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THE RANCHO GRANDE HARLEQUIN TOAD (*Atelopus cruciger*): HUSBANDRY AND BREEDING GUIDELINES

Photo: Jaime Culebras

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The Rancho Grande Harlequin Toad (*Atelopus cruciger*): husbandry and breeding guidelines

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Summary

Harlequin toads are among the most diverse amphibian genera, but the majority of its species are threatened with extinction. In Venezuela, The Rancho Grande Harlequin Toad (*Atelopus cruciger*) is the only one of ten described species that has known stable populations. Because it currently exists in relictual form and has a very small range in the wild, this species is listed as Critically Endangered and recommended for *ex-situ* rescue by the International Union for the Conservation of Nature (IUCN). Here, we described guidelines for the husbandry and breeding of the Rancho Grande Harlequin Toad, based on in-house experience in captive rearing of this species, information on its biology, habitat requirements and behaviour gathered from relic populations and protocols published for other harlequin toad species.

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1 Introduction

Harlequin toads (*Atelopus*) are among the most threatened group of amphibians in the world, with 83% of the species at risk of extinction. In Venezuela, The Rancho Grande Harlequin Toad (*Atelopus cruciger*) is the lone species of the 10 described Venezuelan *Atelopus* species to have known stable populations. Despite continuing efforts to find these toads in their former habitats, only two populations have been discovered. *Atelopus cruciger* is currently listed as Critically Endangered and recommended for *ex-situ* rescue by the International Union for the Conservation of Nature (IUCN).

Captive breeding efforts for harlequin toads began two decades with astonishing advances. Panama, United States and Ecuador have secured *ex-situ* populations of at least ten species of harlequin toads: *A.certus*, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, *A. balios*, *A. elegans*, *A. ignescens*, *A. bomolochos*, *A. nanay*, *A. spumarius* and *A. sp* "Limón". However, many other harlequin toad species remain on the brink of extinction. Although most harlequin toad species have similar biology, preferred habitats, and behavior, the range of altitudinal distribution between them can lead to subtle differences in climate or environment. As a result, species may respond differently to environmental cues, particularly those triggering breeding. Therefore, breeding and husbandry protocols need to be adjusted for each species.

The husbandry and captive breeding guidelines described here were modelled after the *ex-situ* operation of the Panama Amphibian Rescue (PARC) [1] and Centro Jambato in Ecuador, the protocols developed by the Dallas World Aquarium for *Atelopus balios* [2] and the National Aquarium in Baltimore [3], with modifications according to in-house experience and information gathered during the last 17 years from two relic populations. As new knowledge is continuously emerging and processes are optimized, this document will be updated annually.

2 The species

Taxonomy

Order: Anura
Family: Bufonidae
Genus: Atelopus
Species: cruciger
Common names: Veragua Stubfoot Toad, Rancho Grande Harlequin Toad, Sapito Rayado.

The Rancho Grande Harlequin Toad, *Atelopus cruciger* (Lichtenstein & Martens, 1856), is a bright yellow and black coloured toad, with a pointed snout and marked sexual size dimorphism, both distinctive characteristics of harlequin toads (**Figure 1**) [4]. Adult males can have a body size (snout-vent-length, SVL) of 22–35 mm and females 32–50 mm, although adults from post-epidemic relic populations tend to be smaller than those from pre-epidemic populations [5]. Sex of adults can be determined by the shape of forearms (long and thin in females vs. short and broad in males) and the presence of brownish horny pad at the base of the thumb (only in males) [6]. Because each individual has an unique and invariant dorsal pattern once it reaches ~25 mm in SVL, these patterns can be used to unequivocally identify individuals. Mark-recapture studies suggest that, in wild populations, only few adults survive beyond their first breeding season [7, 8]. However, adults have been maintained in captivity for more than 52 months.

As in most other harlequin toad species, males of *A. cruciger* show an elaborated display of behaviours including various vocalizations and hand waving. Females tend to form small groups when migrating from the forest to the stream, while males are more frequently observed in solitary [9].

