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# THE RANCHO GRANDE HARLEQUIN TOAD (*Atelopus cruciger*): CONSERVATION ACTION PLAN 2023-2033

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ZOOLOGICO LESLIE PANTIN

IVIC
INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTÍFICAS

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# The Rancho Grande harlequin toad (*Atelopus cruciger*): Conservation Action Plan 2023–2033

Margarita Lampo[*1,2], Celsa J. Señaris[3], Onil Ballestas[1,2], Ingrid Márquez[4], and Fernando J.M. Rojas-Runjaic[4]

[1]Fundación para el Desarrollo de las Ciencias Fiśicas, Matemáticas y Naturales (FUDECI), Caracas, Venezuela.
[2]Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela.
[3]Estación Biológica de Doñana (EBD-CSIC), Sevilla, España.
[4]Museo de Historia Natural La Salle (MHNLS), Fundación La Salle de Ciencias Naturales (FLASA), Caracas Venezuela.

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## Executive Summary

Ranging from Bolivia to Costa Rica, harlequin toads (*Atelopus* spp.) are among the most diverse amphibians, but the majority of its species are threatened with extinction. According to the International Union for Conservation of Nature (IUCN), 83% of described species are listed as Endangered or Critically Endangered. In Venezuela, The Rancho Grande harlequin toad (*Atelopus cruciger*) is the only one of nine described species with known stable populations. However, because it currently exists in relictual form and has a very small range in the wild, this species is listed as Critically Endangered by the IUCN.

Rancho Grande harlequin toads disappeared from most of their former habitat, on the central portion of the Cordillera de La Costa in northern Venezuela, in the late 80's presumably due to chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*). This fungal pathogen continues to be highly virulent for this toad in remnant populations, but a high recruitment of juveniles and low *Bd* transmission allow this species to coexist endemically with the fungus in lowlands. Although most of its former habitats remain pristine and protected by national parks, environmental degradation due to agriculture related activities, urban expansion, fires and river pollution have been documented in some lowland habitats.

Following almost two decades of research on the Rancho Grande harlequin toad in relict populations, this document brings together the knowledge of a team of experts to identify priority conservation needs and design an action plan to rescue this species. This plan is based on the concept of "One Conservation", which integrates sustainability with *in-situ* and *ex-situ* conservation for the restoration of ecosystems, and was framed under the Amphibian Conservation Action Plan (ACAP) of the IUCN SSC Amphibian Specialist Group 2022 (`https://www.iucn-amphibians.org/`), and the Conservation Action Plan of the Atelopus Survival Initiative- HarleCAP (`https://www.atelopus.org`).

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Figure 1: **The Rancho Grande harlequin toad *(Atelopus cruciger)***. An adult female from Cuyagua river (Estado Aragua) Photo: Jaime Culebras | Photo Wildlife Tours.

# 1 THE CONTEXT

## 1.1 The species

*Atelopus cruciger* (Lichtenstein & Martens, 1856). Sapito Rayado de Rancho Grande, Rancho Grande Harlequin Toad, Veragua Stubfoot Toad (**Figure 1**).

## 1.2 Conservation status


Figure 2: **CRITERIA:** B1ab(iii)c(iv)+2ab(iii)c(iv)[1]

## 1.3 Distribution, population size, and trends

Until the 80's, the Rancho Grande harlequin toad was one of the most abundant and conspicuous amphibians in the montane forests and streams of the central portion of the Cordillera de La Costa in northern Venezuela [2, 3, 4]. Records of this harlequin toad exist from 77 localities distributed in most of the Cordillera de La Costa, from sea level to 2,400 meters of elevation [5, 6, 7, 8] **(Figure 2**). One individual was also sighted in Guatopo National Park, on the Serranía del Interior in 1984 [9]. Its range was estimated to be 16,500 $km^2$. During the late 1980's it disappeared from most of its range, a phenomenon that coincided with the appearance of the chytrid fungus *Batrachochytrium dendrobatidis* in museum specimens collected in the area [10].

In 2004–2005, 15 localities with past records of the species were searched (246 person/hour), but the species was found at only two localities between 120–322 meters of elevation on the Cata and Cuyagua rivers [8] (**Figure 2**). In 2023–2024, seven localities -three with past records and four with no past records but within the historical geographic range- were explored (90 person/hour) in hope of identifying populations that may have rebounded beyond previous detection thresholds. However, we found no evidence of additional populations of *A. cruciger*.

