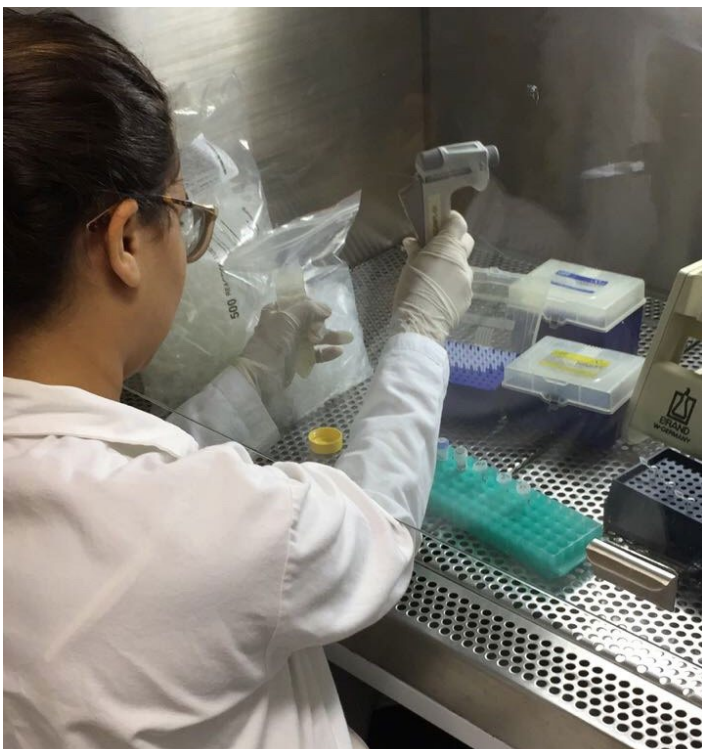
The team at Pernambuco sampling bromeliads and frogs; part of the team is looking at frogs and bromeliads correlations. Photo: Ana Paula Tavares.



phylogenetic analysis, it was possible to group different anuran species with significant statistical power and support, indicating that the 16s region of mitochondrial anuran DNA can identify any species present within the phytotelm through molecular and bioinformatics techniques. Based on the aforementioned phylogenetic analysis, a pair of degenerate primers specific to the 16s region of the mitochondrial DNA were designed so as to amplify the DNA of any of the anurans previously analyzed and expected to be in contact with the phytotelm water in any way through a monitoring program. A 265 base pair fragment will be amplified using primers



A pair of White-striped Tree Frogs (*Phyllodytes wuchereri*) detected inside a tank bromeliad. In the breeding season there is a higher amount of DNA in the water. Photo: Julien Bourgeois.



The lab team working on sample trials for eDNA analysis. So far, at least ten researchers from two states have been trained to work on eDNA. Photo: Clara Ribeiro.



The Moustached Tree Frog (*Phyllodytes melanomystax*), an endemic bromeligenous frog inhabitant of tank bromeliads, which has been monitored since 2009. Some bromeliads are listed as endangered in Bahia. Photo: Moacir Tinoco.

after PCR reactions, conducted at the lab. For species identification, each sequence is aligned with the dataset initially analyzed. After alignment, a phylogenetic tree will be reconstructed. The sequence is considered of the species if the bootstrap value for the group monophyletic is above 60% in the tree.

The main outcomes

Currently, our search is looking for over twenty anuran species we suppose are using different tank bromeliad species in three different Brazilian ecoregions: the Atlantic Forest, the Brazilian savannah (Cerrado), and the Dryland (Caatinga). In the near future we will be able to say if species such as the Green Tree Frog (*Phyllomedusa bahiana*), the Moustached Tree Frog (*Phyllodytes melanomystax*) or the White-striped Tree Frog (*Phyllodytes wuchereri*) are bromeliad species specific, using for instance species such as the Tank Bromeliad (*Hohenbergia litoralis*) or the Spear Bromeliad (*Aechmaea multiflora*), both of which are endemic to the Restinga and Atlantic Forest.

