



Amphibian Ark

Rescuing amphibians in crisis



Telmatobius culeus © D. Alarcón/D. Grunbaum

December 2025

Newsletter No. 72, ISSN 2640-4141



This giving season, you can make all the difference between “too late” and a second chance for the most threatened amphibians.



Your generosity helps us mobilize the *ex situ* conservation programs these species urgently need: prioritizing the most at-risk species, working with partners in range countries, and providing specialist training and funding where it matters most.

With your help, we can continue rescuing amphibians in crisis.

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Amphibian Ark

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Amphibian Ark News

Welcome to Issue No. 72 of the Amphibian Ark Newsletter!

Here's what's been keeping us busy this quarter:

New IUCN Resolution highlights the urgency for amphibian conservation financing

At the 2025 World Conservation Congress, a new IUCN Resolution was approved which calls on the urgent need for governments and donors to step up with coordinated action and long-term investment to prevent further amphibian extinctions.

For Amphibian Ark, the new IUCN resolution acknowledges the essential contribution and need of effective *ex situ* programs to amphibian recovery. Around 25% of threatened amphibian species require *ex situ* conservation. Since 1980, *ex situ* measures have helped decrease the extinction risk of 63 amphibian species, and nearly 100 additional threatened species are now being maintained in assurance colonies.

We recently co-authored a Conservation Note in *Oryx* magazine about this resolution: you can [find it here](#).

"The IUCN's call to action comes at a critical moment. Many amphibians are teetering on the brink of extinction. As the most threatened of all vertebrates, they need urgent support. By urging governments and funders to step up, the IUCN is drawing welcome attention to one of the world's most pressing conservation crises."

— Jonathan Wilcken
Executive Director of Amphibian Ark



Oryx

Adoption of new IUCN resolution guiding the conservation of amphibians

Published online by Cambridge University Press: 14 November 2025

Amaël Borzée , Sally Wren , Caroline Lees ,
Gina della tognà , Janice Chanson , Jonathan Wilcken ,
Louise Hobin , Pria N. Ghosh  and Simon N. Stuart

[Read more here](#)



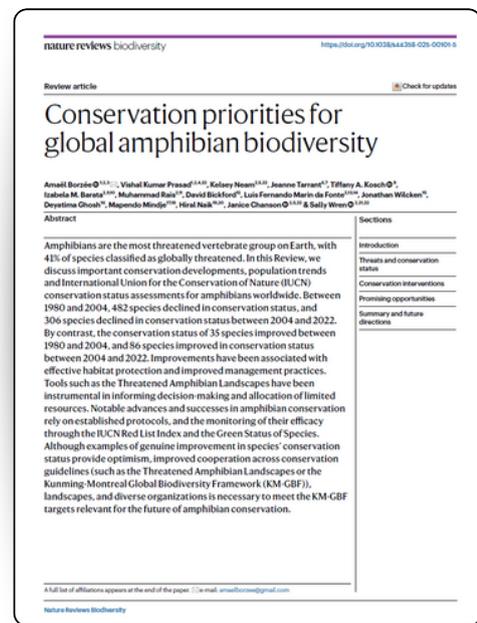
Amphibian Ark News

A new global synthesis highlights the funding gap for amphibian conservation and the measures that can keep amphibians afloat

A recent publication in Nature Reviews Biodiversity delivers a clear and urgent message: past conservation efforts and their results are a source of hope, but the ongoing amphibian crisis demands additional tools—and addressing its widening funding gap.

While past efforts have helped some species recover, nearly 40% of amphibians remain threatened—yet the gap between what's needed and what's currently funded seems to be widening. The paper details important changes in amphibian conservation since 1980, including threats and their potential solutions.

The paper also reinforces a familiar message to Amphibian Ark's mission: *ex situ* conservation breeding remains essential, especially for species facing imminent extinction before threats in the wild can be reduced.



“Conservation breeding can be an effective stopgap in the face of urgent threats (...) before meaningful threat mitigation can occur.”

— Borzée et al. 2025

[Read more here](#)



Amphibian Ark News

Have we normalized the amphibian crisis?

This October, our Executive Director, Jonathan Wilcken, spoke at the WAZA 80th Annual Conference in Cali, Colombia, highlighting a sobering trend: amphibians remain the world’s most threatened vertebrate group, yet they receive only a small fraction of the conservation attention and funding they urgently need. This imbalance is also reflected in internet search trends: while interest in biodiversity conservation has increased over the past 15 years, interest in amphibian conservation has steadily waned.

As Jonathan notes, Amphibian Ark and other amphibian conservation organizations share similar concerns about the global support for amphibians.



Has the world forgotten about the amphibian crisis? Help us spread the word by watching and sharing this video with your networks and keeping amphibians top of mind!

Thanks to Cali Zoo and WAZA, we have been able to make this presentation available for you to watch on our YouTube channel.

[Watch it here](#)

In case you missed it: our website is now available in Spanish!



Is there another language we should consider? We’d love to hear from you! Email us at info@amphibianark.org and let us know which languages would best support your work and community.

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***Telmatobius* action planning workshop: *ex situ* will play an important role for the conservation of the Andean water frogs**

Luis Carrillo | Amphibian Ark

Patricia Mendoza-Miranda | Co-coordinator ASG-Telmatobius Task Force and Bolivian Amphibian Initiative

Luis Castillo | Co-coordinator ASG-Telmatobius Task Force and Grupo RANA

Currently, there are 61 species described of Andean water frogs (genus *Telmatobius*) distributed along the high Andes of Argentina, Bolivia, Chile, Ecuador, and Peru. Unfortunately, 85% are threatened with extinction, placing them as the second most threatened amphibian genus worldwide.

In October 2025, the *Telmatobius* Task Force organized a three-day multi-species action planning workshop, hosted by the Universidad Andrés Bello in Santiago de Chile. The workshop was facilitated by the IUCN SSC Conservation Planning Specialist Group (CPSG) and brought together 47 people from different sectors: field biologists, private sector, NGOs, academia, government,

and communities.

One of the main goals identified during the workshop was to increase the number of *ex situ* conservation centres for *Telmatobius* species. Currently, there are just three institutions managing *ex situ* conservation programs for *Telmatobius* species within the region: two in Chile (Zoológico Nacional-Parquemets and Centro de Biodiversidad y Conservación El Loa, holding one species), and one in Bolivia (Centro K'ayra-Museo de Historia Natural Alcide d'Orbigny, holding five species). The Bioparque Municipal Vesty Pakos-Hampaturi also receives and manages *Telmatobius* species, especially *Telmatobius culeus*, mainly for exhibition and education.





In preparation for this workshop, the IUCN Amphibian Red List Authority (ARLA) and Amphibian Ark (AArk) carried out joint assessment to establish the current conservation category (ARLA) and the conservation needs of all the species within the genus. During the AArk's Conservation Needs Assessments, 35 species were identified in need of *ex situ* conservation as one of the tools to achieve the effective conservation of these species.

The two most *Telmatobius*-diverse countries—Peru and Bolivia—are also the countries with a greater number of species identified for *ex situ* rescue (fig. 1). Sadly, when analyzing the percentage of species per country recommended for *ex situ* rescue, we identified a very high proportion of Ecuadorian and Bolivian species (Fig. 2).