Subpopulations at Cata and Cuyagua appear to occupy areas less than 4 $km^2$ on the lower basin of the river, between 100–320 meters of elevation [11, 12, 13, 14]. However, we cannot rule out that they extend over a slightly larger area, since steep terrain and cliffs has limited explorations upstream. Based on a minimum cell size of 4 $km^2$, its area of occupancy (AOO) has been estimated as 8 $km^2$. The extent of occurrence (EOO) of its extant range is estimated to be 80 $km^2$ [1].

Abundance of reproductive individuals in both subpopulations has been estimated based on capture histories and mark-recapture models. In Cata river, its abundance varied between 24 and 119 during 2005–2012, with no increasing or decreasing trend [12, 13]. The average abundance in this subpopulation was 77 reproductive individuals during 2005-2013. The subpopulation at Cuyagua appears to be larger. The number of reproductive individuals varied between 274 and 494 between December 2013 and May 2014, with an average of 356 reproductive individuals at this locality (capture histories from [14] reanalyzed). Based on the sizes of both subpopulations, we estimated the population to contain 433 reproductive individuals. However, a tenfold variation was observed between 2010 and 2013. The number of reproductive individuals increased from 35 to 350, due to a peak in recruitment [13].

Although mark-recapture studies stopped in 2015, monitoring continued in 2016–2018, 2020 and 2022–2024 using visual counts. In 2023, 38 adults were counted during a six-hour session (5 persons) in a 350 m transect in Cata. Based on the number of detected adults and their detection probability estimated for the dry season from capture-recapture models [13], we approximated the total population size in Cata in 110 adults in 2023. In 2024, we sighted 20 individuals (4 persons) -13 females, 4 males and 3 unsexed- in Cuyagua. Higher encounter

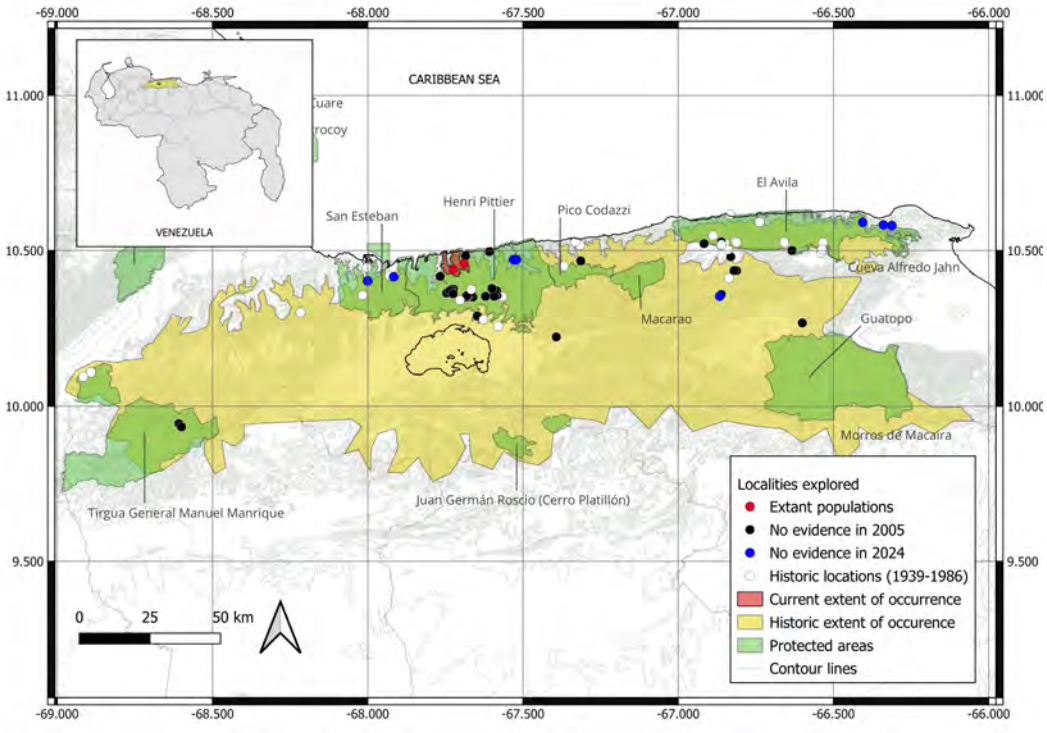


Figure 3: **Geographic distribution of the Rancho Grande harlequin toad (*Atelopus cruciger*).** Most known locations lie within protected areas.

rates in females, compared to males, suggest the presence of late breeders. We observed no amplexus, but females showed no eggs and a poor body condition characteristic after eggs have been laid. After correcting for detection probability, we estimated the current adult population in 34—162 individuals. In addition, emerging juveniles have been observed on the river beaches at the end of the dry season (April) in 2022, 2023 and 2024. We have no evidence indicating drastic changes in any of these subpopulations. However, as epidemic outbreaks can rapidly decimate entire populations and we do not fully understand what triggers them, precaution is warranted.

