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Amphibian Ark News

Welcome to Issue No. 71 of the Amphibian Ark Newsletter!

Here's what's been keeping us busy this quarter—and what's coming up!

Welcoming Devin Edmonds to the team!

We are happy to have Devin join our team as our new Program Director for Africa and South Asia starting October!



decade of experience

in amphibian conservation and research. He has worked extensively in Madagascar, where he helped establish the country's national amphibian conservation breeding facility and collaborated on action plans for threatened species. His work has integrated field and ex situ efforts, population modeling, and community-based initiatives to support longterm strategies for species survival. At AArk, Devin will be working with regional partners to identify priority species, build capacity, and strengthen in-country programs across Africa and South Asia.

Connect with Devin on Linkedin here. Google Scholar.

In case you missed it: **AArk 2024 Annual Report**

Catch up on a year of amphibian rescue highlights—updates from our Brazil National Program. Conservation Needs Assessments from 2024, and other species updates from past AArk Grantees.



In 2024:

- Our signature Conservation Needs Assessment (CNA) process continued, but for the first time, we translated CNA outcomes into dedicated CNA Reports tailored for decision-makers and conservation partners.
- We awarded three program grants, two extension grants, and one workshop grant
- Our Brazil National Program entered its second year, with exciting updates to share about the species that are part of the program—plus news on other species we've supported worldwide.

Read it here.

Subscribe to our email list or follow us on social media to never miss an update:









Amphibian Ark News

Coming up:

Telmatobius genus-wide Conservation Action Plan workshop in Chile

The IUCN SSC <u>ASG Telmatobius Task Force</u>, along with Amphibian Ark and the Amphibian Red List Authority—and with the support from Re:Wild, Synchronicity Earth, Universidad Andrés Bello, and Centro de Ecología Aplicada—are organizing a genus-wide conservation action plan workshop for all the *Telmatobius* species from Ecuador, Bolivia, Perú, Chile, and Argentina. This comprises 63 species in total.

This multi-species, multi-country workshop will be facilitated by the IUCN SCC <u>Conservation Planning Specialist</u> <u>Group (CPSG)</u>, and aims to develop the first genus-wide conservation action plan for *Telmatobius* species.

The workshop will be held at the Universidad Andrés Bello in **Chile** from **October 15-17. 2025.**







Amphibian Ark News

Coming up:

Implementing the ASI Harlequin Toad Conservation Action Plan in Colombia

Amphibian Ark and the <u>Atelopus Survival Initiative (ASI)</u> are convening a workshop during the Annual Meeting of the IUCN SSC <u>Conservation Planning Specialist Group (CPSG)</u> in Cali, Colombia, from 23-26 October.

The ASI developed the Harlequin Toad Conservation Action Plan (HarleCAP) in 2021, outlining range-wide priorities to be implemented between 2021-2041. This workshop aims to operationalize the HarleCAP for implementation in Colombia.

Find the workshop description here.

<u>Contact us</u> if you want more details about the workshop. You can find more information and register to the CPSG Annual Meeting <u>here</u>.



Atelopus spumarius

© Jaime Culebras





Busy August for Amphibian Conservation in Brazil

Renata Ibelli Vaz | AArk Brazil National Coordinator Cybele Sabino Lisboa | AArk Brazil National Coordinator Luis Carrillo | AArk Program Director for the Americas & the Caribbean

August was a particularly active month for amphibian conservation efforts in Brazil, marked by capacity-building initiatives, technical visits, and scientific exchanges.

Conservation Medicine Workshop at São Paulo Zoo

From August 20 to 22, the São Paulo Zoo hosted the first Amphibian *ex situ*Conservation Medicine Workshop. Organized by Amphibian Ark (AArk), the São Paulo Zoo, Amphibian Specialist Group (ASG) Brazil, and The Wild Place, the event featured lectures and practical sessions led by 11 specialists. The event gathered 22 participants, representing zoos, aquariums, universities, NGOs, and independent professionals from different regions of Brazil. Over the three days, they received intensive training on a wide range of topics, including conservation

strategies, anatomy, clinical examination, nutrition, diseases, diagnostic imaging (ultrasound and X-ray), quarantine, anesthesia, surgery, and animal welfare.

As one of the invited speakers, Luis Carrillo, AArk's Program Director for the Americas & the Caribbean, traveled to Brazil to participate in the workshop. Following the event, he joined Renata Vaz in conducting technical visits to the São Paulo Zoo, where they toured the facilities and learned about ongoing projects with *Atelopus manauensis*.

The zoo team is currently developing husbandry protocols using the analog species *A. hoogmoedi* to gain experience and refine methods before working directly with the target species.







