

Metamorphosis and Effects of Enrichment on the Survival of *Polypedates leucomystax* and *Duttaphrynus melanostictus* Tadpoles in Captivity

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Abstract

Amphibian populations, particularly those of the order Anura, are experiencing global declines due to habitat destruction, climate change, and emerging pathogens, such as *Batrachochytrium dendrobatidis* fungus. Captive rearing and enrichment activities have been proposed as conservation tools, however the specific effects of enrichment on anuran development and post-metamorphosis survival remain underexplored. This study investigated the metamorphosis, physiological development, and survival rates of *Polypedates leucomystax* (Four-lined Tree Frog) and *Duttaphrynus melanostictus* (Common Toad) tadpoles in captivity, with and without enrichment activities. Sixty tadpoles (30 per species) were collected from Sabah, Malaysia, and reared in controlled tanks. Half of the specimens were then subjected to environmental and feeding enrichment following metamorphosis, while the other half were maintained without enrichment. Results shows that *D. melanostictus* completed metamorphosis faster, while *P. leucomystax* exhibited higher post-metamorphosis survival rates. Enrichment activities initially led to slightly lower survival in captivity, likely due to environmental disturbance, but significantly improved performance during environmental stress trials. Enriched individuals hunted prey more efficiently and showed higher resilience to food deprivation. These findings suggest that early enrichment enhances behavioural adaptability and survival in variable environments. This study highlights the importance of incorporating enrichment strategies in captive rearing programs to improve anuran survivability during and after reintroduction.

Keywords: captive rearing, *Duttaphrynus melanostictus*, enrichment, metamorphosis, *Polypedates leucomystax*, survival rate

1 Introduction

Amphibians are small vertebrates that can sustain in aquatic environments as well as in terrestrial habitats. This dual lifestyle is reflected in their name. The name amphibian is derived from the Greek *amphibios*, which means “living a double life”. Amphibians were believed to first appear around 368 million years ago based on the discovery of *Elginerpeton* during the late Devonian period (O'Rourke, 2007). Amphibians are divided into 3 orders; order Anura which represents frog and toad, order Caudata, newts and salamander and lastly order Gymnophiona which makes up of caecilians. Anurans, particularly frogs and toads, are the most diverse and widespread, and can be found on every continent except Antarctica. In Sabah, Malaysia, biodiversity richness includes seven anuran families: Bufonidae, Ceratobatrachidae, Dicroglossidae, Microhylidae, Megophryidae, Ranidae, and Rhacophoridae (Inger et al, 2017).

Due to their permeable skin and ectothermic physiology, amphibians are highly sensitive to environmental fluctuations. Among them, Anura is especially susceptible to habitat loss, pollution, climate change, and infectious diseases, contributing to global population declines (O'Rourke, 2007). Moist environments, such as streams, swamps, ponds, and rivers, can serve as vital refuges because excessive exposure to sun or wind can risk dehydration and cellular damage. Amphibians are among the first to be impacted by anthropogenic stressors like global warming, habitat devastation, and pollution. Frog populations have declined drastically since the 1980s.

Around one-third of recognized amphibian species are currently classified as threatened, with many having gone extinct due to habitat degradation and disease susceptibility (Cushman, 2006). In response, conservationists have turned to captive rearing to protect these species and support population recovery. This strategy involves raising amphibians from eggs or larvae in controlled environments and reintroducing them to natural habitats. For example, a study in southern Arizona successfully bolstered the endangered *Rana tarahumarae* population between 2000–2001 (Rorabough, 2005). Despite its success, captive rearing faces challenges. Survival rates during captivity or post-release are often low due to poor adaptation. A study on the Fijian ground frog *Platymantis vitianus* at the University of the South Pacific reported early mortality due to predation by invasive ants (Narayan et al., 2009). Another program at Kula Ecopark reared only a single froglet over three years. These findings highlight the complexity of captive conservation (Narayan et al., 2008).

Still, captive rearing offers benefits beyond species recovery. It generates surplus animals for behavioural research, disease studies, collection sustainability, and enrichment activities (Pigliucci, 2005; Réale et al., 2007). The key to success often lies in understanding and supporting the metamorphosis process, which governs survival and development.