## 1.4 Habitat and ecology

The Rancho Grande harlequin toad is a terrestrial species with diurnal habits. Adults are found on the margins of the streams with clear neutral pH waters surrounded by gallery, cloud, deciduous and semi-deciduous montane forests (**Figure 3**). During the dry season, adults are commonly found on sand or gravel beaches, on rocks, or on *Cyclanthus bipartitus* plants that grow between rocks [15, 12]. Adults are occasionally seen on the vegetation climbing up to two meters above the ground. Males tend to occupy fixed territories that can partially overlap but no physical aggressive or territorial defence behaviour between adult males have been

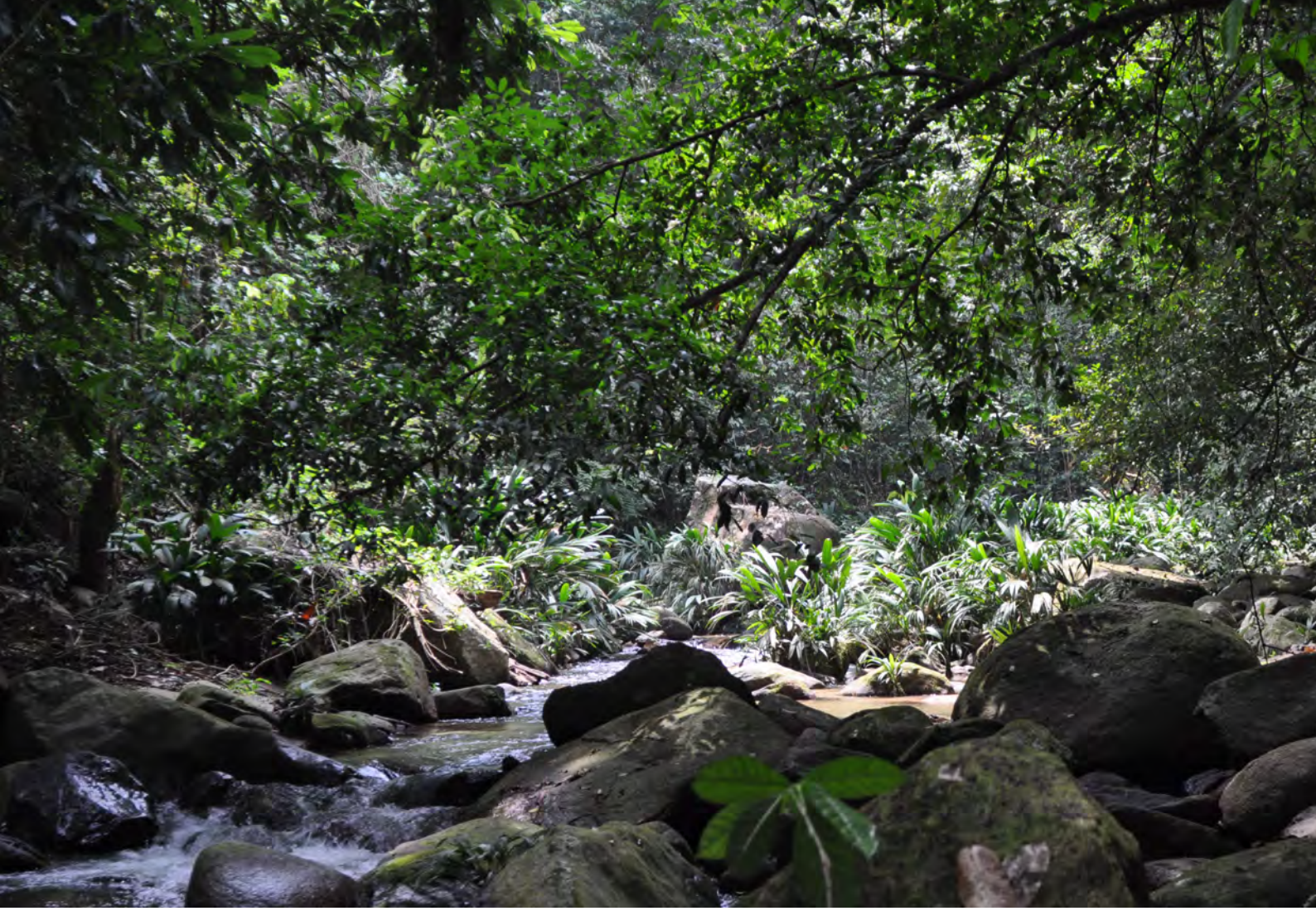

Figure 4: **Habitat of the Rancho Grande harlequin toad (*Atelopus cruciger*).** Lower section of Cata river, Aragua State, Venezuela. Foto: Margarita Lampo

observed [16]. During the rainy season, the water level may rise two meters and most adults retreat into the forest [12].