AArk's Partner Projects

During this visit, the AArk team also visited two of AArk's partner projects. At BioParque do Rio, the team is advancing a conservation program for the critically endangered *Physalaemus soaresi*, while maintaining the analogue species *P. signifer* to optimize husbandry practices.

At the Butantan Institute's Museu Biológico, they observed the facilities and the progress of the *ex situ* project with the critically endangered *Melanophryniscus setiba*. All visits provided valuable opportunities to assess infrastructure, discuss challenges, and exchange approaches. You can read more about both of these projects in <u>our previous</u> newsletter or on <u>our website</u>.



Amphibian Ark News



XI Brazilian Congress of Herpetology

Finally, in the last week of August, Renata and Luis traveled to Manaus to attend the XI Brazilian Congress of Herpetology. There, they co-led an 8-hour training course on *ex situ* amphibian husbandry for research and conservation, attended by 13 students. They also contributed to an ASG Brazil symposium on the steps of species conservation, highlighting both *in situ* and *ex situ* initiatives.

In addition, they participated in the workshop that launched the Conservation Action Plan for *A. manauensis*, a critically endangered species.

The congress was a fruitful occasion to share advances, strengthen national and international collaborations, and discuss new research and conservation initiatives.

Together, these activities reflect the growing activities being developed for amphibian conservation in Brazil, with institutions, professionals, and partners working side by side to advance knowledge, strengthen capacity, and implement concrete actions for the survival of threatened species.





Part of ASG team and Luis Carrillo, at the XI Brazilian Congress of Herpetology

Training course on *ex situ* amphibian husbandry for research and conservation, at the XI Brazilian Congress of Herpetology



Action Plan Workshop for the species Atelopus manauensis, at the XI Brazilian Congress of Herpetology



2025 Grantees

Each year, AArk provides grants to help AArk partners launch amphibian rescue programs for at-risk species. These grants provide up to three years of support for *ex situ* projects within range states, where rescue efforts are critical to prevent their extinction.

Establishment of an *ex situ* population of the Pehuenche frog (*Alsodes pehuenche*) - Ecoparque Buenos Aires, Argentina

Borja Baguette Pereiro, Gabriela Díaz, Liliana Moreno, Vanesa Pellegrini, Andrés Capdevielle

The critically endangered Pehuenche frog (Alsodes pehuenche) is endemic to the meltwater streams of the central Andes in Argentina and Chile, occupying barely 4.8 km², entirely outside protected areas. Adults are almost entirely aquatic and endure extreme conditions: from summer water temperatures of 19°C to winter ice and intense UV radiation. Tadpoles take at least four winters to metamorphose, and the species faces multiple threats: predation by introduced trout species, road construction and winter



de-icing salts, climate-driven droughts and flash floods, livestock impacts, growing tourism, and the presence of chytrid fungus. Despite ongoing *in situ* conservation efforts, monitoring in 2023-2024 documented a population decline, prompting the launch of an *ex situ* conservation program.

With AArk's three-year grant, the Ecoparque Buenos Aires team will establish a captive-breeding program to secure a genetically diverse *ex situ* population while continuing habitat restoration and trout-exclusion trials. Year 1 will focus on outfitting facilities and collecting founders from several streams to preserve genetic diversity; Year 2 on refining husbandry and initiating breeding; and Year 3 on expanding the captive population, maintaining genetic health, and identifying release sites for future reintroductions.





2025 Grantees

Ex situ conservation of five endemic amphibians in Guatemala - La Aurora National Zoo, Guatemala

Rowland Kingsley Griffin, Jennifer Michelle Hernandez Muralles

Guatemala is a mega-diverse country with 146 amphibian species, of which 99 are threatened with extinction, making it the country with the second-highest proportion of endangered amphibians in mainland Latin America. As part of AArk's 2024 Conservation Needs Assessment (CNA) for Guatemalan amphibians, we worked with local experts and identified 11 species urgently needing *ex situ* conservation. Yet, no *ex situ* projects currently exist. This new initiative addresses that gap by launching the country's first amphibian breeding research facility.

Habitat loss and degradation are the main threats to all species proposed for this project. We can mitigate these effects by working with local NGO and private reserves.





Habitat protection efforts are already underway through these partners, and these will be the locations for future reintroductions.

With AArk's three-year grant, La Aurora National Zoo and partners will first gain husbandry expertise using two analog species (*Bolitoglossa helmrichi* and *Plectrohyla guatemalensis*), then expand to three focal species: *B. morio, B. kaqchikelorum,* and *Craugastor inachus.* Year 1 will focus on training with Detroit and St. Louis Zoos and designing the new facility; Year 2 will focus on the husbandry and breeding attempts of analog species, collecting founders, and refining care protocols; and Year 3 will include the addition of *B. morio* and *B. kaqchikelorum*, first breeding attempts of *Craugastor inachus*, and potential analog releases.