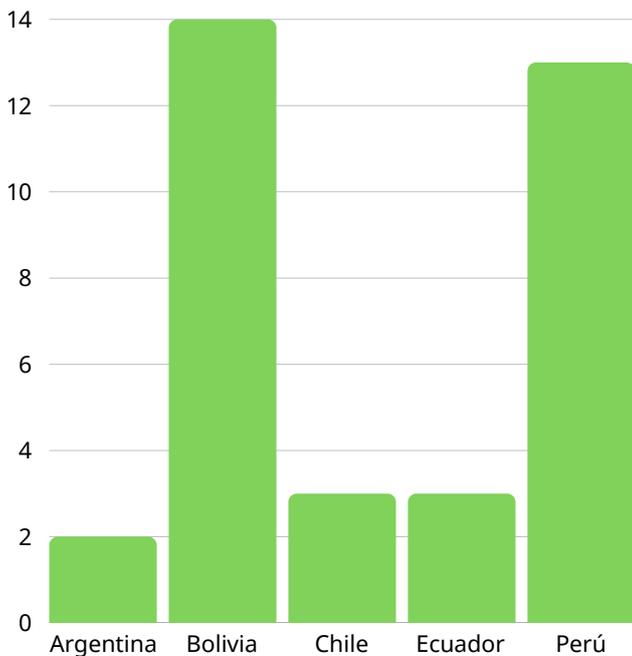


Figure 1. Number of species identified for *ex situ* conservation per country. Bolivia (14) and Peru (13) have the larger number of species identified as in need of *ex situ* rescue.

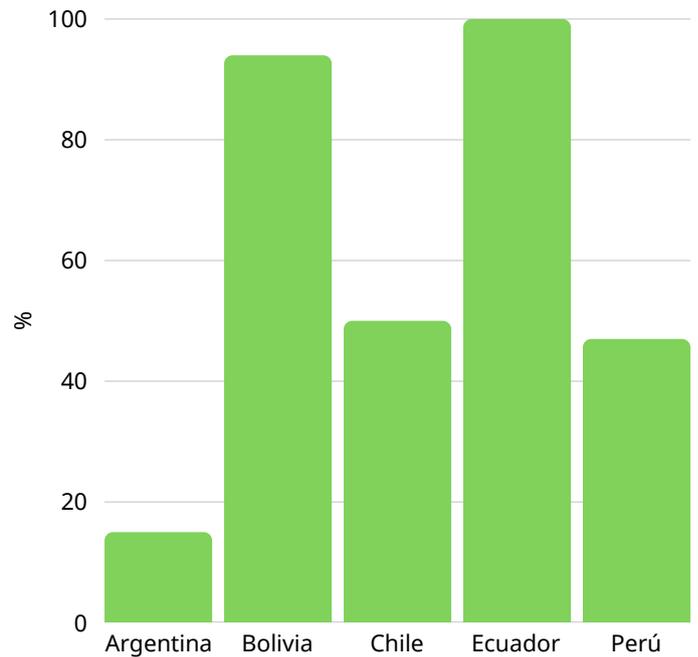


Figure 2. Percentage of *Telmatobius* species within each country that have been recommended for *ex situ* rescue through AArk's CNA.



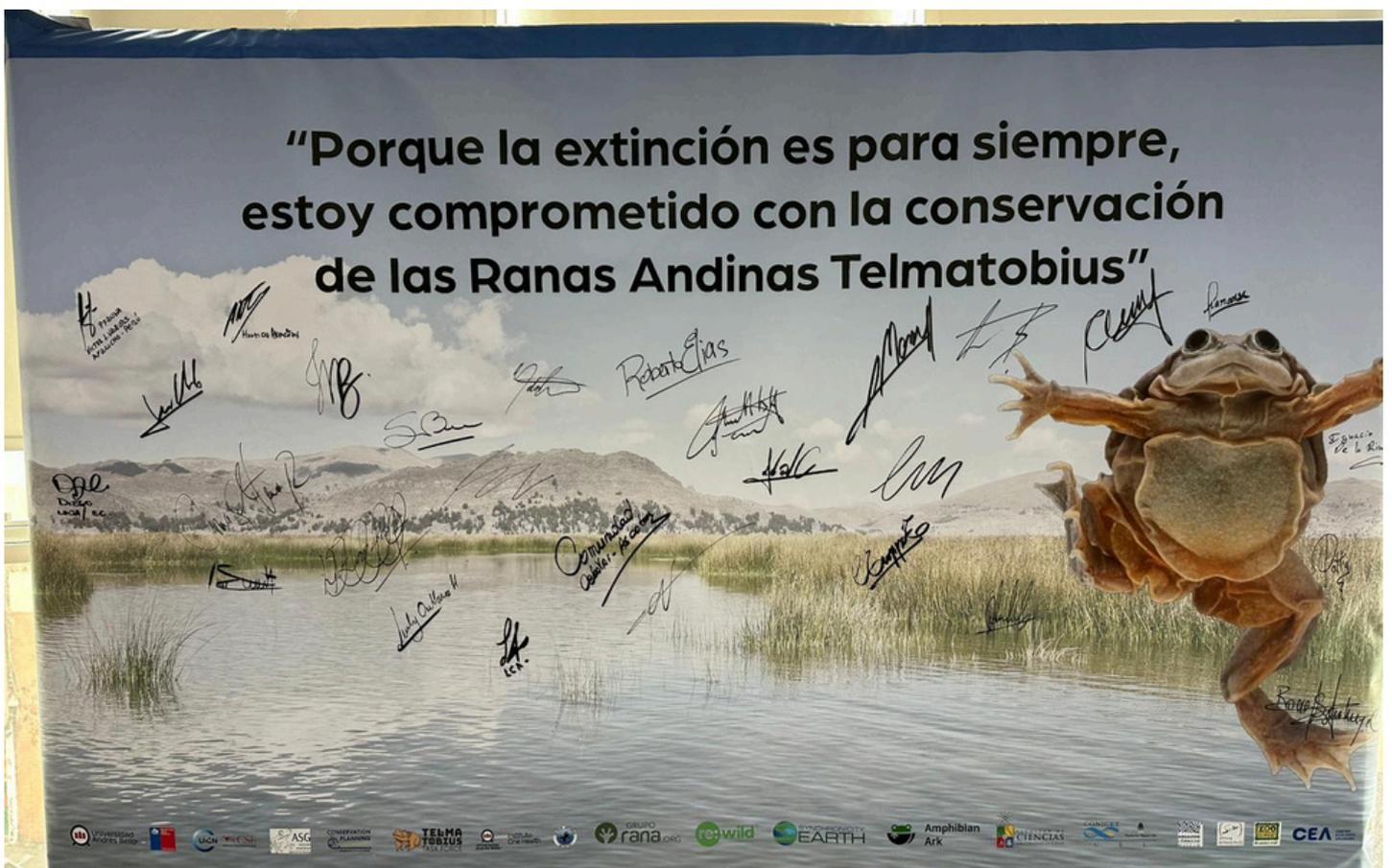
Figure 3. Participants of the multi-species planning workshop at Universidad Andrés Bello, Chile.