This species shows indirect development with a larval stage represented by rheophilic benthic tadpoles. Between 400 and 1,200 oocytes have been counted in mature females. In captivity, females have laid 350–800 eggs in a single spawn. Tadpoles, although difficult to detect in the wild, can be found attached by their abdominal sucker underneath and on the side surfaces of the rocks in fast-flowing waters. Recently metamorphosed tadpoles, with snout-vent length of 0.5–0.7 cm, have been observed during April–May nearby the water. However, they disappear into the forest until they reach a size of 2.0–3.5 cm. Mature individuals congregate on the stream shoreline during the dry season to breed. Mark-recapture data suggests that few adults survive to the next dry season in natural population, therefore, generation time is estimated in 2–3 years. However, one adult male have been kept in captivity for 52 months. Adults feed on insects, with a predominance of ants and beetles [17]. Parasitology studies from museum specimens suggest adults are often infected with nematodes and cestodes [18]. A reduction in the average body size of reproductive individuals in post-epidemic populations compared to pre-epidemic populations suggest that Rancho Grande harlequin toads

are breeding earlier to compensate for the high chytrid-induced mortality [19].

## 1.5 Primary threats

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, is currently considered its major threat. The disappearance of *A. cruciger* from most historic localities during the late 80's and early 90's is attributed to an epidemic event caused by this pathogen [10]. *Batrachochytrium dendrobatidis* has been detected on other amphibian species in several localities on the northern and southern slopes of the cordillera de La Costa [20]. The lower elevation of localities where *A. cruciger* still survives suggest that lowland subpopulations have better chances to recover from disease outbreaks [8]. Nonetheless, the fungus is highly virulent for this species. Infected adults have a life expectancy of few weeks [13]. Persistence of wild populations in the presence of this fungus has been attributed to a reduced transmission in lowland warm localities [13, 21]. The potential effects of global climate change on the epidemiology of chytridiomycosis remain uncertain. While an increase in temperature may reduce transmission rates and promote endemic coexistence of toads with the fungus, an increase in the severity of droughts can have the opposite effect on transmission triggering epidemic outbreaks and severe fluctuations in the population size. Rapid recruitment appears to play a key role in the persistence of *A. cruciger* populations. Therefore, climatic events that reduce recruitment could compromise the ability of population to persist with enzootic infection [21].

Although currently known subpopulations are located within seven protected areas (**Figure 1**), pressure from nearby inhabitants to use these habitats for recreational purposes continuously grow and local law enforcement capabilities are insufficient. Also, environmental degradation due to agriculture related activities in some of its former habitats on the Cordillera de la Costa may prevent the recovery of undetected populations. Satellite imagery analyses project a loss of 30% and 84% of semi-deciduous and deciduous forests, respectively, by 2036 [22]. In 2023, a crop smallholding was detected within one kilometre of the relict population at Cuyagua river.

## 1.6 Current protection

Relict populations of Rancho Grande harlequin toads are located on the northern limit of the Henri Pittier National Park and most of its former habitats lie within this and four other protected areas, namely: Waraira Repano, Macarao, and Guatopo national parks, on the central portion of Cordillera de La Costa (**Figure 2**). While most habitats on this mountain range remain pristine, there is an increasing pressure to expand agricultural activities and urban settlements into protected areas. Satellite imagery suggests that 13% and 30% of semi-deciduous and deciduous forests were lost between 1986 and 2001 [22]. Semi-deciduous and deciduous forests are located at low elevation, where the Rancho Grande harlequin toads have thrive and appear to have a better chance to coexist with *Bd*. In addition,

some streams with past records of this species have recently shown signs of contamination with nitrites and phosphates (unpublished data). Current protection is insufficient to guarantee the preservation of these lowland habitats.