Craugastor inachus
© Alejandra Zamora



Bolitoglossa kaqchikelorum © Manuel Acevedo



Ex situ conservation for the critically endangered tree-frog Nyctimantis pomba

Cybele Sabino Lisboa | AArk Brazil National Coordinator

In 2019, we launched a conservation program to develop a captive maintenance and breeding protocol for *Nyctimantis pomba*. The primary goal of *ex situ* management for this species is to establish an insurance population while *in situ* threats are being addressed. Additionally, captive-bred individuals may support future population reinforcement efforts. Beyond these conservation roles, *ex situ* management also plays a key part in education, training, and scientific research, particularly by generating much-needed data on the species' reproductive biology, which remains largely unknown.

To initiate the captive breeding program, we received financial support from Amphibian Ark in both 2019 and 2021. The *ex situ* population was first established at the Conservation Center of Fauna of São Paulo State (CECFau), and by 2024, a successful breeding protocol had been developed. The population has since expanded, with some individuals transferred to the São Paulo Zoo and Butantan Institute.

As the number of frogs grew, expanding our facilities became essential to meet their care requirements. In response to this need, we secured a third AArk grant in 2024, aimed at acquiring additional equipment and materials

to support the growing *ex situ* population.

Program updates

By now, the *ex situ* population counts with 280 individuals distributed across three institutions: the CECFau (237), São Paulo Zoo (33), and the Butantan Institute (10). The populations are managed through ZIMS for studbooks.

In the field, an expedition supported by Stiftung Artenschutz brought together researchers from Federal University of São Paulo (UNIFESP), CECFau, and the Federal University of Viçosa (UFV) to monitor the species and advance an ongoing study of its thermal ecology.





Strategic planning and policy for this species also advanced. In June 2025, ASG Brazil coordinated a monitoring meeting to review progress and chart the next steps for the species' conservation plan (PECAn - Nyctimantis pomba). Two months later, Brazil's federal biodiversity agency RAN/ICMBio officially published the Nyctimantis pomba Population Management Program, formally endorsing the ex situ efforts coordinated by all the members of this project.

Next steps

The team's immediate priorities are to allocate the remaining grant funds for additional laboratory equipment, optimize the breeding terrarium at São Paulo Zoo to encourage reproduction, and finalize the husbandry guidelines for this species.





A Leap Forward: CRIA's 1,000th Toadlet Marks Conservation Success

Margarita Lampo | Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales; Instituto Venezolano de Investigaciones Científicas
Onil Ballestas | Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales; Instituto Venezolano de Investigaciones Científicas
Katiuska González | Instituto Venezolano de Investigaciones Científicas
Ingrid Márquez | Universidad Central de Venezuela
Jhon Espinoza | Universidad Central de Venezuela
Isabella Lara | Universidad Simón Bolívar
Ana Sofía González | Universidad Metropolitana
Samuel Beomon | Zoológico Leslie Pantin
Federico Pantin | Zoológico Leslie Pantin
Tuenade Hernández | Zoológico Leslie Pantin

In march 2025, the Centro de Reproducción e Investigación de Arlequines (CRIA) reached a major milestone: the emergence of its 1,000th captive-born toadlet, healthy and strong. This achievement represents a significant step in the conservation program launched in 2022 to safeguard the Rancho Grande Harlequin Toad (Atelopus cruciger), a critically endangered species endemic to Venezuela. The species disappeared from most of its range in the late 1980s and, despite frequent searches in its former habitats, is currently known only from two populations.

Following nearly two decades of research on the relict populations, we launched a <u>conservation action plan</u> with six main goals:

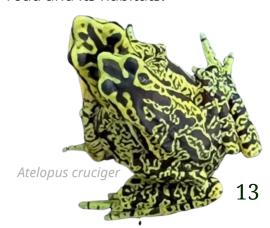
- (1) secure an assurance colony
- (2) increase the number of extant populations and expand the species' current distribution

by reintroducing captive-bred individuals into suitable sites within their historical range; (3) mitigate threats by prioritizing lowland habitats for reintroduction and strengthening protected area management to maximize

(4) search for additional, as yet undetected populations;

juvenile recruitment;

(5) engage local communities and integrate them into harlequin toad conservation efforts; and (6) raise awareness at local, national, and global levels about the Rancho Grande Harlequin Toad and its habitats.





CRIA began in 2022 with 22 founder toads and a grant from Amphibian Ark. Two 16 m² facilities were built, providing capacity for 280 adults and six breeding pairs/tadpole rearing units. Early challenges included inadequate egg-laying conditions and cases of spindly leg syndrome. By refining tank design, improving water quality, and optimizing diets, we significantly increased breeding success, survival rates, and the overall health of the toadlets. Medical histories, husbandry data, and studbooks for all founders and captive-born individuals are maintained in the Zoological Information Management System (ZIMS).