As presented above, on average more than half (60%) of the assessed *Telmatobius* species for their conservation needs require *ex situ* support (Rescue). Amphibian Ark has previously supported all the current *ex situ* *Telmatobius* programs either with funding or specialist advice and technical guidance.

We remain committed to keep supporting these and new *ex situ* components of conservation programs for these magnificent frogs!

AArk has supported Centro K'ayra in Bolivia through veterinary guidance, grant-writing support, and access to ZIMS (thanks to the generous donation form Species 360); the Centro de Biodiversidad y Conservación El Loa in Chile through veterinary guidance; and the Zoológico Nacional in Chile through an emergency grant for the *ex situ* establishment of rescued individuals of *Telmatobius halli* (formerly *T. dankoi*).





Building on top of the ASI's HarleCAP: multi-species conservation planning for Colombian *Atelopus* species

Beatriz Velásquez | Amphibian Ark

Luis Carrillo | Amphibian Ark

Luis Marin Fonte | Atelopus Survival Initiative

From October 23-26, the workshop for the formulation of an Action Plan for the Conservation of Colombia's Harlequin Toads (*Atelopus*) was held in Cali, Colombia. It was organized by Amphibian Ark and Atelopus Survival Initiative, in partnership with the IUCN Conservation Planning Specialist Group and the Amphibian Specialist Group - Colombia. It took place within the framework of the 2025 CPSG Annual Meeting, and brought together 30 participants—experts, researchers, officials from national environmental authorities, NGOs, institutions dedicated to *ex situ* management, and members of local communities where *Atelopus* populations still exist—from 19 different institutions.

The objective of this action plan is to define strategies for Colombia that will enable the achievement of the goals set forth in the Harlequin Toads Conservation Action Plan (HarleCAP), proposed as a planning tool for the conservation of *Atelopus* species throughout their distribution range over the next 20 years.

Thanks to the facilitation of CPSG and the implementation of its planning approach, the broad 5 goals established in HarleCAP were reviewed, analysing their progress, relevance to the country, and whether there is a need to propose new objectives in accordance with the national context. Subsequently, the objectives were prioritized through a voting





system, considering their feasibility within a five-year timeframe, as outlined in the action plan for Colombia.

Four lines of action were prioritized:

1. Data policy and strengthening of ReCRAC (*Red de Conservación de Ranas Arlequín de Colombia*).
2. National program for the search for lost species.
3. *Ex situ* breeding as a conservation tool.
4. Identification, prevention, and mitigation of threats.

Based on these prioritized objectives, participants were then divided into four working groups according to their expertise, experience, and thematic affinity. Each group was tasked with identifying potential projects to be implemented in Colombia and developing them by defining the necessary actions, points of contact, collaborators, and timelines. The proposed projects are:

Project 1: National Program for the Search of Lost Species

Description: A coordinated national program to rediscover and document *Atelopus* species through museum research, fieldwork, prioritized surveys, integration of genomic tools, threat assessments, and long-term monitoring.

Summary of Objectives: Rediscovery of lost species; Taxonomic and systematic studies; Threat evaluation for remnant populations; Natural history and behavioural data; Genomic banking; Financial sustainability strategies; and Integration with institutional mandates and national policy.

Project 2: Identification, Prevention, and Mitigation of Threats and Their Effects

Description: Identify key threats and develop, implement, monitor, and evaluate mitigation strategies across Colombia.

Summary of Objectives: Characterize threats affecting *Atelopus*; Assess population status; Evaluate disease dynamics and interactions with other threats; Restore key habitats; Develop innovative responses to emerging diseases; Strengthen public awareness and policy alignment.

Project 3: Ex Situ Reproduction as a Conservation Strategy

Description: Strengthen *ex situ* breeding capacity for *Atelopus* in Colombia, including infrastructure and expertise.

Summary of Objectives: Build national capacity for *ex situ* programs; Integrate *ex situ* knowledge into *in situ* management.

Project 4: Data Policy and Strengthening of ReCRAC

Description: Develop a national data-use policy aligned with Colombian regulations to remove barriers, fill knowledge gaps, promote collaborations, and reestablish ReCRAC, the Colombian Harlequin Toad Conservation Network.

Summary of Objectives: Establish data-sharing guidelines; and Reactivate ReCRAC as a collaborative platform.



A direct product for this workshop was the facilitation of inter-institutional collaboration and allowed for the reactivation of ReCRAC, an initiative that emerged a few years ago and is comprised of individuals and organizations working collaboratively to improve the conservation status of Harlequin toads in Colombia. Its work seeks to foster knowledge exchange, promote technical collaboration, and raise awareness of the high risk of extinction faced by this group of amphibians.

This Action Plan constitutes a roadmap that will guide actions aimed at protecting *Atelopus* toads in Colombia over the next five years. Furthermore, it is expected to be linked to the National Amphibian Conservation Program, currently under review by the Ministry of Environment, to contribute to the conservation of one of the most threatened amphibian groups in the world.



Atelopus laetissimus © Duvan F. Zambrano





Strategic Amphibian Conservation Plan (PECA_n) for *Melanophryniscus setiba*, a Threatened Brazilian Toad

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Mariana Pontes | Universidade Estadual de Campinas; Instituto Boitatá; Melanophryniscus Task Force (IUCN SSC Amphibian Specialist Group)

Quezia Ramalho | Departamento de Ecologia, Universidade de São Paulo; IUCN SSC Amphibian Specialist Group Brazil

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Iberê Machado | Instituto Boitatá; IUCN SSC Amphibian Specialist Group Brazil

Iago Junqueira | The Wild Place; IUCN SSC Amphibian Specialist Group Brazil

Janaína Serrano | McGill University, Department of Biology; IUCN SSC Amphibian Specialist Group Brazil

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Melanophryniscus setiba is a micro-endemic species known only from a small restinga ecosystem at the Paulo César Vinha State Park, located in the state of Espírito Santo, Brazil (Peloso et al., 2012). Although the species occurs within a protected area, it is exposed to increasing pressures from expanding tourism, surrounding land development, and recurrent wildfires. Due to its small geographic range and significant threats, the species is listed as Critically Endangered (CR) by the IUCN Red List and classified as Vulnerable (VU) in the current Brazilian assessment (IUCN Red List; MMA, 2022; Bastos et al., 2023). Despite its discovery and formal description in 2012, research groups have only recently conducted *in situ* studies focusing on its distribution, habitat use, ecology, and threats, alongside education and outreach initiatives within the state park.

In 2020, *M. setiba* was assessed through Amphibian Ark's Conservation Needs Assessment (CNA). The results highlighted a set of conservation priorities for the species, including *in situ* conservation, *in situ* research, education strategies and the establishment of an *ex situ* response, as *M. setiba* was classified as a rescue priority species. Following these recommendations, the *ex situ* conservation program for *M. setiba* was initiated in 2024 as a partnership between Amphibian Ark and the Museu Biológico of the Instituto Butantan, with formal support from the *in situ* research team. The program became one of the first institutional initiatives integrated into Amphibian Ark's Regional Coordination Program in Brazil. In this structure, Amphibian Ark assists the program by providing strategic guidance, technical support,





and alignment with international best practices, while the Museu Biológico leads the operational implementation of the program, including husbandry, monitoring, protocol development, and day-to-day coordination.