# 2 ACTION PLAN

## 2.1 Rationale and goal

Our Theory of Change (ToC) consists of increasing the species capacity to self-sustain without the need for intensive conservation, by increasing its population size, the number of subpopulations and its extent of occurrence. *Atelopus cruciger* currently exists in relictual form, which makes this species particularly prone to extinction due to environmental fluctuations and demographic noise. Theory and practice have shown that the extinction risk of rare species decreases as the number of independent subpopulations increases. Our five-year goal is to downlist the current species threat category from Critically Endangered to Endangered by increasing its extent of occurrence (EEO) and the area of occupancy (AO) (**Figure 4**)

Rancho Grande harlequin toads are currently listed as Critically Endangered (CR) under criteria B1ab(iii)c(iv)+2ab(iii)c(iv) [1]. The species' estimated extent of occurrence (EOO) is only 80 $km^2$ and its area of occupancy (AO) is 8 $km^2$. Reintroduction strategy will aim to increase EOO and AO beyond the threshold for Critically endangered, 100 $km^2$ and 10 $km^2$ respectively.


Figure 5: **Downlisting the Rancho Grande harlequin toad (*Atelopus cruciger*).** The 2023—2028 Action Plan goal.

This action plan aims to 1) increase the number of extant populations and the current geographic distribution of the species by reintroducing captive bred individuals into habitats within their former range, 2) mitigate threats by selecting lowland habitats for reintroduction and improving the management of protected areas to maximize juvenile recruitment, 3) explore both historic and new lowland localities, looking for other relict subpopulations in order to incorporate them to *ex-situ* breeding program and to increase the genetic diversity preserved, 4) engage with local communities and integrate them into harlequin toad conservation and 5) raise local, national, and global awareness of Rancho Grande harlequin toads and their major threats.

# 3 PRIORITY ACTIONS

The current plan is based on five priority actions aimed at 1) increasing the number of extant populations and the current geographic distribution of the species by reintroducing captive bred individuals into habitats within their former range, 2) mitigating threats by selecting lowland habitats for reintroduction and improving the management of protected areas to maximize juvenile recruitment, 3) exploring both historic and new lowland localities for other relict subpopulations to incorporate them to *ex-situ* breeding program and to increase the genetic diversity preserved, 4) integrating local communities into harlequin toad conservation and 5) raising local, national, and global awareness of Rancho Grande harlequin toads and their major threats (**Table 1**).

| | OBJECTIVES | ACTIONS | TIME FRAME |
|---|---|---|---|
| 1 | Secure back-up colonies and captive bred individuals for reintroduction | Establish ex-situ captive breeding facilities<br>Build husbandry and breeding capacity | 2 years |
| 2 | Identify undetected populations | Exploration of former and new habitats | 10 years |
| 3 | Increase the geographic distribution of the species | Reintroduction of captive bred toads into selected habitats<br>Monitor fate of introduced toads | 10 years |
| 4 | Assess of the size and health of relict populations | Determine abundance and infection prevalence | 5 years |
| 5 | Raise public awareness and engage local communities in conservation actions | Create conservation awareness<br>Build local capacity for monitoring species | 5 years |

# 4 CURRENT CONSERVATION ACTIONS

## 4.1 *Ex-situ*

The primary goal of the *ex-situ* component is to establish a captive breeding colony as an insurance population to 1) reduce the extinction risks due to stochastic threat-

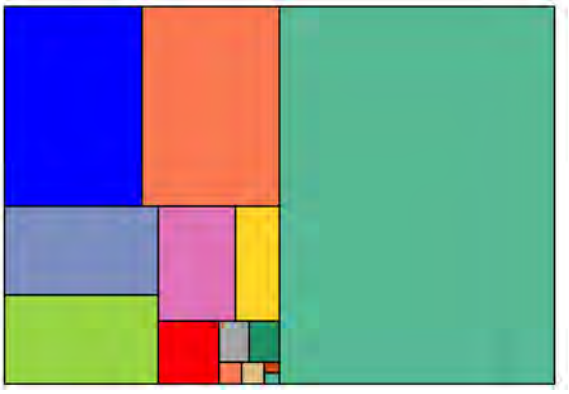

Figure 6: **Founder Contribution to Current Colony at Centro de Reproducción e Investigación de Arlequines (CRIA ).** As of July 9 2025, the colony comprises 625 adults/juveniles originating from 13 parental lines, each represented by a distinct color.

ening processes associated with its small populations and reduced extent of occurrence, 2) buy time until an effective strategy for minimizing the impact of chytridiomycosis in wild populations is developed, and 3) restore wild populations through reintroduction of captive bred individuals into suitable habitats (*e.g.*, thermal refuges). The immediate goal is to maintain a genetically and demographically viable population in captivity and produce sufficient fit and healthy toads for release in the wild.