With the support of trained volunteers and assistants, we developed Guidelines for the Husbandry and Breeding of the Rancho Grande Harlequin Toad, available and

regularly updated <u>here</u>. Today, CRIA maintains an assurance colony of more than 200 adults from 13 parental lines, along with over 300 juveniles and F_2 toadlets that have just begun emerging.

Reintroduction trials began in 2024, starting with the release of 207 tadpoles in January, followed by 200 adults in April 2025. Since reintroductions in *Atelopus* species are still highly experimental, and no self-sustaining populations of reintroduced individuals have been documented, our strategy prioritizes systematic data collection to guide adaptive management. All juveniles and adults were photographed, measured, and weighed before release. Dorsal photographs provide a unique record of individual markings, enabling reliable identification during post-release monitoring. In a subset of individuals,





passive reflectors (RECCO®) were externally attached to track their movements after release. We designed and implemented semienclosed mesocosms to limit dispersion and promote establishment at the release site. These structures also facilitate post-release monitoring by increasing the likelihood of recaptures and enabling the use of capture histories to estimate survival, track body condition, and monitor Bd infection status of released individuals. As of 25 of August, 2025, more than 15 adults released in April and May of this year have survived, maintaining good body condition and showing no evidence of Bd infection.

None of these advances would have been possible without the sustained support of Amphibian Ark, whose initial investment and continued funding provided the foundation for infrastructure, husbandry protocols, and the first successful breeding and reintroduction efforts for this species. Their partnership remains essential to securing a future for the Rancho Grande Harlequin Toad in the wild.



Captive-born juveniles of the Rancho Grande Harlequin Toad (*Atelopus cruciger*) at the Centro de Reproducción e Investigación de Arlequines (CRIA). © Jaime Culebras





Clarifying the protocol for the successful rearing of Ambystoma dumerilii larvae

Rodolfo Pérez Rodríguez | Universidad Michoacana de San Nicolás de Hidalgo, México

The salamander *Ambystoma dumerilii* is a paedomorphic amphibian, microendemic to Lake Pátzcuaro in central Mexico, and has historically been subject to traditional exploitation in the region. Today, the species faces a suite of pressures: overharvesting, pollution, land-use change, the introduction of exotic species, global warming, and the emergence of infectious diseases. These have led to a severe decline in its natural populations.

Under this scenario, *ex situ* management has become a key strategy for its conservation. However, the early larval stage has been identified as a critical period, during which survival rates rarely exceed 50%, primarily due to inadequate diet, poor water quality, extreme temperatures, and a high incidence of density-related aggression. Therefore, it is imperative to establish standardized protocols and best husbandry practices to improve survival and ensure optimal larval development.

Since receiving the AArk grant in 2024, the team has made notable advances:

- 1. Survival rates range from 85% to 95% by the fifth week of life, coinciding with the first dietary transition.
- 2. An appropriate diet was established for the first three months.

- 3. Water quality was improved through the use of filtration and aeration, leading to a reduction in nutrient concentrations.
- 4. A water quality monitoring system was consolidated, establishing weekly monitoring frequency, minimum maintenance volume, and a 25% water exchange rate during the first five weeks of larval stage.
- 5. Pathogen diagnostics using real-time PCR detected the presence of *Batrachochytrium dendrobatidis* in juveniles, whereas *B. salamandrivorans* and Ranavirus tested negative.

Collaborations

Collaboration with local fishers from San Jerónimo Purenchécuaro has been key for *in situ* monitoring of natural populations in Lake Pátzcuaro and for planning soft releases that will follow IUCN translocation protocols. The team is also considering a rescue program for eggs incidentally caught in fishing nets, which





would be reared under controlled conditions.

International support continues as well: Dr. Jaime Bosch of the Spanish National Research Council provides ongoing technical assistance and pathogen diagnostics. Building on this momentum, the project has been incorporated into Mexico's "Participatory Action Plan for the Socio-environmental Restoration of the Lake Pátzcuaro Basin", led by SECIHTI (Mexican Government).

Next steps

The focus now shifts to refining larval management and scaling up capacity. The team will determine optimal larval densities for the 7-8 week pre-juvenile stage, when activity and aggression increase, testing structural enrichment such as substrates to reduce encounters and injuries. A prototype recirculating aquaculture system will be designed and constructed to accommodate larger numbers of larvae, reduce daily

maintenance demands, and conserve water while maintaining high water quality and growth rates. Finally, comprehensive sanitary protocols will be implemented to eradicate Bd and monitor for other pathogens such as helminths and protozoans. A veterinary specialist in amphibians will guide diagnostic procedures, preventive measures, and treatments.