Our goals and outcomes are ambitious and complex. We first want to determine the eDNA as a valid method for monitoring amphibian species at new tropical systems. By doing this we want to determine the different rates in which eDNA degrades on the three different ecoregions as they bare completely different environmental conditions. Moreover, we aim to determine which genetic protocol is the most efficient for this usage and positively detect amphibian cryptic species. In the end we believe the project will produce a consistent genome bank for those species, making it available for future research and conservation strategies. Our project also aims to reinforce the use of eDNA for *in situ* conservation strategies such as monitoring and management, in order to detect and protect populations from different portions of the three ecoregions. Overall the project will benefit frogs, bromeliads and the entire ecosystem of Restinga.

Acknowledgments

We want to thank the Amphibian Ark for the opportunity to publish this progress report on our amphibian conservation and management initiatives and our outcomes to date. The partner institutions: UCSAL, UFRPE, FIOCRUZ, the Botanic Gardens of Recife, the Parque das Dunas in Salvador, Bahia, the Parque Klaus Peters in Mata de São João, the Fazenda Milagres in Conde and the management council of several state protected areas such as the North Coast APA and the Joanes



The Bahian Green Tree Frog (*Phyllomedusa bahiana*) is an arboreal frog that occasionally uses bromeliad species as shelter, although its relationship with the plant is not clearly defined.
Photo: Moacir Tinoco.

Ipitanga APA. We also want to thank our lab task force, the undergrad and graduate students from the HNLB/ECOA and LEHP/GEHP who are all contributing on reaching the goals: Icaro Ströbel, Rafaela Calil, Jose Augusto Souza, Letícia Azevedo, Julien Bourgeois and Ubiratã Souza, We also want to thank the INDUSLAB Ltd. company for the support it gives to our project by supplying extra materials for the genetic analyses.

Endangered tank bromeliads are home for a high number of bromeligenous frogs but the frogs are in most cases are listed as Least Concern. Photo: Moacir Tinoco.



Collecting live food with a light trap to feed salamander larvae

Johan Auwerx, Bruno Picavet, and Daniel De Charleroy, Instituut voor Natuur- en Bosonderzoek, Linkebeek, Belgium

Ex situ breeding of new species is like opening a box of chocolates: you never know what you're going to get. To obtain a workable protocol with a good survival rate, there are lots of problems to be solved. One of the biggest challenges when rearing the Great Crested Newt (*Triturus cristatus*) was to deal with extremely cannibalistic behavior of the larvae. If they don't swim in an over-supply of food, they will start to attack each other just a few weeks after hatching.

In 2018, we succeeded in breeding an extremely high number of these highly threatened species (Auwerx J., 2018). Early in Spring, males and females were placed in two artificial ponds decorated with a natural submerged and floating vegetation (e.g. *Myosotis scorpioides*, *Veronica beccabunga*, *Mentha aquatic* and *Rorippa nasturtium*). After fertilization, the females deposited single eggs on the leaves, folding and gluing the leaves into a firm envelope. Twice each week we collected eggs from this 'mesocosm'. No predators (e.g. dragonflies, beetles, frogs etc.) were present in these ponds.

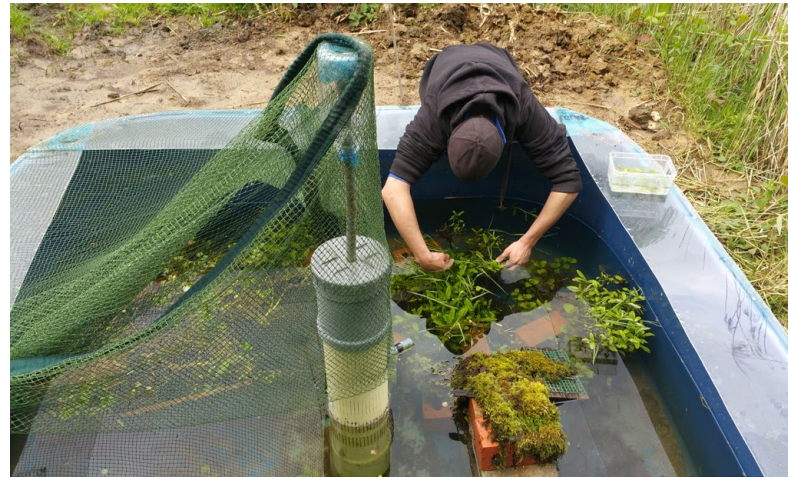
We harvested a total of 2,563 eggs, over a period of sixty-eight days (21 April 2018 until 28 June 2018). The majority of the eggs (around 90 %) were deposited on *Myosotis scorpioides* leaves. Afterwards, these eggs were transferred into a hatchery, where they could develop in a safe and controlled environment. Due to a chromosome abnormality, 50% of the Great Crested Newt eggs died within a week.