Metamorphosis is a post-embryonic phase marked by dramatic physiological changes. In anurans, the cycle spans four stages: egg, tadpole, froglet, and adult (Etkin, 1964). During breeding season, females lay jelly-coated egg masses in wetlands, with toads forming ribbon-like strings and frogs producing clusters. Within weeks, eggs hatch into tadpoles (Duellman & Trueb, 1994). Driven by thyroid hormones, metamorphosis restructures tadpole organs and tissues (Kaltenbach, 1996). Tadpoles grow lungs, lose gills, and begin surfacing for air. Limbs emerge, tails shrink through apoptosis, and skin thickens to support terrestrial life (Duellman & Trueb, 1994). Digestive systems adapt from herbivory to carnivory, with true stomachs forming and denticles replaced by functional mouths and teeth.

Environmental conditions, like temperature, food availability, and pond evaporation can affect metamorphic rates in the wild (Hayes, 1997). In captivity, enrichment activities aim to simulate natural stimuli to support developmental outcomes. These include habitat modifications, dietary variation, and low-level stress exposure (Narayan et al., 2009), yet comparative data on their impact remain limited.

This study aimed to investigate the effects of environmental and feeding enrichment on the metamorphosis and post-metamorphic survival of two common Southeast Asian anurans: *Polypedates leucomystax* (four-lined tree frog) and *Duttaphrynus melanostictus* (Asian common toad). These species differ in ecological niches and developmental rates, making them ideal models for evaluating enrichment outcomes.

2 Materials & Methods

2.1 Study area

The study was conducted in Kota Kinabalu, Sabah, located on the northern coast of Borneo Island, Malaysia. Tadpole specimens were collected from two rural areas: Kampung Dambai and Kampung Rampayan (Figure 1). These sites were chosen due to their semi-natural aquatic environments which serve as breeding grounds for local amphibian populations.

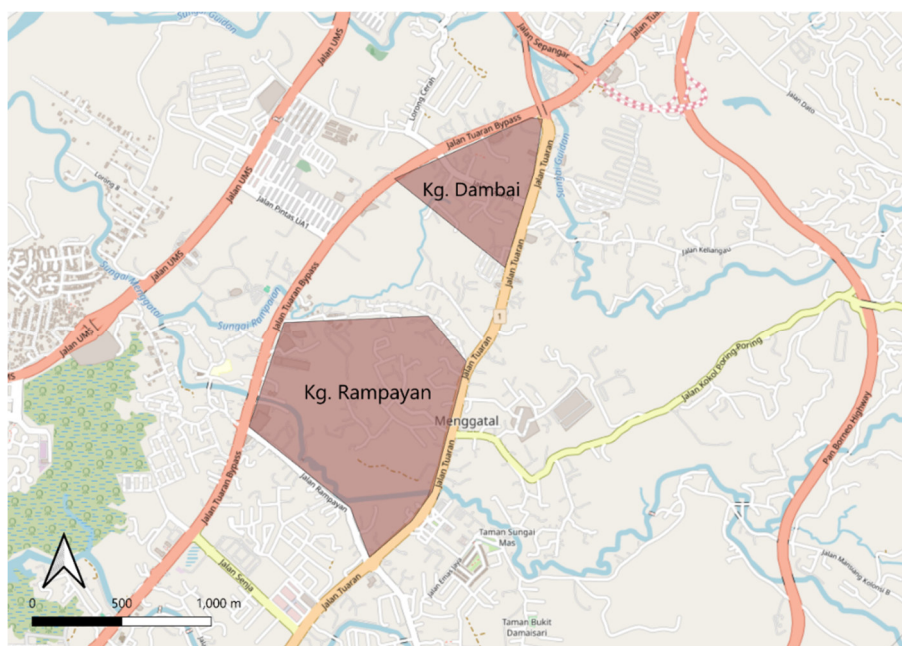


Figure 1: Map of Kampung Dambai and Kampung Rampayan

2.2 Tadpole Collection and Species Identification

A total of 60 tadpoles were collected, where 30 from the Bufonidae family (*Duttaphrynus melanostictus*) and 30 from the Rhacophoridae family (*Polypedates leucomystax*). Collection was performed using dip nets, and tadpoles were transported in containers filled with water from their original habitats to minimize stress. Species identification was initially based on morphological features and confirmed post-metamorphosis using key diagnostic traits such as skin texture, coloration, limb proportions, and presence of webbing.