With these targeted actions, the project aims to strengthen captive-breeding success, reduce disease risks, and create the capacity needed to support reintroduction efforts and secure the long-term survival of this iconic Mexican salamander.





Advances in *ex situ* conservation of *Hyloscirtus tigrinus* (Anura: Hylidae)

Diego P. Almeida-Reinoso | Laboratorio de Herpetología, Departamento de Biología, Facultad de Ciencias, Escuela Politécnica Nacional; Centro de Conservación de Anfibios SAR-RANA

L x situ conservation of endangered amphibians is presented as an emergency measure in the face of the accelerated decline of many amphibian species around the world. Since 2005, it has gained significant momentum with the creation of the first ex situ conservation centers, with Ecuador being one of the first countries to adopt this measure in response to the amphibian crisis.

Hyloscirtus tigrinus was described in 2008 for Colombia (Mueses-Cisneros et al. 2008) and is listed as an endangered species. The first record of the species for Ecuador was reported in 2012 (Coloma et al. 2012), where it was listed as critically endangered. However, according to the IUCN, H. tigrinus is today listed as endangered.

Conservation efforts began in 2013 with the support of the AArk grant to the Gustavo Orcés Herpetological Foundation in Quito. Given the difficulty in finding adults, tadpole rescue has been the only means to initiate conservation actions for this species.

Unfortunately, after the first attempts at breeding and handling larvae under laboratory conditions, the first individuals died shortly after metamorphosis due to chytridiomycosis—despite receiving treatment against the fungus *Batrachochytrium dendrobatidis* (Bd). These observations were reported in the AArk newsletter by

Garzón and Almeida-Reinoso, 2014. That same year, the SAR-RANA Center for the Conservation of Amphibians at Risk of Extinction was established, with an emphasis on the ex situ conservation of the tiger-banded torrent frog. Since then, a juvenile collected as a tadpole in Quebrada Corazón, Sucumbíos Province, has been housed. Its ontogenetic development and changes in coloration patterns have been documented (Almeida-Reinoso and Proaño-Viteri. 2017).





In 2019, an adult female individual was rescued and admitted to SAR-RANA, and continues to be housed there to this day.

On December 10, 2022, 10 Hyloscirtus spp. tadpoles were rescued for ex situ conservation purposes from the only locality in Ecuador known to date. The individuals were found during the day, at three different points at an average altitude of 2,675 meters above sea level, and in various stages of development. The youngest was at stage 28 (Gosner, 1960) with a total length of 70 mm and the most advanced stage being stage 30 (Gosner, 1960) with a total length of 100 mm. The tadpoles were transported to the SAR-RANA facilities as part of the ex situ conservation program for endangered amphibians in the city of Quito. They were maintained in individual 10gallon aguariums with an open water inlet and outlet system, with an average water temperature of 16.5°C (max. 22°C and min. 11°C). The aquarium water was renewed 50% every five days. The tadpoles were fed daily with food exclusively for herbivore and detritivore tadpoles called SAR type I

(Super Tadpole Food) (Almeida-Reinoso, 2008; Garzón and Almeida-Reinoso. 2014; Almeida-Reinoso et al., 2023) and raised until metamorphosis. Under these conditions, the last tadpole to complete metamorphosis was transferred to a smaller container on January 26, 2024, to complete tail resorption. Once their tails were completely reabsorbed, the metamorphs were transferred to individual terrariums.

All juveniles received preventative treatment against chytridiomycosis using a 0.01% itraconazole solution bathing protocol, modified and adapted exclusively for this species (protocol reported by Almeida -Reinoso & Proaño 2017).

Five of the tadpoles completed their metamorphosis at the end of August 2023, eight months after being rescued. However, these specimens turned out to be Hyloscirtus pantostictus, a species sympatric to Hyloscirtus tigrinus. In their early stages, the tadpoles of the two species are very similar, making them difficult to differentiate. However, as they develop, certain differences can be detected in the pattern and coloration: a silver iris and the presence of golden dots on the lateral area of the body and on the caudal fin of *H. tigrinus* (Figure 1), or a gray iris and a brown body mottled with gray in *H. pantostictus* (Coloma et al. 2012).



Figure 1. Tadpoles of *Hyloscirtus tigrinus* (Gosner 41) SAR-R 0011 © Diego P. Almeida-Reinoso



The adults of the two species are completely different. The remaining five tadpoles turned out to be *H. tigrinus* and completed their larval development in an average of 12 months (Table 1). The larval development time of *H. pantostictus* is shorter than that of *H. tigrinus*, even when both species were maintained under the same conditions.

Currently, 19 months after metamorphosis, three juvenile *H. tigrinus* remain (Figure 2). One Gosner stage 42 tadpole of *H. tigrinus* died before completing the reabsorption of its tail, and another juvenile died three weeks after completing metamorphosis. The two individuals entered the scientific collection of the National Polytechnic School,

along with the five juvenile *H.* pantostictus that were preserved.