#### 4.1.1 Securing colonies and producing surplus individuals for reintroductions

The first *ex-situ* program for Rancho Grande harlequin toads was launched in 2022, with the opening of the **Centro de Reproducción de Investigación de Arlequines (CRIA)**. CRIA consists of two facilities, one at Leslie Pantin Zoo in Turmero (Aragua state) and the other at FUDECI in Caracas, for holding a back-up captive colony and producing captive bred harlequin toads for future release into the wild [23]. CRIA initiated with 22 founders and has already produced over 1000 juveniles/adults from 13 different parental lines (**Figures 6**). A total of 207 tadpoles and 198 adult/juveniles have been released to the wild (See *In-situ* section). As of July 9 2025, CRIA currently holds 625 juvenile/adults and over 300 tadpoles. The parental lines exhibited highly heterogeneous contributions, with three lines accounting for 75% of the progeny generated (Figure 6). In May 2025, we produced the first F2 progeny.

### 4.2 *In-situ*

#### Monitoring relict populations

Since 2005, relict populations have been systematically monitored using mark-recapture methods (2005–2014) [12, 13, 14] or visual counts (2015–2024) [unpublished]. Estimates of detection rates based on mark-recapture data, have im-

Figure 7: **Río Cuyagua.** Javier Mesa, Onil Ballestas and Margarita Lampo (left to right) swabbing an adult toad. Photo: Jaime Culebras | Photo Wildlife Tours.

proved significantly our ability to approximate the abundance of reproductive individuals from visual counts. Regular visits to these population during the dry season every year are used to update the abundance of reproductive individuals and the prevalence of infection with *Bd* (**Figure 4**). Also, the detection of juveniles at the end of the dry season is used as an indicator of breeding success. Habitat quality is also monitored yearly to detect potential threats to relict populations.

### Searching for undetected populations

Since the survey of 15 historical locations in 2008 [8], only few explorations have been conducted by trained field personnel. Between 2023 and 2024, however, we explored the lower sections of seven rivers on the southern slope of Cordillera de la Costa. No evidence of additional populations of *A. cruciger* beyond those already detected in 2004 were found. To aid with the location of undetected subpopulations, local guides at three localities with past records were trained and a citizen science campaign was also initiated.

### Suitability assessment for reintroduction

Our primary objective was to identify optimal sites for future reintroduction, prioritizing pristine, protected areas at low altitudes, similar to the Cata and Cuyagua sites. We evaluated forest integrity, water quality, human disturbance, and researcher accessibility (unpublished data). One location in the Sabana River, at the easternmost extent of the distribution, was determined to be the most suitable for reintroduction.

### Releasing tadpoles – A pilot trial

Amphibian translocations are still largely experimental [29], and to date, reintroduction efforts for harlequin toads have not succeed in establishing populations [30, 31]. Raising captive bred tadpoles and metamorphs until they reach a size when they can better fend for themselves, is typically done to circumvent periods of high mortality during early life stages [29], but one potential drawback is that captive raised juveniles may lose their natural skin defences from pathogens [31]. Juveniles released as tadpoles that survive to adulthood are likely to be better immunologically equipped to fight diseases. Furthermore, if fecundity is very high, raising tadpoles to juveniles can become labor-intensive and costly. Thus, Releasing surplus tadpoles may offer an complementary strategy for restoring populations.

On January 11 2024, the first pilot release trial of captive bred *A. cruciger* was conducted. Two-hundred and seven tadpoles from a 800-egg batch laid in July 2023 at CRIA were released in La Sabana river. All tadpoles were in developmental stages 40–44, just before initiating metamorphosis. Tadpoles were packed in plastic bags with 50/50 proportion of water and oxygen enriched air, in a thermal insulator bag at 22°C (**Figure 10**). Tadpoles were acclimatized by placing the bags in water for 20 minutes and slowly mixing the water. As tadpoles left the bag, they immediately swam and attached to the rocks.

The first post-release monitoring was conducted in January 2025. The release area was explored by three persons for three hours, but no individuals were detected. Two audiomoth recorders were placed at the release point for three days but we detected no vocalizations.

### Juveniles and adults – A pilot trial

Two hundred juvenile and adult toads were released into a 220 $m^2$ mesocosm in a section of the La Sabana River in two batches: one in April 2025 and the other in May 2025. Prior to release, all toads were photographed, measured, and weighed. Post-metamorphic individuals were transported in plastic boxes lined with paper towels and filled with ample moss. To facilitate acclimatization, toads were initially placed in 1 × 1 × 40 cm cages filled with leaf litter. After 24 hours, their condition was assessed, and they were released into the mesocosm. Ten percent of the toads were fitted with diode tags. Post-release monitoring began the day following release to construct capture histories for estimating survival using

Figure 8: **First release trial.** Preparing tadpoles for the journey. Acclimatizing tadpoles to their new habitat in La Sabana river. Margarita Lampo, Onil Ballestas and Federico Pantin (left to right) Photo: Javier Mesa.

capture-recapture models. The elevated survival rates of released toads in the first month yielded promising results.