Figure 1: **Sexual size dimorphism in the Rancho Grande Harlequin Toad (*Atelopus cruciger*).** The male, on top, is about 70% the size (SVL) of the female. Photo: Jaime Culebras

3 The natural habitat

Atelopus cruciger is a terrestrial, diurnal species with adults living along streams in gallery forests and cloud forests (**Figure 2**). Its was formerly found between 100–2,200 m asl, although only lowland population are currently known [10]. In the lower section of Cata river, where one relic population exists, air temperature varies from 19 to 32°C and humidity from 62–100% (**Figure 3**). The lowest temperatures are registered during December–January and the highest in July–August. Historic data suggests that daily rainfall may vary from 10 mm during the dry season (November–April) and 120 mm during the wet season (May–December). Water temperature at 300 m asl varies from 19–21°C.

During the dry season, adults are commonly seen on sand or gravel beaches, perching on rocks, or on *Cyclanthus bipartitus* plants that grow between rocks. During the rainy season, most adults retreat into the forest as water level may rise two meters. Adults are occasionally seen climbing on vegetation up to two meters above the ground. Each breeding season, males and females tend to return to the same territories.

Although previously described *A. cruciger* tadpoles were raised in captivity [4], a single tadpole was found in the wild, attached to a rock submerged in a river section where the water flow was very high. Metamorphs have been observed during April–May around the river margins. However, they disappear into the forest until they reach a size of 20 mm. Mature individuals congregate along the streams during the dry season where they feed primarily on ants and beetles in preparation for reproduction [11].

4 Distribution, population size and trends

Until the 1980s, 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 [12, 13, 14]. Records of this harlequin frog exist from 77 localities distributed in most of the Cordillera de La Costa, from sea level to 2,400 meters of altitude [15, 16, 17, 10]. One individual was also sighted in Guatopo National Park, on the Serranía del Interior, in 1984 [18]. 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 [19].

In 2004—2005, 15 localities with past records of the species were searched (246 person hours), but the species was found at only two localities between 120–322 meters of altitude on the Cata and Cuyagua rivers [10]. Subpopulations at Cata and Cuyagua appear to occupy areas less than 4 km^2 on the lower sections of the river, between 100–320 meters of altitude [20, 7, 8, 21], although the presence of 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