Preliminary results

The three surviving juvenile *H. tigrinus* are kept in individual outdoor terrariums enriched with plants, logs, and sphagnum moss. They have a mesh top that allows rainwater or scheduled irrigation during dry days to enter. The mesh top allows the entry of ultraviolet rays from the sun, which are essential for the frogs' absorption of calcium and vitamin D.

The structure, design, and layout of the terrariums, as well as their internal conditions, maintain an ideal temperature and humidity for the frogs even on the hottest







Figure 2. Juveniles of *H. tigrinus*. A) SAR-R 010; B) SAR-R 011; C) SAR-R 013 © Diego P. Almeida-Reinoso

Table 1. Ex situ development table in SAR-RANA, of Hyloscirtus tigrinus and H. pantostictus tadpoles.

Species	No. Individual	Rescue date	Entry to SAR-RANA	Condition	Metamorphosis completed	Current state
H. pantostictus	S-R-0017	10.Dic.2023	12.Dic.2023	Medium tadpole, without hind legs	02.Jul.2023	Preserved juvenile
H. pantostictus	S-R-0018	10.Dic.2023	12.Dic.2023	Small tadpole, without hind legs	03.Sep.2023	Preserved juvenile
H. tigrinus	S-R-0010	10.Dic.2023	12.Dic.2023	Medium tadpole, without hind legs	07.Nov.2023	Living juvenile
H. tigrinus	S-R-0011	10.Dic.2023	12.Dic.2023	Medium tadpole, without hind legs	10.Nov.2023	Living juvenile
H. tigrinus	S-R-0012	10.Dic.2023	12.Dic.2023	Medium tadpole, without hind legs	24.Nov.2023	Preserved juvenile
H. tigrinus	S-R-0013	10.Dic.2023	12.Dic.2023	Medium tadpole, without hind legs	16.Nov.2023	Living juvenile
H. tigrinus	S-R-0014	10.Dic.2023	12.Dic.2023	Small tadpole, without hind legs	/V 12 11 1/	Tadpole in Gosner 41, preserved



days, with outside temperatures exceeding 25°C.

The frogs are fed every three days with a varied diet of invertebrates (crickets, moths, mealworms, and woodlice). The mainstay of the live food is crickets (two species), which are dusted with calcium and vitamin D3 supplements once a week before being placed in the terrariums. Intake of these enriched crickets prevents bone malformations and general osteopenia in the frogs (Whitaker B. and Yaw T. 2021).

Since Hyloscirtus tigrinus was first reported in Ecuador in 2008, only six adult wild individuals have been observed in the country to date. Only one of these has been rescued and transferred to SAR-RANA. However, several tadpoles (now juveniles) have been rescued, allowing the first ex situ colony of the species to be established. A total of five individuals remain: one adult female rescued in 2019; one male raised ex situ from its larval stage, rescued in 2014; and three juveniles rescued as tadpoles in 2022.

We first heard the male's song in May 2024, so we assume that the individual rescued in 2014 took ten years to reach adulthood.

Conclusions

Hyloscirtus tigrinus is among the longest-lived anuran species known to date, with metamorphosis times of more than a year at average temperatures of 16°C in ex *situ* conditions. The average water temperature in its habitat ranges between 5 and 9°C, leading us to assume that in the wild, an individual could remain a tadpole for several years before metamorphosis and would take several more years to reach the reproductive adult stage. This extremely long developmental time further compromises the status of its populations and constitutes another cause that puts the species at risk in the wild. Therefore, ex situ conservation is, for now, the best option to ensure the survival of the species and avoid its imminent local extinction.

Ex situ management is an effective tool for the conservation of at-risk amphibians, like *H. tigrinus*.

Since the first trials in 2013, we have learned several aspects of its development, demonstrating the continuous ontogenetic changes as it transitions from juvenile to sub-adult to adult. However, a viable ex situ conservation program requires action planning that includes a minimum of 15 years of work, the securing of funding, increased infrastructure, ex situ conservation infrastructure. and the commitment of several national stakeholders. including academia, local government, and central government. Likewise, the action plan for the conservation of the species must contemplate in situ actions, such as the recovery of its habitat, training and education of people in the localities where the species is found, eradication of exotic species such as the trout (Oncorhynchus mykiss) that, together with logging, loss of forest, expansion of agriculture and livestock, and emerging diseases such as chytridiomycosis, are the main causes that threaten the existence of the tiger-striped torrent frog in Ecuador.