The *in situ* research team contributes by supplying essential biological and ecological information, ensuring that *ex situ* decisions are informed by field knowledge and conservation priorities. In 2024, the program also received partial financial support through an Amphibian Ark grant, and two breeding pairs have been maintained at the Museu Biológico, supporting the development of species-specific husbandry protocols and contributing to a better understanding of reproductive biology, behavior, health, diet, growth, and development.

Although these studies and initiatives have significantly advanced our knowledge and conservation efforts, important gaps remain, especially regarding key biological traits and threat mitigation strategies. For this reason, the Brazilian regional Amphibian Specialist Group (ASG Brazil) initiated the development of a Strategic Amphibian Conservation Plan (PECAn) specifically dedicated to the species.

ASG Brazil has successfully led two previous PECAn processes for *Nyctimantis pomba* and *Pithecopus rusticus* (Vaz et al. 2024). These processes have proven valuable in helping researchers identify knowledge gaps, prioritize next steps, coordinate responsibilities, and strengthen collaboration.



Melanophryniscus setiba
© José Felipe Batista da Silva

The *M. setiba* PECAn process began with a series of preparatory meetings between ASG Brazil and Mariana Pontes, the *in situ* focal researcher and head of the Sapo Setibinha-da-restinga project. On November 24th, 2025, ASG Brazil, supported by Amphibian Ark, the Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios (RAN) of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), and Instituto Boitatá, held a full-day workshop.

This workshop brought together approximately 30 participants from universities, governmental institutions, NGOs, environmental agencies, and conservation networks. The group included representatives from the Instituto Estadual de Meio Ambiente e Recursos Hídricos (IEMA) and managers of the Paulo César Vinha State Park, as well as researchers, independent specialists, students, and other conservation practitioners. During this session, stakeholders consolidated all current information on the species. They then discussed remaining needs in research, natural history, threat assessment, education, and stakeholder engagement.



One of the actions formally included in the plan is the continuation and strengthening of the *ex situ* program as a core component of conservation. The plan emphasizes the need to maintain a strong connection between the *ex situ* husbandry program and the *in situ* field data. Rather than being treated as separate or competing lines of action, the *ex situ* and *in situ* approaches are understood as complementary and interdependent. The knowledge generated in the field informs and improves the development of the *ex situ* program. In contrast, observations of the animals under human care generate new perspectives and questions for *in situ* studies. Together, both strands guide decision-making, optimize protocols, and support long-term planning, including the potential future reinforcement of wild populations, if scientifically justified.

The PECAn represents an important milestone for *M. setiba*. It strengthens the connection between government agencies, *in situ* and *ex situ* stakeholders, and institutions, thereby creating a unified strategic vision and providing a structured path to guide research, management, education, and institutional coordination. The immediate next steps for this PECAn include consolidating all material generated during the workshop, finalizing the action plan, and publishing it so that partners, institutions, and stakeholders can easily access the current information on the species. The published document will also present actions that currently lack assigned coordinators or require initiation, thereby fostering collaboration, transparency, and shared responsibility within the conservation community.



Participants of the PECAn workshop for *Melanophryniscus setiba*, held on November 24th, 2025



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Building Capacity for Amphibian Health: Highlights from Brazil's First Conservation Medicine Workshop

Renata Ibelli Vaz | AArk Brazil National Coordinator

Cybele Sabino Lisboa | AArk Brazil National Coordinator

Luis Carrillo | AArk Program Director for the Americas & the Caribbean

Brazil holds the greatest amphibian diversity on the planet, yet specialists continue to warn that more species are becoming threatened every year. While zoos, research centres, and NGOs have made enormous progress in recent years in *ex situ* conservation, one particularly persistent barrier in Brazil remains the scarcity of veterinarians trained to work specifically with amphibians under human care.

To start changing this scenario, a unique initiative from Amphibian Ark (AArk), the Amphibian Specialist Group (ASG) Brazil, the São Paulo Zoo, and The Wild Place was launched in August 2025: an intensive three-day workshop designed to build and

strengthen the country's capacity for amphibian *ex situ* conservation medicine.

The first Amphibian *ex situ* Conservation Medicine Workshop, hosted by São Paulo Zoo, brought together 22 veterinarians and early-career professionals from several Brazilian states, as well as colleagues from Chile and Argentina. Supported by a US\$2,500 AArk grant and funds from each involved institution, the workshop was designed to allow participants to immerse themselves in the world of amphibian health.

Over three intensive days, the program featured 17 presentations delivered by a diverse team of national and international





experts, creating an opportunity for participants to interact closely with professionals who are actively engaged in amphibian research, clinical medicine, field conservation, and program coordination. The speakers included veterinarians from the São Paulo Zoo (Fabrício Rassy, Carolina Nery, and Robertta Nogueira), partner veterinarians Miriam Halász Vac and Ticiane Zwarg, Iago Junqueira (The Wild Place/ASG Brazil), and international specialists Dr. Allan Pessier (Washington State University) and Dr. Luis Carrillo (Amphibian Ark). Additional talks were delivered by Renata Ibelli Vaz (ASG Brazil/AArk), Cybele Sabino Lisboa (São Paulo Zoo/ASG Brazil/AArk), and Prof. Dr. Felipe Toledo (University of Campinas – UNICAMP).

On day 1, the workshop opened with an overview of global amphibian conservation and the role of Amphibian Ark, followed by updates on amphibian conservation efforts in Brazil. Presentations explored essential topics such as amphibian welfare, anatomy, physiology, and clinical examination. The afternoon concluded with discussions of case studies and program presentations from participants, which encouraged knowledge exchange and peer learning.

The second day focused on the major diseases affecting amphibians in *ex situ* environments. Instructors addressed non-infectious conditions, chytridiomycosis, and other important infectious diseases. Participants then moved to diagnostic approaches, including imaging (X-ray and ultrasound), microbiology, and hematology. A guided visit to the SP Zoo Veterinary Hospital allowed participants to see the diagnostic

workflow in practice, followed by hands-on demonstrations with real clinical cases.

The final day explored anesthesia, surgical procedures, and the fundamental role of quarantine in *ex situ* programs. Participants visited “Pulo do Sapo,” the zoo’s dedicated amphibian exhibit, and other facilities, gaining insight into husbandry practices, environmental control, and long-term management strategies. The workshop concluded with a practical clinical examination, ultrasound, and X-ray, using specimens from the zoo collection, ensuring participants had the chance to apply newly learned clinical and diagnostic techniques in a guided, supportive setting.

A key highlight of the workshop was the enthusiasm and engagement of the participants. Many arrived with backgrounds in wildlife rehabilitation, zoo medicine, academia, or early-stage conservation projects. Others were motivated by a longstanding interest in amphibians but lacked opportunities for practical training. The workshop provided a space where experience, curiosity, and scientific rigor could meet.