### 4.2.1 Building capacity and developing protocols

Capacity building focuses on training individuals with skills for specific tasks, organizing teams for carrying on projects, developing institutional leadership, sharing knowledge, and experiences with other groups working in the conservation of harlequin toads. As part of the HarleCAP (https://www.atelopus.org/the-initiative), a broader action plan aimed at improving the range-wide conservation of harlequin toads, this the Rancho Grande harlequin toad Action Plan benefits from the collaborative effort of 38 experts from eleven countries. Through training course, workshops, and a communication network for consulting on specific topics HarleCAP maximize the probability of success of the specific action plans. All research results, protocols or methodologies derived from the *ex-situ* and *in-situ* activities of this action plan will be freely available.

Husbandry and breeding guidelines for the The Rancho Grande harlequin toad developed at CRIA were modelled after the *ex-situ* operation of the Panama Amphibian Rescue (PARC) [25] for *Atelopus varius/zeteki* and of Center Jambatu in Ecuador [26] for several *Atelopus* species, the protocols developed by the Dallas World Aquarium for *Atelopus balios* [27], and the National Aquarium in Baltimore [28], with modifications according to in-house experience and information gathered during the last 17-years from two relict populations (`https://fudecive.org/protocolos-y-manuales-cria/`). As our program is in its initial phase and new knowledge is expected to emerge, this first version will be adjusted and updated.

Figure 9: **Captive bred Rancho Grande harlequin toads**. Juuveniles produced at at Centro para la Reproducción e Investigación sobre Arlequines (CRIA). Photo: Jaime Culebras | Photo Wildlife Tours.

## 5 ENGAGING COMMUNITIES AND RAISING AWARENESS

Raising awareness about the need to preserve the biodiversity is a priority of this action plan. Research indicates that children's constructive engagement with the natural world contributes to the development of environmentally conscious individuals. While school workshops have been effective in reaching children, their experiences in extracurricular settings have also demonstrated efficacy. Interventions conducted within amphibian zoos have shown considerable success in enhancing students' scientific knowledge and motivation.

In 2024, we conducted educational activities at the local elementary school in Cuyagua and the Leslie Pantin Zoo (**Figure 9**). At both institutions, five posters with informative infographics about the RG Harlequin Toad and its primary threats were displayed, and notebooks produced by our partner Rio Verde, featuring this species, were distributed among students. These notebooks include a QR code linking to a YouTube questionnaire about the Rancho Grande Harlequin Toad.


¡Hola!


RÍO


Figure 10: **The Rancho Grande Harlequin Toad (*Atelopus cruciger*)at school.** Children from Cuyagua's elementary school and Leslie Pantin Zoo engaged in educational activities. Photo: Jaime Culebras | Photo Wildlife Tours.

The primary stakeholders for this action plan are:

- Leslie Pantin Zoo: Federico Pantin and Tuenade Hernández.
- Academic institutions - researchers and captive breeding specialists.
- Conservation groups: *Atelopus Survival Initiative*, Luis Fernando Marín.
- Communication organizations: Río Verde, Diego Bilbao.
- Education ONG organisations: AMBLEMA.
- Local communities close to sampling sites: Cuyagua and Cata.
- Local environmental and park authorities: Ministerio de Ecosocialismo and INPARQUES.

# 6 KNOWLEDGE GAPS

## 6.1 *In-situ*

Because tadpoles are rarely seen in the wild, little is known about their habitat, diet or behaviour. Also, the role of tadpoles in *Bd* transmission is unknown. We do not know if they get infected by *Bd* in the wild, and are vulnerable to developing the disease. Mathematical models suggest that tadpoles could act as a *Bd* reservoir for the infection of post-metamorphic individuals or as banks for recruitment of healthy juveniles [24]. Infection of tadpoles with *Bd* in rapid-water habitat where they live seems unlikely, but the distribution of free zoospores in the environment is unknown.

To date, no successful reintroductions of harlequin toads have been documented [30, 31]. In previous trials, released toads typically disappeared shortly after release, likely due to dispersal or mortality, and failed to establish populations at the release sites [31]. Little is known about environmental cues used by toads to move select habitats. Adults, for example, return to the same locations at each breeding season. The learning mechanisms involved in selecting locations or establishing routes are not well understood and may include interactions between juveniles and adults (*e.g.*, social learning). Understanding these mechanisms is crucial for designing reintroduction strategies [16].