2.3 Experimental Design and Tank Setup

Tadpoles were divided evenly into five tanks. Twelve *D. melanostictus* tadpoles each in Tank A and B, 12 *P. leucomystax* each in Tank C and D, and 6 tadpoles each from *D. melanostictus* and *P. leucomystax* in Tank E (Figure 2), measuring 20 cm × 20 cm × 9.5 cm. Each tank was filled with three-quarters pond water and natural substrates, including gravel, aquatic plants, and rocks to simulate natural habitat conditions. Water temperature was maintained between 18–26 °C, and tanks were subjected to a 12:12 hour light-dark cycle. The tadpoles were fed daily with a combination of algae, spinach, lettuce, zucchini, mosquito larvae, and frozen bloodworms. As tadpoles approached metamorphosis (appearance of limb buds), water levels were gradually reduced, and tanks were partially filled with moist sand to support the transition to terrestrial life.

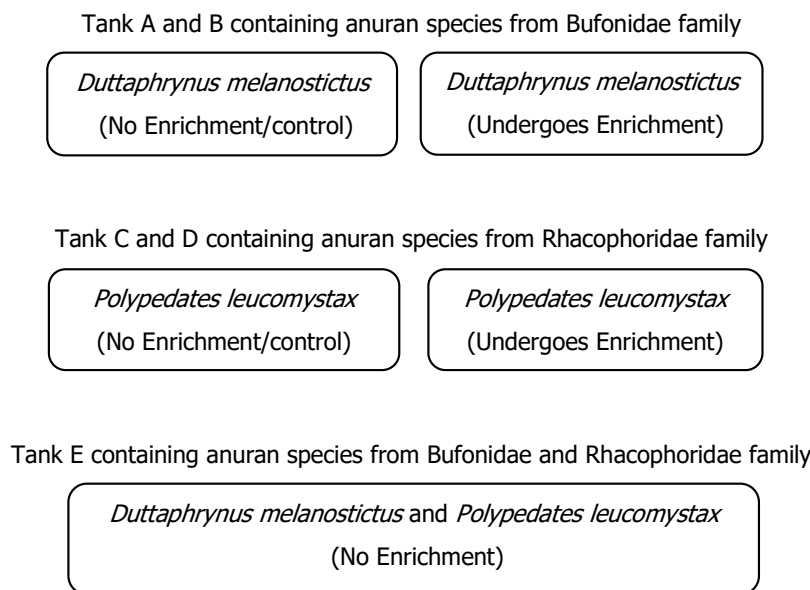


Figure 2: Distribution of the tadpoles in the rearing tank

2.4 Enrichment Protocol

Following metamorphosis, juvenile anurans were divided into two groups. The first group is the Enrichment Group, which was subjected to both environmental and feeding enrichment. Another is the Control Group, where the specimen was maintained in static conditions with constant food and environment.

Tanks for enriched individuals were periodically modified with changes in substrate, rearranged plant and rock structures, and rotation between larger and smaller enclosures to encourage exploratory behaviour and adaptation to a variable environment. The habitat of captive animals was changed occasionally to enhance their exploratory nature and ease of adaptation to new environments (Michaels et al., 2014). Besides that, to compare the efficiency of the exhibit and the change in environment, the froglet/toadlet will be shifted from a larger exhibit to a smaller exhibit for 2 days. The total number of anurans alive was noted.

Feeding was varied daily in terms of prey type, location, and accessibility. Live prey (e.g., fruit flies, ants, worms) was hidden under substrate, placed inside feeding tubes, or scattered around the tank to simulate natural foraging conditions. Control animals received the same food (fruit flies) at fixed intervals in predictable locations. This activity was conducted after the tadpoles had metamorphosed. The foods were hidden in a puzzle feeder and were not easily accessible. For instance, when feeding the animal with live prey, instead of feeding them directly, the prey was placed in a tube, and the tube was placed in the tank (Caitlin, 2014). The animal must wait for the prey to come out of the tube and capture the prey for their meal. Besides that, food will also be buried underneath the sand and placed between the rocks and plants (Caitlin, 2014). There will also be food placement variation where different types of food will be introduced, and the feeding timing and quantity may also vary daily (Caitlin, 2014).