Acknowledgments

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First record of successful reproduction of the mountain stream axolotl under professional care: a substantial advance for its *ex situ* conservation

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mong vertebrates, amphibians are the most threatened with extinction according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2025). Among the nearly 9,000 species described worldwide to date, one group in particular has sparked interest in both academia and the general public in the last decade: axolotls, tailed amphibians belonging to the genus *Ambystoma*. There are 18 species of this genus inhabiting Mexico, but, unfortunately, 17 are classified as endangered according to national environmental legislation (Ávila-Akerberg et al., 2021). There are various threats that

these organisms face, such as the excessive growth of urban sprawl and changes in land use, in addition to the introduction of exotic species, pollution, and illegal trade, among others (Frías-Álvarez et al., 2010; SEMARNAT, 2018; IUCN, 2025). These threats normally intensify when the species are subjected to pressure factors in megalopolises such as Mexico City, where there are still records of wild specimens of three species of Ambystoma: Mexican axolotl (A. mexicanum), altiplano axolotl (A. velasci), and mountain stream axolotl (A. altamirani) (CONABIO, 2016; Lemos-Espinal & Smith, 2020).

In the case of the Mexican

axolotl, research has achieved notable success, from the knowledge of the characteristics of its habitat. its conservation status in the wild, and its management in captivity (SEMARNAT, 2018). In contrast, knowledge about the living conditions, behavior, and management of the mountain axolotl is scarce, despite it being listed as endangered according to CONABIO (2025) and classified as Endangered according to NOM-059 (DOF, 2010). Studies with wild populations have increased in the last decade, but management under professional care is almost nonexistent.



Because of this, the local government, through the Ministry of the Environment (SEDEMA), designed the "Strategy for the Comprehensive Conservation of the Mountain Axolotl (Ambystoma altamirani Dugès, 1895) in Mexico City," which has involved key stakeholders from academia and civil society organizations.

Among the components of this strategy is *ex situ* conservation, through which the first active Wildlife Conservation Management Unit (UMA) was designed and implemented for the maintenance of colonies under professional care of A. altamirani (Figure 1), which was inaugurated on September 1, 2022 with authorization and registration key DGVS-UMA-IN-1014-CDMX/2021. Following the request and obtaining of the extractive use permit from the General Directorate of Wildlife (DGVS) of the Ministry of Environment and Natural Resources (SEMARNAT), a colony was formed for its maintenance within the UMA, which is located within the Desierto de los Leones National Park; a protected natural area co-managed by the local government through the General Directorate of the System of Protected Natural Areas and Areas of Environmental Value and the federal government through the National Commission of Protected Natural Areas (CONANP), which is located within the natural distribution area of the species, west of Mexico City.

During the first year of operation, species management presented significant challenges due to the lack of available information. Documented data from specialized literature and testimonies from previous working groups indicated that maintenance under confined conditions was unfeasible.

Initial experience confirmed

this trend, as several specimens failed to survive, a situation that persisted despite the availability of specialized personnel, including biologists and veterinarians with experience in herpetofauna. In the second half of 2023, and with the accumulated experience of the staff, a first phase of standardized protocols was consolidated. These quidelines began to be formally applied at the UMA, currently known as the "El Pantano Conservation Center."

That same year, due to the lack of successful colony reproduction and the considerably reduced number of available specimens, a strategy was designed and implemented to stimulate the





natural reproduction of the species, avoiding the use of assisted reproduction mechanisms such as hormonal induction.

The strategy included the following actions: the spectrum of food items offered was expanded, incorporating macro- and microinvertebrates previously identified as part of the species' natural diet under free-living conditions, and the frequency of food supply was doubled. In addition, a progressive transition was made in the environmental enrichment of the enclosures, transforming them from conventional aquariums to aquaterrariums, attempting to simulate as closely as possible the conditions of their natural habitat. These included live vegetation and four types of sediments present in the species' ecosystem (silt, sand, gravel, and rock) (Figure 2).

Preventive medicine protocols were also established, including periodic checkups to detect physical abnormalities, somatometric monitoring (measuring individual growth over time), deworming, and other measures. Finally,

during the reproductive period (cold season), two females and one male were placed in a 200-L aquaterrarium. Individuals were monitored at least three times a week, during which water quality parameters, behavioral data, and signs of reproductive activity were recorded.

In January 2024, the first courtship behaviors were reported, in which atypical movements of the male with respect to one of the females were observed: always remaining behind her and bringing his head very close to the female's tail and cloaca for several moments (this process is known as the mating ritual and it is commonly said that the female and the male dance,

executing these and other types of movements between them). A few weeks later, the presence of spermatophores (small whitish cone-shaped packets containing sperm and normally characteristic of axolotls) was recorded. Spermatophores are naturally placed on the rocky or plant substrate and are subsequently absorbed by the female through the cloaca to fertilize her oocytes. Spermatophores were observed arranged on sand and rocks. However, we were unable to record the exact moment at which these packets were absorbed by the female (this process usually takes place a few hours after the spermatophores are arranged).