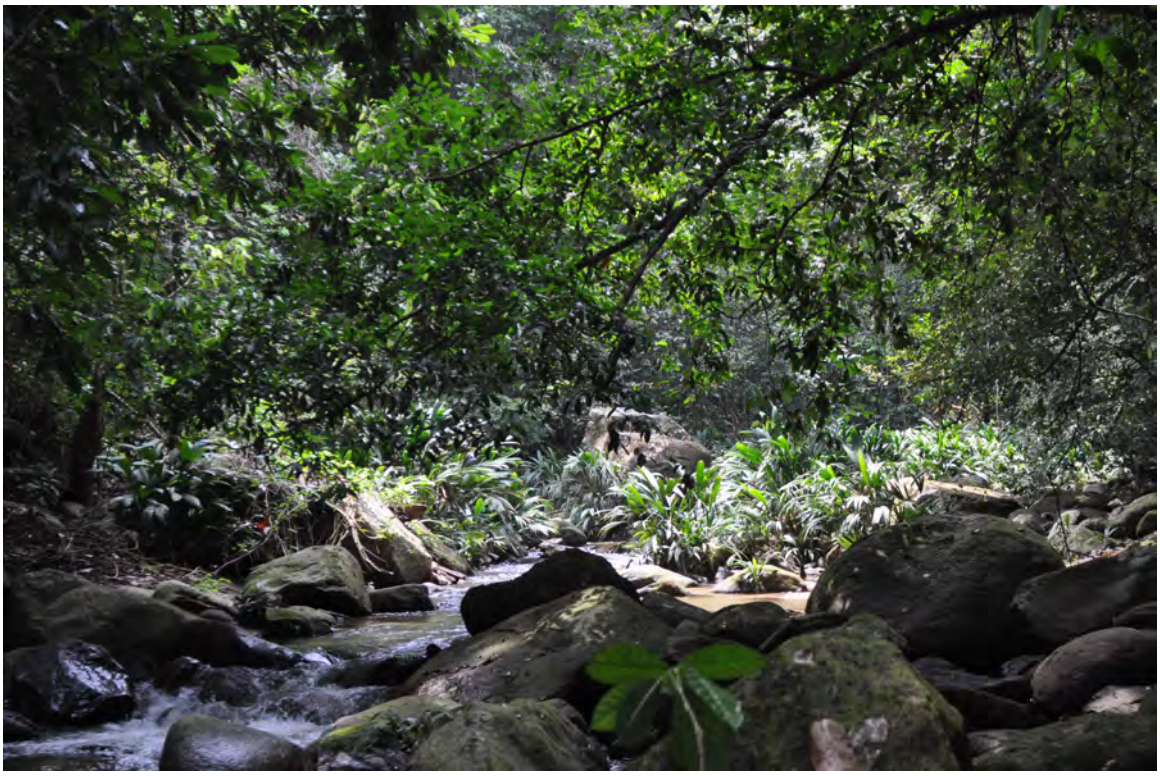

Figure 2: **Habitat of the Rancho Grande Harlequin Toad (*Atelopus cruciger*).** Lower section of Cata river, Aragua State, Venezuela.

km^2. The extent of occurrence (EOO) of its extant range is estimated to be 80 km2 [22].

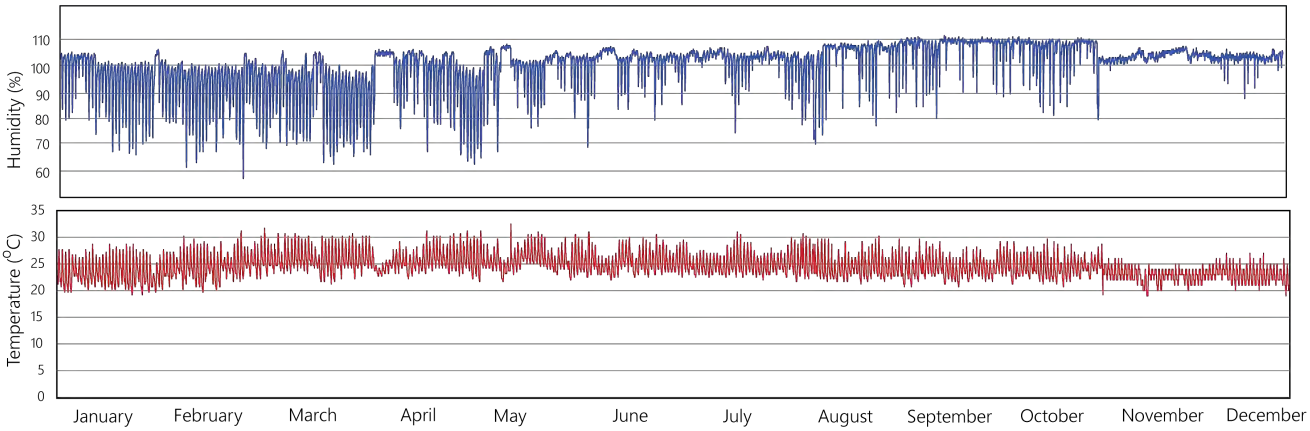

Figure 3: **Temperature and humidity in Cata river, Aragua state, Venezuela** Hourly measures between January and December of 2010.

5 Conservation status

According to the most recent assessment by the IUCN (2022), the Rancho Grande Harleqion Toad is listed as Critically Endangered (CR) under criteria B1ab(iii)c(iv)+2ab(iii)c(iv). Its estimated extent of occurrence (EOO) is only 80 km^2 and its area of occupancy (AOO) is 8 km^2. The population is severely fragmented with no evidence of connectivity between subpopulations, one of which has shown tenfold variations in the number of reproductive individuals over short periods. In addition, the habitats suitable for this species have been declining during the last decades,

despite most being located within the limits of three national parks [22].

6 Major threats

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, is currently considered its major threat. The disappearance of the species from most historic localities during the late 80's and early 90s is attributed to chytridiomycosis [19]. *B. dendrobatidis* has been detected on other amphibian species in several localities on the northern and southern slopes of the cordillera de La Costa [23]. The lower altitude of localities where this species is currently known suggest that lowland subpopulations have better chances to recover from disease outbreaks [10]. Nonetheless, this fungus is highly virulent for this species. Infected adults have a life expectancy of few weeks [8]. Persistence of wild populations in the presence of this fungus has been attributed to a reduced transmission in lowland warm localities [8, 24]. 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 and fungi, 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 populations. Therefore, climatic events that reduce recruitment could compromise the ability of population to persist with endemic infections [24].