2.5 Environmental Stress Test

This activity was implemented after they transitioned into a young frog or toad. To evaluate the resilience of enriched versus control groups, all juvenile anurans were subjected to a mild environmental stressor, which was food deprivation for two

consecutive days, followed by the introduction of live prey on the third day (Cabrera-Guzmán et al., 2013). Survival was monitored daily, and the time taken for individuals to successfully capture their first prey item was recorded. Food was reduced once a week, with the amount of food being reduced every week increasing (Flores-Nieves et al., 2014). The survival rate of the toad and frog was noted down. The group that is entitled to enrichment activities undergoes them for a month. After one month, the groups that receive enrichment and the group that doesn't both undergo environmental stressors and hunting activities to observe which group can overcome the hurdle and adapt better to the environment. The environmental stressor that was applied was slightly advanced than what one of the group faces during enrichment activities. The group that undergoes enrichment occasionally undergoes a shortage of food supply and has been devoid of food for a day while another group received a constant food supply. So, during the environmental stressor, both groups were not fed for 2 days. The number of anurans that survived and perished was recorded. On the third day, the anurans were fed with ants, worms, and roaches. The time taken for the anurans to hunt the animals was recorded along with the survival rate.

2.6 Data Collection and Analysis

Morphological development (limb appearance, tail regression) was observed and recorded every two days during the tadpole phase. Post-metamorphosis survival was recorded daily for 33 days. Time to capture prey during the environmental stress phase was recorded using a stopwatch, measuring the interval from prey introduction to successful capture.

Data were analysed using a two-sample t-test assuming unequal variance to determine significant differences between groups. Survival rates, metamorphosis duration, and hunting efficiency were compared across species and treatment groups. A significance level of $p < 0.05$ was used throughout the analysis

3 Results

3.1 Metamorphosis Duration and Morphological Development

The metamorphosis process differed between the two species. *Duttaphrynus melanostictus* tadpoles completed metamorphosis in approximately 30 days, while *Polypedates leucomystax* took about 33 days. The onset of hind limb development occurred between day 5–9 for both species, but *D. melanostictus* progressed to full metamorphosis slightly faster, likely due to their adaptation to ephemeral aquatic habitats.

Morphologically, *P. leucomystax* tadpoles were slimmer, had longer tails, and developed into froglets with smooth, moist skin and long, webbed hind limbs (Figure 3). In contrast, *D. melanostictus* tadpoles had chunkier bodies, shorter tails, and metamorphosed into toadlets with dry, warty skin and shorter, unwebbed hind limbs.