In small, heated tanks (20-23°C), the embryonic development took between 250-280 day-degrees (D°) till hatching. A total of 1,196 larvae (46.7%) hatched, and after resorbing the yolk-sac, the larvae were between 10–12 mm in length. Some of the deposited eggs were probably overseen, as an extra 203 free swimming larvae were caught in the mesocosms. Together with the hatched larvae from the picked leaves, a total of 1,399 larvae had to be nurtured with exogenous food.

During the first ten days, we gave the newt larvae freshly-hatched Brine Shrimp (*Artemia nauplii*). The eggshells of the Artemia were very accurately removed to prevent the blocking of the digestive system. The newt larvae grew well on this diet until a size of 18-20 mm. At this age, the first signs of cannibalistic behavior occurred. From then on the need for a daily supply of large quantities of living plankton was very high.

A light-activated trap to collect zooplankton

Light traps are used regularly in aquaculture to collect live food. Many species are attracted to light and are thereby called positive phototactic. We developed a light trap (Vught et al., 2015) based on old technical data sheets in WRP Technical Note FW-EV-3.1 from 1994, and the Quatrefoil trap of Floyd et al. (1984). The bottom of the trap consists of a white board (Trespa 8 mm) panel giving the construction the necessary firmness. Four transparent PVC pipes (300 mm length x 200 mm diameter) are glued perpendicular on the bottom. A rectangular slit of 240 mm by 80 mm was cut in the long side of each tube. These four openings lead to the central space, that in turn can be reached via narrow gaps of 6 mm between the cylinders. The styrodur panel (40 mm thickness) at the top allows the structure to float. A second styrodur panel is used as the lid. This lid supports two intense LED lights (Philips / Paulmann, 1W), hanging in the middle space between the four PVC pipes. The LED lights themselves are placed in a glass testing tube so that they can function underwater. We placed the lights in parallel to compensate for any defects. A 12-volt (7 Ah) battery is fixed on top of the lid. The whole trap has a size of 600 x 600 x 350 mm. The volume in the light trap is about

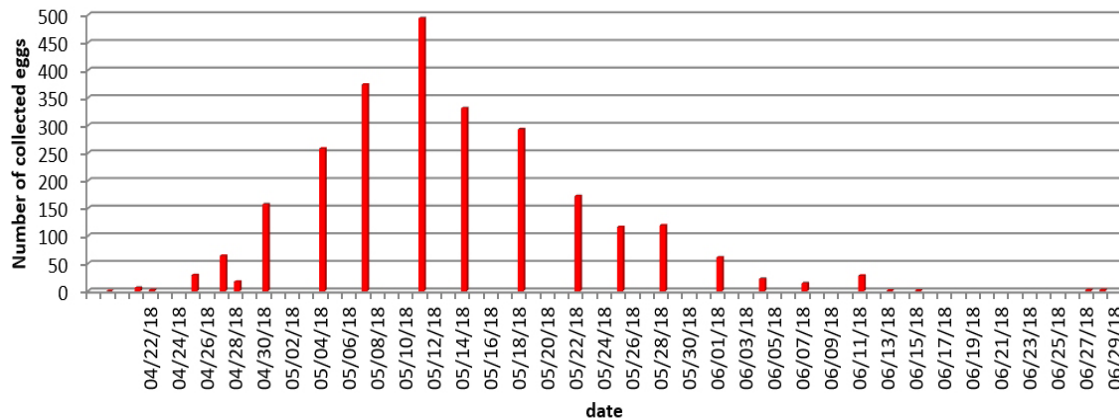


One of the mesocosms at the Instituut voor Natuur- en Bosonderzoek in Belgium. The protected and controlled environment imitates a natural pond. Photo: Johan Auwerx.