Six days after this recording,





103 eggs were laid (Figure 3) with a fertility rate greater than 90% (9 eggs were nonviable, also known as abortive). Embryonic development lasted approximately two and a half months, from the time the female laid the eggs until the hatching of the young (Figure 4 and 5). Subsequently, total length and wet weight of the specimens were monitored bimonthly (Figure 6).

Currently, we have annual information on the development of size and survival of the young. To our knowledge, this event represents the first reproductive success of the species under professional care, an important achievement for the *ex situ* conservation of this species classified as Endangered by the IUCN.

Finally, within the framework of the mountain stream axolotl conservation project, narrated reproduction is a strategic tool for preserving wildlife resources in the city, while also advancing the conservation of a species included since 2014 as part of the List of Priority Species and Populations for Conservation in Mexico.



Figure 3. Female of *A. altamirani* next to the eggs she deposited underneath the rocky substrate.

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Figure 4. Monitoring the embryonic development under professional care.

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Figure 5. Newly hatched *Ambystoma altamirani* under professional care. © José Juan Torres Anguiano



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Figure 6, left: team member measuring the length of the young with a Vernier. Right: team member measuring the weight of the young of *A. altamirani* with a scale. © Valeria Alejandra Perdomo Zúñiga

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Vertical gardens: an alternative microhabitat for amphibians at the Santa Cruz Zoo Foundation

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🕇 ristimantis renjiforum (Endangered), Rheobates palmatus (Least Concern), and Dendropsophus padreluna (LC) are three endemic species to Colombia which are part of the conservation and education program at the Santacruz Zoo Foundation. The initial ex situ conservation program for *Pristimantis sp* at the Santacruz Zoo Foundation received an AArk grant in 2015, and today this program includes more species and combines education, ex situ, and in situ conservation. We care for these species with microhabitats favorable to their survival, adequate nutrition, medical checkups, and appropriate maintenance for each species.

From the moment they arrive or are born, each species receives the necessary management. Data such as hunting methods, use and preference of different spaces and substrates, activity cycles, and other information are obtained through behavioral monitoring, an important process for their adaptability and generating knowledge about the natural history of these species, which is scarce. Likewise, alterations in their natural behavior are detected, evaluated, and mitigated through improvements.

Microhabitats established in aquariums offer each individual resting areas, hiding places, hunting grounds, and other opportunities. It is important to provide different vegetation layers, as frogs require and utilize them depending on their activity, age, or species. As a result of the ethograms created by biology interns and/or volunteers, we identified the

need to increase plant stratification for all species. Different models of vertical gardens were designed and installed, and their acceptance or rejection was monitored.

Pristimantis renjiforum, an arboreal species, preferred vertical gardens placed on the walls, designed with PVC pipes and plants, while ignoring the models located in the center of the aquarium or those that did not have contact with any of the walls. A clear sign of this preference was the successful egg-laying observed within eight days after the installation, followed by parental care and the full development of the embryos until hatching. Individuals of this species currently use the upper part of established gardens more consistently than the substrate vegetation.



Dendropsophus padreluna preferred the garden model that completely covered one wall of the aquarium, made with a mesh with tree bark. plants, and moss. Like the previous species, they ignored the gardens located in the center of the aquarium. They spent most of their time in these wall gardens, which provide good shelter, and they only descend to the substrate to hunt for food. Although they place their eggs in the water, they anchor them to the wall garden to prevent them from floating away.

Rheobates palmatus was the only species that selected gardens made of PVC pipe with plants placed in the center or without contact with walls, utilizing the lower or middle parts, but rarely the upper parts. This corroborates that this species prefers low substrate and very little tall vegetation. It is important to emphasize that gardens for this species should have abundant, thick, and preferably hanging vegetation. They use gardens for resting, hiding, and hunting, although they prefer to lay eggs on the moss used as a substrate underneath abundant vegetation that



Left: *Pristimantis renjiforum.* Right: vertical garden model preferred by *P. renjiforum*—PVC pipes and plants near the walls. © FZS



Left: *Dendropsophus padreluna*. Right: vertical garden model preferred by *D. padreluna*—bark and moss wall with plants © FZS



Left: *Rheobates palmatus.* Right: vertical garden model preferred by *R. palmatus*—PVC and plants, away from walls © FZS

allows them to hide, rather than in the gardens.

Through behavioral observations carried out by students and volunteers that support our staff at the Foundation, we have

documented how these frog species use the different vertical gardens, which structures they prefer to use and occupy, and how these features contribute to their well-being. This information has guided enclosure



adjustments to enhance the adaptability of each species and improve overall management conditions, as described in this article.

We continue to implement improvements that support the conservation of amphibians in our program, aiming for positive outcomes and sharing these results with visitors and followers through various channels.



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