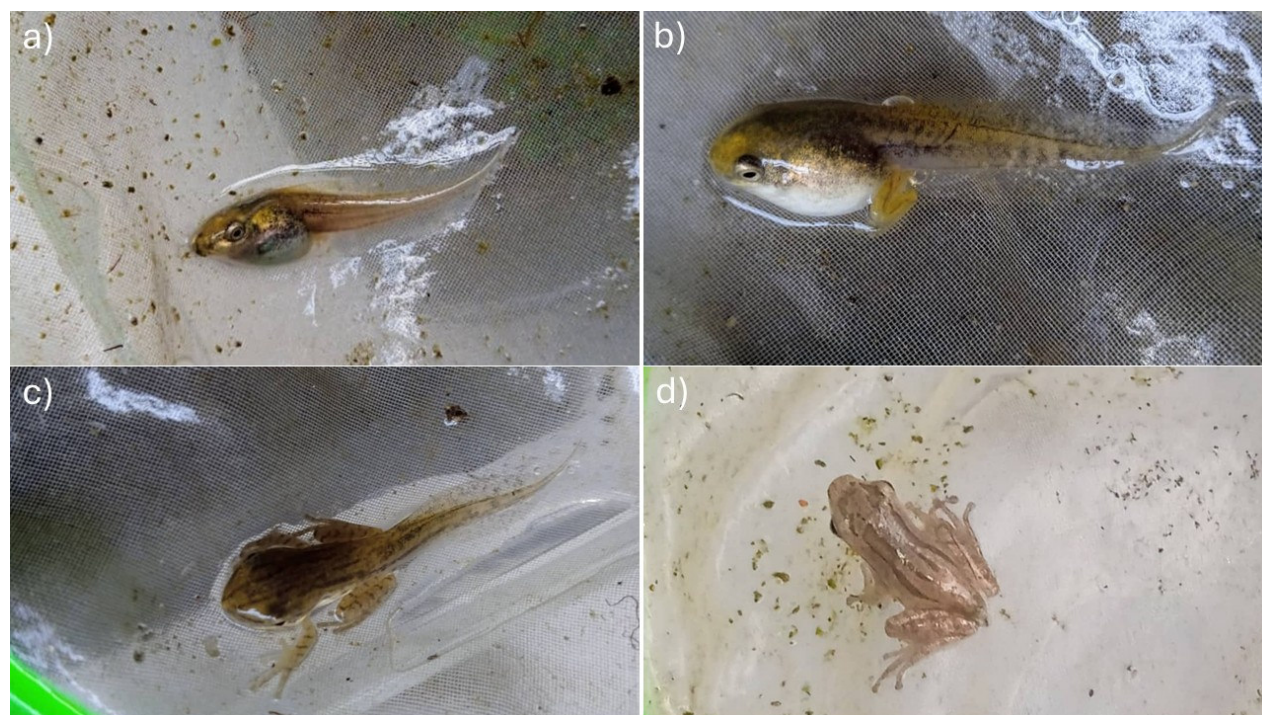


Figure 3: Metamorphosis stage of *Polypeates leucomystax*: a) tadpole, b) emergence of back leg (Day 5-9), c) emergence of front leg (Day 16-18), d) juvenile (Day 30-33)

3.2 Survival During Metamorphosis

Out of 60 tadpoles (30 per species), 48 (80%) successfully completed metamorphosis. Table 1 summarizes the survival rates during this stage.

Table 1: Tadpole survival during metamorphosis (combined across tanks)

Species	Tadpoles collected	Survived	Died	Survival Rate (%)
<i>P. leucomystax</i>	30	24	6	80
<i>D. melanostictus</i>	30	24	6	80

3.3 Survival Post-Metamorphosis

Juvenile *P. leucomystax* had a higher survival rate (79%) in captivity compared to *D. melanostictus* (58%) over the 29-day observation period (Table 2). However, a t-test revealed that this difference was not statistically significant ($p = 0.37$), due to small sample size of 30 individuals per group.

Table 2: Survival number of the juvenile *P. leucomystax* and *D. melanostictus* in captivity

Tank	Total no. of Anuran	No. of Anuran Alive	No. of Anuran died
A (<i>D. melanostictus</i>)	10	7	3
B (<i>D. melanostictus</i>)	9	5	4
C (<i>P. leucomystax</i>)	10	7	3
D (<i>P. leucomystax</i>)	8	6	2
E (<i>P. leucomystax</i> + <i>D. melanostictus</i>)	11	8	3
Total	48	33	15

3.4 Effects of Enrichment on Survival

During the enrichment phase, survival rates varied slightly by treatment and species. *P. leucomystax* with enrichment has 75% survival rate, *P. leucomystax* without enrichment is 70%, *D. melanostictus* with enrichment is 56% and *D. melanostictus* without enrichment is 70%.

These results indicate that enrichment had no clear advantage in captivity and may have slightly reduced survival for toads due to environmental disturbance.

3.5 Effects of Enrichment During Environmental Stress

When subjected to food deprivation (2 days), where juvenile frogs were intentionally not fed for 48 hours followed by prey introduction, all enriched juveniles of both species survived. This standardized period clears their digestive tracts and ensures that every individual starts the next phase at a uniform, heightened level of hunger. In contrast, 2 *P. leucomystax* and 4 *D. melanostictus* individuals from the non-enriched group died. T-test comparisons showed significantly lower mortality in enriched groups (mean = 0) versus control ($p < 0.05$).

3.6 Feeding Efficiency After Starvation

Time taken to capture the first prey item was significantly lower in enriched individuals (Table 3):

Table 3: Average time (seconds) to capture prey post-starvation

Species	Treatment	Avg. Time (s)	Std. Dev	p-value
<i>P. leucomystax</i>	Enrichment	23.4	±16.7	0.036
	Control	49.2	±16.6	
<i>D. melanostictus</i>	Enrichment	47.3	±13.7	0.18
	Control	70.0	±17.0	

Only *P. leucomystax* showed a statistically significant improvement in hunting speed with enrichment ($p = 0.036$). While *D. melanostictus* also performed better with enrichment, the difference was not statistically significant ($p = 0.18$).

4 Discussion

The observed differences in metamorphosis duration between *Duttaphrynus melanostictus* and *Polypedates leucomystax* are consistent with ecological adaptations reported in the literature. *D. melanostictus*, which often breeds in temporary, ephemeral pools, completed metamorphosis earlier than *P. leucomystax*. Rapid development in these environments is an adaptive strategy to avoid desiccation and ensure survival before water sources dry up (Pough & Kamel, 1984). Recent work confirms that *D. melanostictus* tadpoles exhibit high phenotypic plasticity, significantly accelerating their metamorphic

climax when detecting decreasing water levels (Mogali et al., 2017). Conversely, *P. leucomystax*, typically found in more stable aquatic habitats, undergoes slower development and may prioritize growth and physiological refinement over speed (Tamuly & Dey, 2014).