Although currently known subpopulations are located within the Henri Pittier National Park, 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 [25]. In 2023, a crop smallholding was detected within one kilometre of the relic population at Cuyagua river.

7 Management in captivity

7.1 The enclosures

Enclosure design results from a compromise between recreating the species' natural habitat and producing an environment that can be easily maintained. Our enclosures were constructed following PARC recommendations with very few modifications [1].

7.1.1 Maintenance tanks

Maintenance enclosures consist of 10-gallon glass tanks (25 x 50 x 30 cms) covered with a screen lid, with a draining bulkhead on the bottom with plants and pebbles used to provide hiding and perch sites (**Figure 4**). Each tank is fitted with an automatic misting system to maintain correct humidity. Groups of 3–5 adult males or females are kept in each maintenance tanks away from the opposite sex when not breeding. An adult may produce 1–2 fecal pellets each day; therefore, well drained false floors elevated from the bottom are preferred for easy washing to prevent wastewater from misting or cleaning from accumulating and coming in contact with animals. Each maintenance tank is cleaned twice a week by spraying down perch sites and sides of the tank to remove feces or other particulates which are removed via the bulkhead drain. Although it is important to recreate the natural environments of this species for breeding, for practical reasons, the maintenance tanks are kept simple to ensure that the adults are healthy come breeding season.

Figure 4: **Maintenance tank.** Groups of 3–5 same sex toads are housed in these tanks when not breeding. Plants and rocks provide hiding places for toads. Automatic misting systems keep humidity high.

7.1.2 Breeding tanks

The breeding tanks consist of 20-gallon glass containers (30 x 75 x 30 cm) with a bulkhead on the bottom, and a screen lid on the top. Breeding tanks are used for housing amplectant pairs and tadpoles (**Figure 5**). Breeding tanks recreate two main features of natural creeks: (i) running water with high levels of dissolved oxygen, produced by three air diffusers and (ii) dark crevices for females to lay eggs, created by placing various size rocks within the tanks. Each breeding tank has a water reservoir associated, from which water is pumped-up into the breeding tank. Water also circulates from the breeding tank to the reservoir through a water level control system (grey PVC pipes). Water level may be switched from low to high by opening or closing the valves. Low water level is used for amplectant pairs and when metamorphs start emerging from water. High water level is used for tadpoles. Partial water changes can be performed without disturbing the animals, by replacing the water in the plastic container. Water is partially (20-30%) changed every other day through siphoning to reduce fouling.

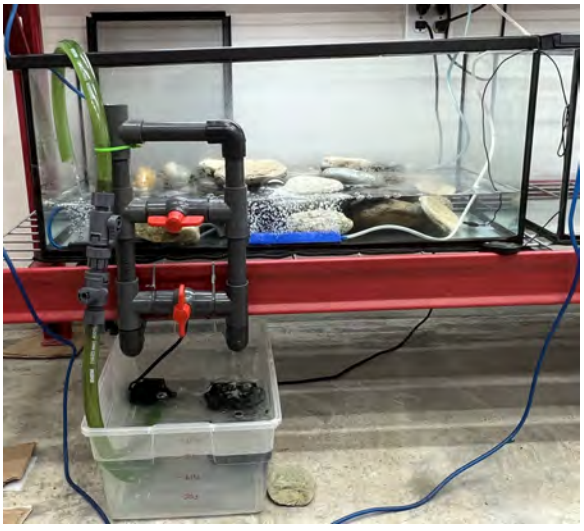

Figure 5: **Breeding tank.** Breeding couples and tadpoles are housed in these tanks. Each breeding tank has three air diffusion stones, an external water storage container with a filter and a pump, and a tubing system that allows for easy changing of water level and replenishing of tanks.