At the end of the workshop, participants completed anonymous evaluation forms assessing each topic and instructor. Participants highlighted the diversity of perspectives and the balance between theory and practice as key strengths of the event. Many expressed a desire for an even longer edition in the future, especially to expand the practical components. One recurring suggestion was to extend the course by at

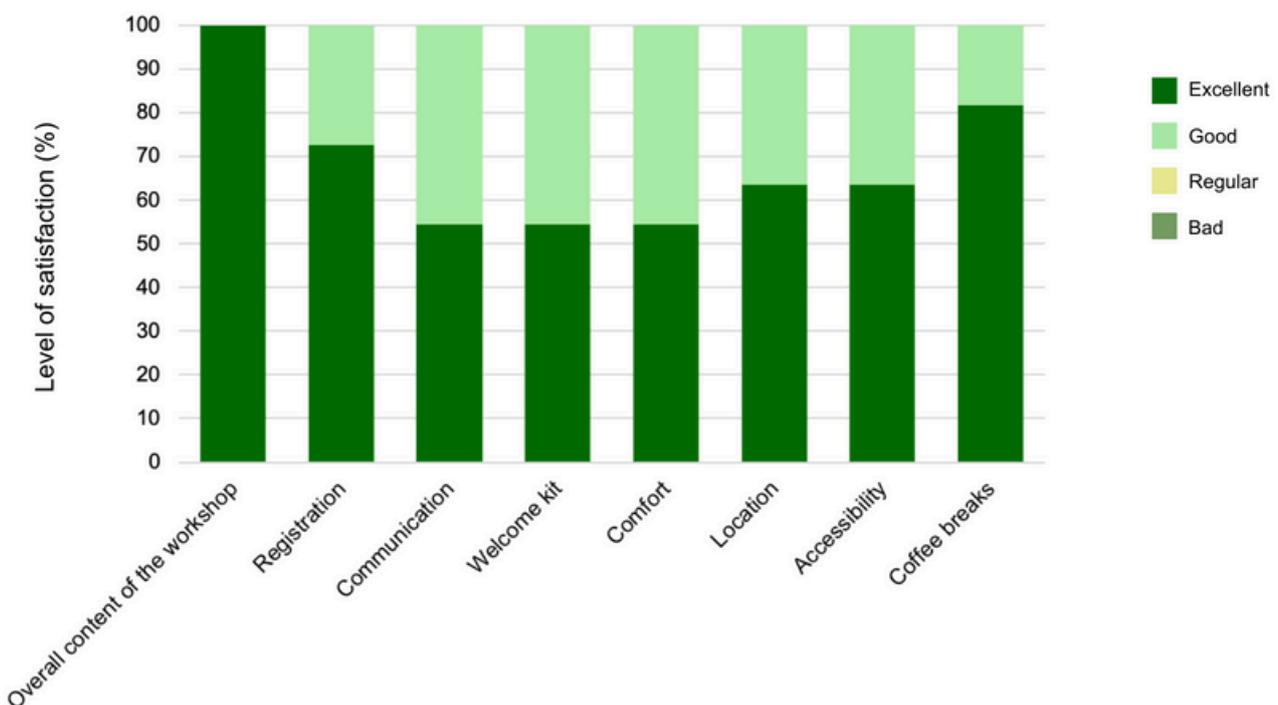


least one extra day, particularly to allow more time for hands-on procedures such as imaging, necropsy, anesthesia, and sample collection. Participants described the workshop as “better than expected,” “exceeded expectations,” and “essential for anyone working with amphibian health,” highlighting in particular “the remarkable diversity of veterinary professionals participating.”

Thanks to efficient financial management and in-kind support from São Paulo Zoo and The Wild Place, a small positive balance remained after the workshop, which is being used to purchase veterinary and husbandry equipment for AArk partner institutions, including Parque das Aves, CECFAU, BioParque do Rio, São Paulo Zoo, and Museu Biológico do Butantan. Tools such as micropipettes, water-testing kits, and humidifiers will strengthen the daily work of teams caring for amphibians across the country.

This gesture also reinforced the sense of collective effort: training is only one part of capacity building, and equipping institutions is another. By reinvesting the remaining funds in Brazil’s *ex situ* network, the workshop created a small but meaningful legacy that extends beyond the classroom.

The first Amphibian *ex situ* Conservation Medicine Workshop marked an important step toward building the specialized expertise required to safeguard Brazil’s amphibians. By bringing together experienced professionals, early-career veterinarians, passionate students, and international experts, the event helped bridge critical gaps in clinical knowledge, diagnostic skills, and professional confidence. As Brazil continues to develop new *ex situ* programs and strengthen existing ones, the need for trained veterinary professionals will only grow.





Workshop Sessions

Average score





Vesty Pakos Municipal Biopark: its contribution to the conservation of two rescued *Telmatobius* species and its role as a natural refuge for native amphibians in La Paz, Bolivia

Daniela Morales Moreno | Bolivian Amphibian Initiative

Fortunato Choque Baptista | Bioparque Municipal Vesty Pakos

Luis Enrique Beltrán | Bioparque Municipal Vesty Pakos

Álvaro Antonio Quispe Flores | Bioparque Municipal Vesty Pakos

Omar Rocha | Bioparque Municipal Vesty Pakos

Grace Ledezma | Investigadora independiente

Javier Aruquipa | Bioparque Municipal Vesty Pakos

Patricia Mendoza-Miranda | Co-coordinador ASG-Telmatobius Task Force & Bolivian Amphibian Initiative

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The genus *Telmatobius* contains some of the most emblematic and threatened amphibian species in the Andes. Among them, the Titicaca giant frog (*Telmatobius culeus*), endemic to the Lake Titicaca water system, faces severe pressures from pollution, habitat degradation, the introduction of exotic species, illegal trafficking for human consumption, and the presence of chytrid. In this context, the Vesty Pakos Municipal Biopark (BMVP), a wildlife sanctuary located

south of La Paz, Bolivia, has been rehabilitating and caring for amphibians rescued from vulnerable situations, such as illegal trade, since 2018, within the framework of an *ex situ* conservation program.

Furthermore, the BMVP serves as a natural refuge for amphibian species of the Inter-Andean Dry Forests ecoregion, contributing to their *in situ* conservation at a local level in the face of habitat loss due to urban sprawl.

The conservation path of *Telmatobius* rescued at the Vesty Pakos Biopark

Between 2018 and 2025, the biopark received a total of 78 individuals belonging to two *Telmatobius* species on 12 separate occasions: *Telmatobius culeus* (N=71) and *Telmatobius cf. marmoratus* (N=7). These individuals, rescued from markets, fairs, and restaurants, entered the program in critical condition: overcrowding, poor water quality, severe stress, and fair to poor health. This was



reflected in poor body condition, skin lesions, ectoparasites, and high bacterial loads, factors that resulted in high mortality rates. In fact, *T. culeus* showed the greatest vulnerability, with a mortality rate of 49.3%, attributed primarily to the deterioration they experienced prior to rescue.

As a first step in the conservation program, specialists from the Bolivian Amphibian Initiative (BAI)—

one of the program's main partners—worked with the biopark's technical team and wildlife caretakers to strengthen their knowledge of amphibian natural history, management, and care. Secondly, an area was specifically prepared for the husbandry of rescued amphibians by a team of professionals in veterinary medicine, biology, and animal care.