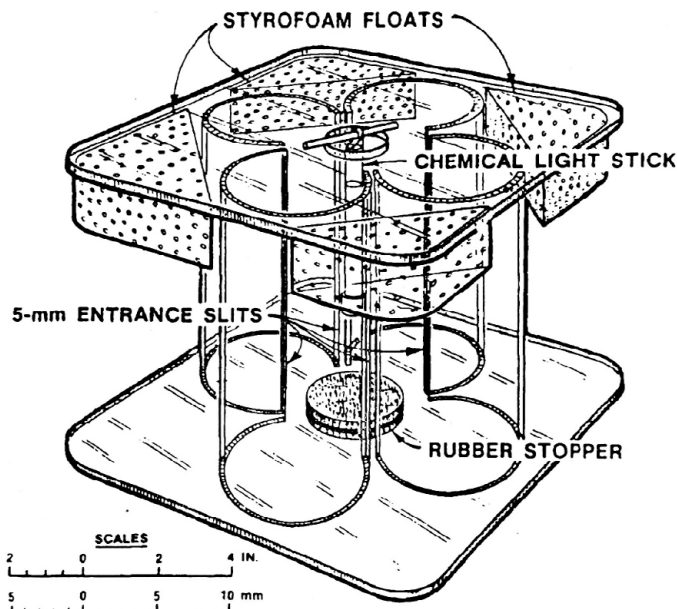


Twice each week eggs were collected by picking the folded leaves. This photo shows the harvest for one day. Photo: Johan Auwerx.

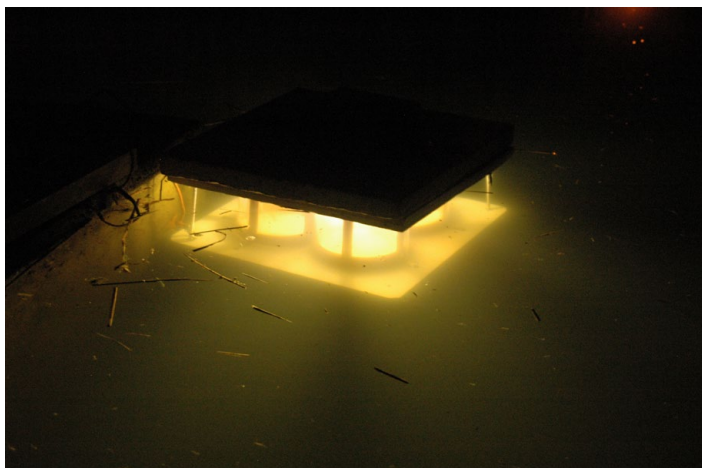
date / number of collected eggs



The number of Great Crested Newt (*Triturus cristatus*) eggs collected during the reproduction season.



A light trap for phototactic fish larvae (WRP Technical Note FW-EV-3.1 (1994)).



The light trap in operation. The intense lighting is visible in clear water for more than five meters. Photo: Johan Auwerx.

50 liters, and the intense lighting is visible in clear water for more than five meters.

The light source in the middle attracts the zooplankton, which then swims through the narrow gaps between the four transparent tubes. These narrow openings prevent the escape of plankton from the trap.

We placed our light trap in the ponds (0.086 ha) in the evening and the next morning the living plankton was scooped out with a fine-mesh aquarium net. The water in the pond was very clear and the aquatic vegetation was not yet developed due to the early time of the year. The attraction of the light source is therefore large.

With one light trap, we were able to collect between 100-120 g (wet weight) of cladocerans and / or copepods each day. As we have twenty-six ponds, plankton with a good size was always available.