The potential effect of climate change in populations of the Rancho Grande harlequin toads is still difficult to predict. Some studies suggest that extreme weather events may play a role in *Atelopus* declines [32]. Temperature is expected to increase and dry seasons will become extreme, but these variables can have opposite effects on different demographic and epidemiological parameters. Mathematical models suggest that *Bd* transmission and juvenile recruitment are the two parameters with greater incidence in the probability of coexistence [21]. Therefore, we need to understand how extreme climate events will affect these two parameters.

## 6.2 *Ex-situ*

We are just beginning to understand how *Atelopus cruciger* adapts to captivity and its requirements to effectively breed outside its natural environment. Although more than ten spawning events have occurred at CRIA, fertilization rates are vary greatly between spawns: one produced over 360 tadpoles while others less than 50 tadpoles. We do not fully understand what determines the fertilization success. Also, mechanisms of calcium incorporation and metabolism in developing tadpoles are not well understood. Although we have been able to reduce the incidence of spindly leg syndrome (SLS) in Rancho Grande harlequin toads, it remains a concurrent problem at CRIA. We are currently exploring different nutrition protocols and their relation to calcium metabolism, to reduce the incidence of SLS.

Information on the genetics of the Rancho Grande harlequin toads is lacking. The genetic diversity in remnant populations, the genetic relatedness between

individuals in our captive breeding program or the level of genetic divergence between subpopulations are unknown. Genetic characterization of both relict subpopulations and captive-bred specimens is required to address these questions and it is crucial for planning future population reinforcements and reintroduction to suitable historical areas of occupancy.

# 7 CHALLENGES

Obtaining funds for research or conservation is the greatest challenge in a country with an economy running on a three-digit inflation rate. Government funds are currently inexistent and local private donors are increasingly scarce. We are currently engaged in an aggressive fund seeking campaign to secure funds to sustain our conservation program.

# 8 SUCCESS INDICATORS

| | OBJECTIVES | INDICATORS |
|---|---|---|
| 1 | Established capacity in *ex-situ* facility | 180 adults (Maintenance tanks)<br>400 juveniles (Small cages)<br>2 amplectant pairs (Breeding tanks)<br>500 tadpoles (Tadpole tanks) |
| 2 | Obtain founders from source populations | 7 females<br>9 males<br>3 juveniles |
| 3 | Trigger spawning | +20 spawning events |
| 4 | F1 juveniles | +1000 from 13 parental lineages |
| 7 | Develop protocols | 1 husbandry and breeding protocol |
| 8 | Identify potential release sites | 1 site identified |
| 9 | Release captive bred toads | 207 tadpoles and 198 adult/juveniles |
| 9 | Survival of released toads | 75% over the first two months |
| 10 | Establishment of new populations | |
| 11 | Community Awareness and engagement | +250 kids from local communities with some knowledge about the species and its threats |

# 9 EXIT STRATEGY

The aim of this action plan is to improve the status risk of Rancho Grande harlequin toads from Critically endangered to Endangered in five years. This action plan will be considered terminated when the species is no longer in the Critically Endangered category. Below are listed the criterion that will trigger the termination of each action of this program.

| | OBJECTIVES | ACTIONS | EXIT TRIGGER CRITERIA |
|---|---|---|---|
| 1 | Identification of undetected populations | Exploration of former and new habitats | Extent of occurrence > 100 $km^2$ threshold for CR<br>Area occupancy > 10 $km^2$ threshold for CR |
| 2 | Regular assessment of the size and health of relict populations | Determine abundance and infection prevalence | Extent of occurrence > 100 $km^2$ threshold for CR<br>Area occupancy > 10 $km^2$ threshold for CR<br>Abundance of reproductive toads in each subpopulations > 250 threshold for CR |
| 3 | Captive propagation of species | Establish an ex-situ captive breeding facility<br>Build hubandry and breeding capacity | Extent of occurrence > 100 $km^2$ threshold for CR<br>Area occupancy > 10$km^2$ threshold for CR |
| 4 | Raise public awareness and engage local communities in conservation actions | Create conservation awareness<br>Build local capacity for monitoring species | Local communities engaged conservation activities |
| 5 | Increase the geographic distribution of the species | Reintroduction of species into selected habitats | Extent of occurrence > 100$km^2$ threshold for CR<br>Area occupancy > 10$km^2$ threshold for CR |

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