In 2020, the BMVP obtained

approval from the National Environmental Authority for the "Program for the Management and Conservation of Amphibians at the Vesty Pakos Municipal Biopark, La Paz, Bolivia." This program encompasses all the pillars for the *ex situ* conservation of this vulnerable group (management, maintenance, and education). Currently, the Biopark houses 30 individuals of *T. culeus* and 7 individuals of *T. cf. marmoratus*, which



One of the 30 *Telmatobius culeus* specimens housed at the BMVP, currently located in one of the four aquariums in the exhibition area. © Arturo Muñoz



are under controlled conditions and biosecurity protocols that include initial clinical evaluation, nutritional management, and behavioral monitoring.

Knowing to conserve: environmental education to raise awareness among visitors

To support the educational and awareness-raising efforts for visitors, we created an area with one exhibit aquarium in 2019. This was renovated in 2024 with the addition of three additional aquariums. This area, which features the giant frog, is a strategic space for disseminating information about the species and the threats it faces in its natural habitat. So far in 2025, the BMVP has received an average of 13,289 visitors per month. The interpretive displays, aquariums, and complementary educational activities allow for communicating issues such as pollution, illegal trafficking, and the importance of conserving high-Andean amphibians. The aim is to raise awareness and foster positive feelings toward the species among visitors, making them part of the

solution for amphibian conservation.

The Biopark as a natural refuge for native amphibians of the La Paz valley

In addition to *ex situ* conservation efforts, the Biopark plays a vital role as a reservoir of herpetological diversity native to the dry inter-Andean valley. Within the Biopark, four native species have been recorded that regularly utilize the water bodies, vegetation, and available microhabitats:

- *Gastrotheca marsupiata* (marsupial frog): indicator of humid microhabitats and shrubby vegetation; its reproduction depends on the maternal dorsal sac and shallow ponds.

- *Rhinella spinulosa* (Andean spiny toad): terrestrial species adapted to arid environments; controller of invertebrate populations.
- *Pleurodema cinereum* (four-eyed frog): associated with temporary ponds; sensitive to the loss of urban wetlands.
- *Boana riojana* (Andean tree frog): closely associated with bodies of water; active day and night; easily recognizable by its metallic call; feeds on insects and arachnids.

The La Paz Valley region has suffered intense urban pressure, loss of wetlands, and pollution, drastically reducing habitats for these species. The BMVP (Bolivian Biosphere Reserve), by



Javier Aruquipa, head wildlife warden of the amphibian area at the BMVP
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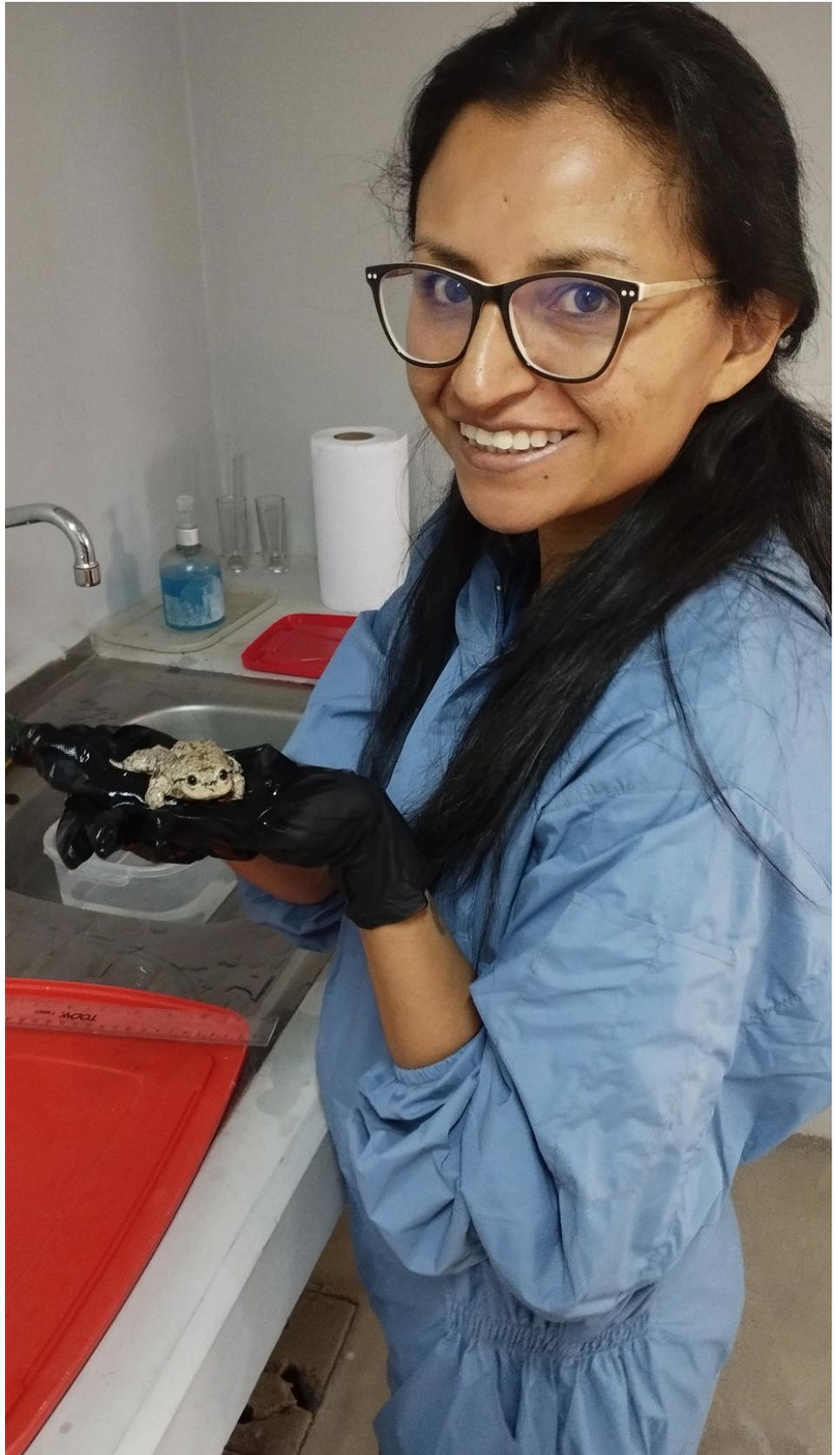


maintaining permanent bodies of water, suitable temporary pools during the rainy season, native vegetation, and shaded microhabitats, constitutes an urban refuge of high ecological value, with suitable habitats for reproduction, contributing to the local persistence of these populations.

This dual role—*ex situ* for critically endangered species and *in situ* refuge for native species—positions the Biopark as a model of conservation integrated into Andean urban landscapes. Furthermore, it is a strategic site for increasing and promoting the conservation of its fauna through environmental education.

Potential and challenges of the amphibian conservation program

Over the seven years of the amphibian conservation program at the BMVP, the technical knowledge for managing the species has been acquired, along with the creation of strategic alliances with institutions such as BAI and BAGO Laboratories of Bolivia S.A., which provide technical and financial



Daniela Morales Moreno, PhD, during the physical evaluation of one of the 37 amphibians housed at the BMVP © Diego E. Maldonado Velarde



support for amphibian care, respectively. Furthermore, the continuous health monitoring by the BMVP's professional team facilitates immediate attention when needed.