Thanks to the food collected with the trap, we could provide an abundance of living food to the salamander larvae on a daily basis. They grew fast during the first four to six weeks and cannibalism was only being detected in a few batches, however losses were high when cannibalism did occur. Shortly before metamorphosis, at lengths between 40-75 mm, the juveniles were transferred to their new habitat in the wild. Finally, 1,229 of the 1,399 larvae were successfully raised and released in nature.

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Effects of animal-based and plant-based protein on larval growth and metamorphosis in Dyeing Dart Frog tadpoles

Webster Cheong and Francis Cabana, Wildlife Reserves Singapore, Singapore

Anurans have been bred in zoos and private collections for decades, however in all that time we are still no closer to fully understanding larval diet. Food items consumed during larval stages of most species are not well-documented and gut content analyses studies have not yet been carried out. Zoos and private enthusiasts have experienced varying degrees of success with captive-bred tadpoles raised on commercial fish feed or tadpole pellets. Unfortunately, the variation of nutrient concentrations and ingredient quality in commercial products make it difficult to ascertain the ideal diet, especially without known nutritional requirements for this species of tadpole. Identifying efficient and cost-effective ways of successfully rearing tadpoles of endangered frogs would prove to be the basis of any *ex situ* conservation program to promote growth rate (Martinez et al. 1994).

The Dyeing Dart Frog (*Dendrobates tinctorius*) is very prevalent both in zoological institutions as well as the pet trade. We chose it as our study species as they are prolific breeders with well-established husbandry practices. We assume the tadpoles of this species to be opportunistic omnivores as they have been shown to rasp on vegetable matter and will cannibalize when the opportunity arises. They also have clearly defined keratinized labial teeth. While it is possible to attain equal crude protein content from both plant and animal-based diets, amino acid profiles will differ between the two. The aim of the study was to determine whether a plant-based, animal-based or omnivorous diet will cause a difference in larval growth rates and rate of metamorphosis.



Tadpole-rearing containers - all tadpoles were reared individually during the experiment. Water color is due to Indian Almond Tree (*Terminalia catappa*) extract. Photo: Webster Cheong.

We used a total of twenty-four tadpoles for the study with four groups of six animals (Control, Herbivore, Carnivore, and Omnivore). All tadpoles were kept individually in plastic containers with Java moss added. Herbivore tadpoles were raised on spirulina powder and spirulina pellets. Carnivore tadpoles were raised on freeze-dried bloodworms and tubifex worms. Omnivore tadpoles were fed a mixture of both Herbivore and Carnivore diets. Control tadpoles were not fed throughout the duration of the study to cancel the effects of Java moss consumption. All treatment diets were chosen based on similar crude protein content. Total length measurements were made on a weekly basis until tadpoles reached stage 42 on Gosner's staging table.

Results revealed a significant difference in both larval growth and rate of metamorphosis between Control and treatment groups. Control tadpoles did not exhibit any growth after two weeks and also failed to metamorphose within the average number of days taken for the other three treatment groups (± 108 days). We can assume that these tadpoles can survive prolonged periods of time with a sub-optimal diet; in this case, Java moss. There was no significant difference of the growth rate between Herbivore and Carnivore ($Z = -1.618$, $P = 0.106$) or Omnivore ($Z = -0.619$, $P = 0.536$) treatments. Carnivore and Omnivore treatments would have had a significant difference if it were not for our Bonferroni corrections ($Z = -2.504$, $P = 0.012$).

Amino acid analyses of the diets showed that spirulina had higher amounts of tyrosine, phenylalanine, and arginine as compared to tubifex and bloodworm. From the results, we can assume that source of protein may not be as important as percentage of protein. Tadpole growth is largely correlated with the percentage of protein in their diet (Kupferberg, 1997); therefore for the Dyeing Dart Frog we would propose that diets of 53-60% crude protein were sufficient to support growth, no matter the source. Determining the exact nutritional requirements for every species is extremely challenging, therefore developing guidelines with the use of model species would be the way to move forward. This is especially pertinent now as the numbers of declining amphibian populations increase; the success of an *ex situ* program could depend on the viability of the larval diet.

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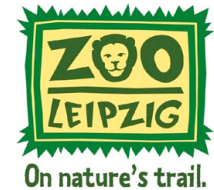
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