Morphologically, the species' traits observed in this study align with their respective ecological roles. Frogs possessed longer, webbed limbs suited for jumping and swimming, while toads had stouter bodies and unwebbed feet adapted for walking and terrestrial life. These differences are important not only for survival strategies but also for predicting how each species responds to environmental pressures in both natural and captive environments. The distinct morphological trajectories observed at the climax of metamorphosis directly justify the varying responses to captive enrichment seen in this study. The development of longer, webbed limbs in *P. leucomystax* requires a stable, structurally complex aquatic and vertical matrix to prevent developmental stress. Conversely, the stout, terrestrial morphology of *D. melanostictus* demands a rapid transition to land.

Although both species had the same survival rate during metamorphosis (80%), juvenile *P. leucomystax* demonstrated higher survivability in captivity after metamorphosis (79%) compared to *D. melanostictus* (58%). While this difference was not statistically significant, it may reflect differences in resilience to captive environmental conditions. Previous studies suggest that smaller-bodied anurans, like juvenile toads, are more vulnerable to fluctuations in temperature, humidity, and enclosure disturbances (Shaw & Holzapfel, 2008; Székely et al., 2020).

Housing design and substrate source may also have contributed to differential mortality. Materials not originating from the toads' native habitat could have introduced microbial threats or failed to meet the species' microhabitat requirements, as documented in similar captive studies (Taylor et al., 2001).

Enrichment initially appeared to reduce survival slightly in captivity, especially in *D. melanostictus*, possibly due to the additional stress caused by environmental changes. However, the benefit of enrichment became evident when individuals were exposed to stressors. All enriched individuals from both species survived the simulated food deprivation period, while several individuals from the control group perished.

This suggests that enrichment activities, despite their potential to temporarily disrupt stable conditions, may significantly enhance the adaptability of juvenile anurans. By promoting exploratory behaviour and variable feeding strategies, enriched environments likely train individuals to cope with unpredictable ecological conditions (Michaels et al., 2014; Kusumaningsih & Rosiana, 2023).

Enriched *P. leucomystax* juveniles hunted more quickly and efficiently after starvation, with a statistically significant reduction in prey capture time. This suggests that feeding enrichment, which mimics natural hunting scenarios, improves responsiveness and foraging skills. Although *D. melanostictus* showed similar trends, the differences were not statistically significant, likely due to sample size and behavioral differences between the species.

These results align with earlier findings that enrichment activities reduce stress and enhance foraging ability in captive amphibians (Caitlin, 2014; Mendelson III & Altig, 2016). Importantly, these behaviours are crucial for survival following reintroduction into wild environments.

This study reinforces the potential of enrichment activities as tools to prepare captive anurans for environmental variability, a critical factor for reintroduction success. Tadpoles and juvenile frogs reared without enrichment may be physically healthy but behaviourally unfit for survival in the wild. Enrichment can bridge this gap by introducing challenges that mimic natural conditions without exposing the animals to lethal risks.

Additionally, introducing enrichment early, preferably at the tadpole stage, may yield even stronger outcomes, as developmental plasticity is higher during early life stages. Future research should examine long-term survival post-release and explore how early-stage enrichment influences hormonal and neurological development in amphibians.

5 Conclusion

This study demonstrates that enrichment activities, though initially associated with slightly reduced survival in captivity, significantly enhance the adaptability and stress resilience of juvenile anurans. *Polypedates leucomystax* exhibited greater overall survival and foraging efficiency than *Duttaphrynus melanostictus*, highlighting interspecific differences in behaviour and physiological tolerance. Importantly, enriched individuals from both species responded more favourably to environmental stressors, suggesting that such interventions may improve post-release outcomes in conservation programs. The results emphasize the need for holistic captive rearing protocols that incorporate enrichment strategies to simulate natural stimuli. Future research should explore the integration of enrichment from earlier developmental stages and assess long-term survival and fitness following release into natural environments. By refining captive breeding techniques through

enrichment, conservationists can better prepare amphibians for the challenges of life in the wild, contributing meaningfully to population recovery efforts.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

HG: Conceptualization, Methodology, Writing- Original draft preparation. **HG:** Data curation. **HG:** Visualization, Investigation. **MFA:** Supervision, Writing- Reviewing and Editing.

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