The main challenges in the short and medium term include:

1. The health stabilization of *T. culeus* after its admission, due to the severe pre-rescue deterioration, and the increase in infrastructure and personnel for a growing population.
2. The need for continuous improvement of biosecurity protocols, infectious disease diagnosis, and diversification of food handling. Regarding this last point, a veterinary student is currently working on the production of other live foods (fish, snails, and *Hyalellas*).
3. Incorporation of the Biopark into in situ conservation actions of *Telmatobius* species.
4. The management of suitable microhabitats for native species, especially during long dry periods.
5. The integration of environmental education with applied conservation actions.

Conclusions

The experience of the Vesty Pakos Municipal Biopark, with technical advice and support from BAI and the collaboration of BAGO Laboratories of Bolivia S.A., demonstrates that *ex situ* conservation is a vital tool for species threatened by illegal trafficking, such as *Telmatobius culeus*. At the same time, the Biopark's recognition as a natural refuge for native amphibians



Educational activity in the information sector about the four amphibian species that naturally inhabit the BMVP. © Daniela Morales Moreno



of the dry valley expands its ecological and educational value, reinforcing its role in the conservation of local herpetofauna.

This integrated approach, comprised of *ex situ* management, *in situ* refuge, and environmental education, demonstrates the commitment of the BMVP and its partners to biodiversity conservation at the local and regional levels. This work involves informing, raising awareness, and mobilizing

the population to reduce the pressures affecting amphibians, especially those impacting the genus *Telmatobius* in Lake Titicaca.

As we consolidate and expand these conservation efforts, we invite organizations, institutions, or individuals interested in collaborating to strengthen our work to establish scientific, training, or operational support partnerships that contribute to the protection of Andean

amphibians. Please feel free to contact us, visit the BMVP website, and [follow us on social media](#).

Exhibition area for the Titicaca giant frog, with the four aquariums set up for individuals of the species. © Daniela Morales Moreno





Successful cryopreservation of amphibian embryonic cells in a common species, striped marsh frog (*Limnodynastes peronii*) and application to the endangered Littlejohn's tree frog (*Rawlinsonia littlejohni*)

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Cryopreservation can prevent the loss of genetic diversity from declining populations and extinctions, allow the long-term storage of genetic material, and reduce costs for captive breeding programs (Kouba, 2022). The cryopreserved genetic material can be thawed and used to recover live individuals via in vitro

fertilization, nuclear transfer (NT), germ line chimeras, intracytoplasmic sperm injection, and artificial insemination (Andrabi & Maxwell, 2007; Bolton et al., 2022). In amphibian conservation, cryopreservation has largely been limited to sperm (haploid genome), which has been successful in several endangered species (Clulow

et al., 2022). However, there are no effective protocols to cryopreserve whole amphibian eggs and embryos due to their large size and high yolk content (ranging between 1 mm to 42 mm in diameter) (Clulow et al., 2022). This prevents cryoprotectants from dehydrating the cells, which is necessary for successful cryopreservation (Clulow et



al., 2022). Previous attempts to cryopreserve whole amphibian eggs and embryos resulted in death due to the formation of lethal intracellular ice that occurs during freezing and thawing (Clulow et al., 2014; Derakhshan et al., 2017; Guenther et al., 2006; Wooi et al., 2018). On the other hand, mammalian oocytes and embryos can be successfully cryopreserved and recovered post-thaw due to their small size (75-200 micrometres in diameter) (Clulow et al., 2022; Prentice & Anzar, 2011).

An alternative approach is to cryopreserve dissociated embryonic cells, for use in NT to produce clones or the production of germ line chimeras, which can allow for

long-term storage of diploid genomes (Lawson et al., 2013). Cryopreserving dissociated embryonic cells has been successful in different species of fish and germ line chimeras have been produced with cryopreserved cells (Kusuda et al., 2002; Yoshizaki & Lee, 2018). Being able to cryopreserve amphibian embryonic cells would expand biobanking for amphibians by allowing the diploid genome to be stored and recovered (Clulow et al., 2022).

Despite *Xenopus laevis* and *Ambystoma mexicanum* being well studied amphibian models (Blum & Ott, 2019; Bölük et al., 2022), there are no reports of successful

cryopreservation of amphibian embryonic cells. Only one study has been conducted on amphibian embryonic cell cryopreservation in a non-model species (*Limnodynastes peronii*) using dimethyl sulfoxide (DMSO) or glycerol, with poor post-thaw recovery (Lawson et al., 2013).

In the current study, we used embryonic cells from different embryonic stages (blastula, gastrula, and neurula) from *L. peronii*, a common Australian ground frog that spawns frequently in captivity. Cells from three embryonic stages (blastula, gastrula, and neurula) were cryopreserved (with a slow freeze protocol) with 10% DMSO paired with different sucrose concentrations (0%, 1%, and 10%).

Cells from all embryonic stages showed significantly higher post-thaw recovery in terms of membrane integrity and cell concentration when cryopreserved with 10% DMSO and 10% sucrose. Gastrula and neurula cells had better recovery than blastula cells due to their smaller cell diameters. Based on the results with *L. peronii*, 10% DMSO and 10% sucrose



Figure 1. Left: Male striped marsh frog (*Limnodynastes peronii*) © Roshan Patel. Right: Littlejohn's tree frog (*Rawlinsonia littlejohni*) © Rose Upton

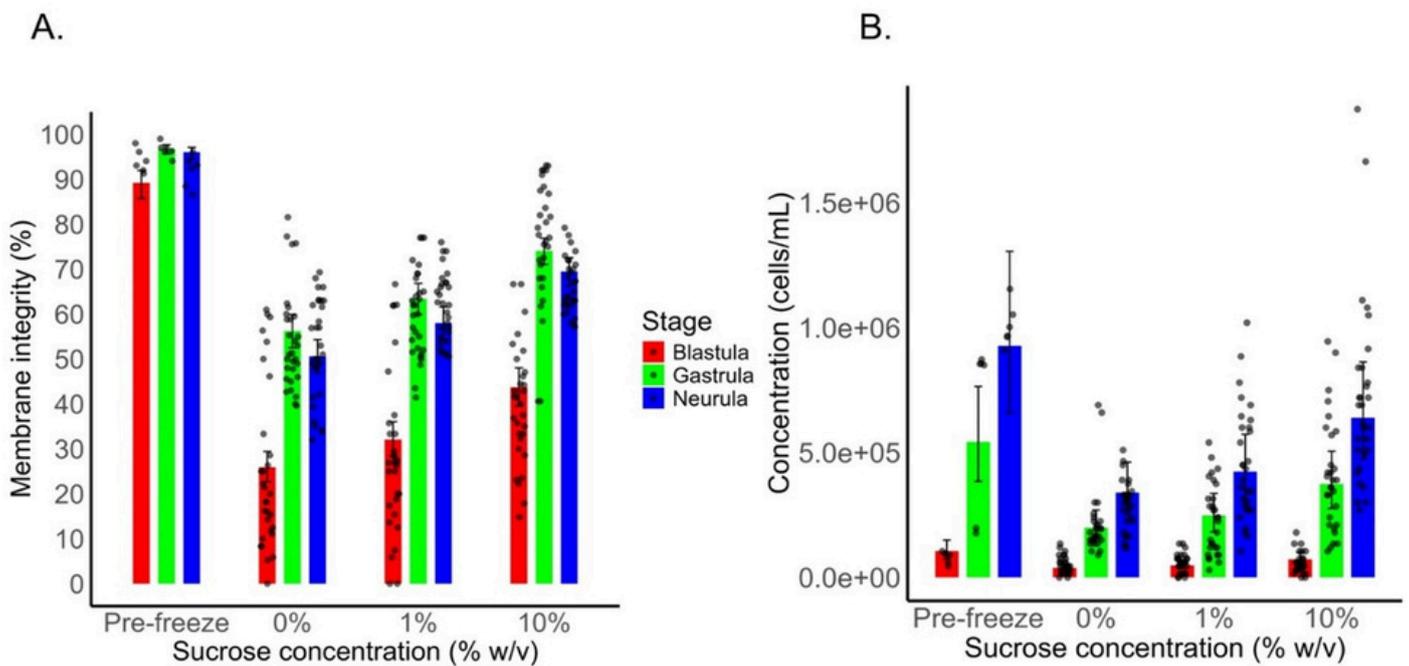


Figure 2: Pre-freeze and post-thaw for membrane integrity (2A) and cell concentrations (2B) for *L. peronii* embryonic cells that were cryopreserved with 10% DMSO paired with 0%, 1%, or 10% sucrose.

was used to cryopreserve neurula cells from *Rawlinsonia littlejohni*, formerly *Litoria littlejohni* (Donnellan et al., 2025), which is endangered (Upton et al., 2024).

Post-thaw recovery resulted in around 50% membrane intact cells (Figure 3). This represents the first report of intact embryonic cell recovery from a threatened amphibian, indicating that the approach may be valuable as a conservation tool for amphibian biobanking.

Future directions

Future research on amphibian embryonic cell cryopreservation should

investigate DNA and epigenetic alterations that happen during cryopreservation. The impetus for such studies reflects the fact that for NT to be successful with cryopreserved cells, the genome must be intact. Studies on fish germ cell cryopreservation have reported DNA damage and epigenetic alterations in post-thaw germ cells (Riesco et al., 2012; Riesco & Robles, 2013). Conducting similar studies on amphibian embryonic cells can help optimize the cryopreservation protocol through the use of additives, such as amino acids or polyamines, to reduce DNA

damage and epigenetic alterations (Kutluyer & Kocabas, 2016; Wang et al., 2025).

The next step is to establish consistent nuclear transfer (NT) protocols in non-model amphibian species using cryopreserved cells. Despite amphibian NT research being successfully conducted during the mid to late 20th century on many species of amphibians with high yields of clones being produced with embryonic cells, the goal of such studies was not conservation (Gurdon, 1962; Signoret et al., 1962). In the wake of these studies, amphibian NT declined and

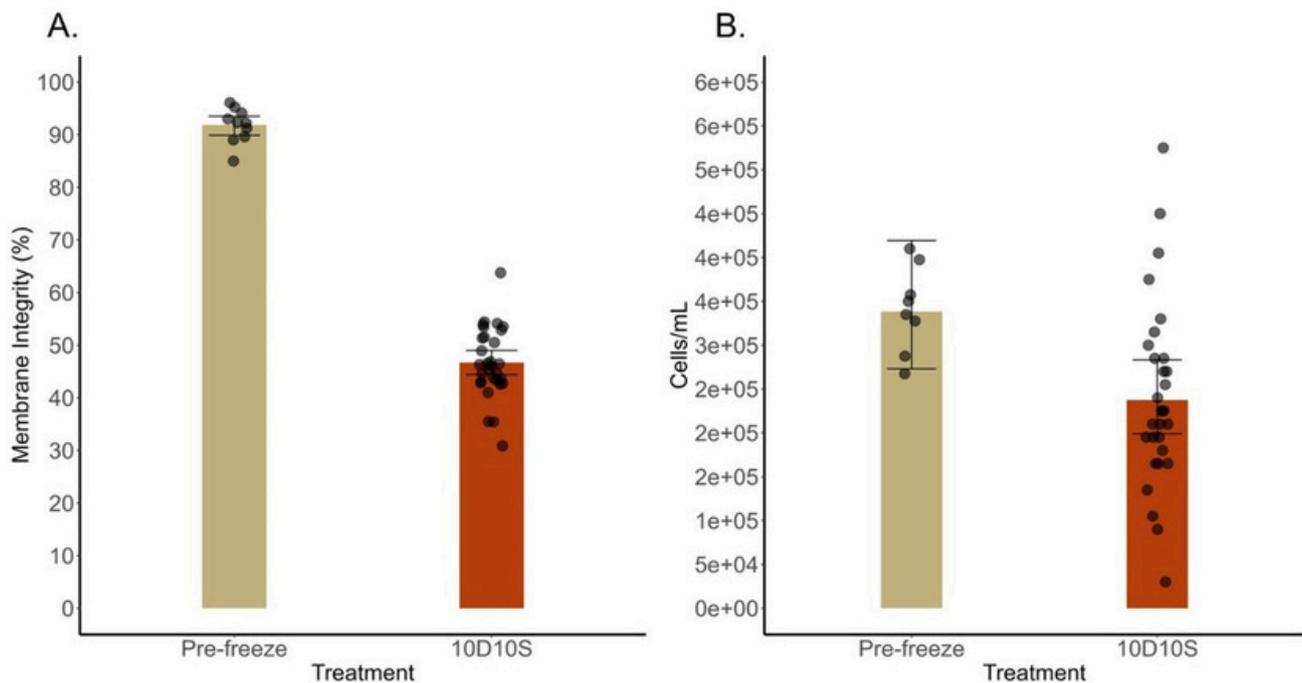


Figure 3: Pre-freeze and post-thaw for membrane integrity (4A) and cell concentrations (4B) for *R. littlejohni* neurula cells that were cryopreserved with 10% DMSO paired with 10% sucrose (10D10S).

was replaced by mammalian NT (Blum & Ott, 2019; Gurdon, 2013; Gurdon & Melton, 2008). Despite amphibians being the most threatened vertebrate group in the sixth mass extinction, NT has not been used in the conservation of endangered amphibians (Kouba & Vance, 2009; Novak, Brand, et al., 2025). Some endangered or extinct amphibians only have frozen diploid cells available (Herrick, 2019; Novak, Ryder, et al., 2025), and NT is the only method to recover genetic diversity from such materials (Novak, Ryder, et al., 2025).

Using *L. peronii* as a model, we attempted NT using both

fresh and cryopreserved cells. Two tadpoles were produced with fresh embryonic cells; however, only one was a true clone (meaning that the development was only due to the transferred cell nucleus). The most advanced stage attained was neurula with cryopreserved cells. This indicates that the NT procedure needs further optimization to increase the yield of cloned individuals, which we are currently working on.

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