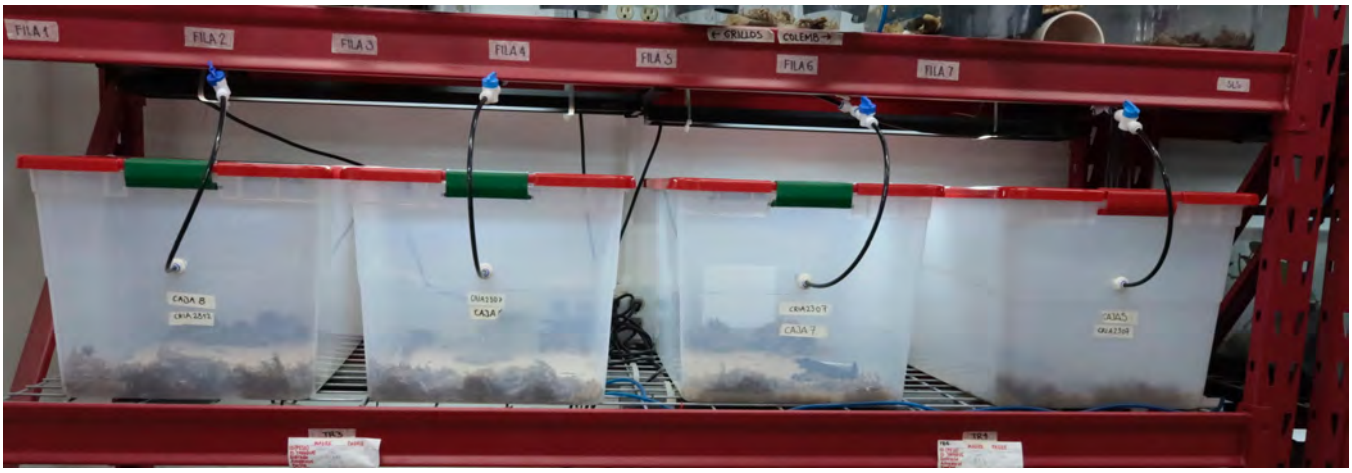

Figure 6: **Juvenile boxes**. Plastic 60 x 40 cm containers with misting systems.

7.1.3 Juvenile boxes

After emerging from the water, the toadlets are transferred to plastic lab mouse cages with a paper substrate and ample water-soaked moss. They are kept in groups of 10-15 individuals in these cages for the first 20 days. Once they are large enough to consume pinhead crickets, the juveniles are transferred to larger boxes measuring 60 x 40 cm in groups of 40-50 (**Figure 6**).

7.2 The environment

7.2.1 Light

The Rancho Grande Harlequin Toad naturally inhabits gallery forests where sunlight filters through the canopy and direct exposure occurs only occasionally, when they bask on the stream shoreline where canopy tends to open. We used Zoo Med Reptisun T5 HO 5.0 UVB High Output Bulb 24 watt to provide UVB and UVA. By placing plants within the tanks, animals can photo-regulate by moving from the shade to exposed areas. Because the species distribution is tropical (10°N), photoperiod is set to 12x12-hour on/off.

7.2.2 Temperature

Because *A. cruciger* can live from 100–2,200 m asl, the species can live in a wide range of ambient temperatures. In lowland habitats (300 m asl), the average ambient temperature is 24.5°C (19–32.5°C) with daily variation of 5–7°C (**Figure 3**). In highland habitats (1,158 m asl), where the species used to exist, the average ambient temperature is 19.4°C (18–21.7°C).

In contrast to air temperature, water temperature in mountain streams varies little along the altitudinal gradient. In lowland habitats, where air temperature can reach 32°C, water temperature rarely exceeds 21°C as they descend from high mountain peaks. This means that, unless water chillers are used to control water independently of air temperature, ambient temperature should not exceed 23 °C to keep water temperature below 22 °C (1° below ambient temperature). Because tadpoles tend to be very sensitive to changes in temperature, redundant air conditioning systems and generators are recommended to avoid heating if any power or equipment failure occurs.

7.2.3 Humidity

Relative humidity in gallery forests alongside mountain streams where *A. cruciger* lives is very high. In lowland habitats, the mean annual humidity is 100%, although it can drop to 55% around midday during the dry season, when toads reproduce (December–February) (**Figure 3**). In the maintenance tanks where we are recreating the rainy season to entice high feeding

and growth in preparation for reproduction, the automatic misting systems are set for three, 1-minute cycles each day to keep relative humidty between 75–85%. In the breeding tanks, where the adults are introduced to dry season conditions to trigger breeding, humidty drops to 65% with air temperatures between 19.9–22.8°C.

7.2.4 Water

Maintaining water quality is key for tadpole survival and the emergence of healthy toadlets. The mountain streams inhabited by Rancho Grande harlequin toads have soft waters, with temperatures of 18–20°C and pH 6.5–7, Ca^{++}<<1mg/L and Mg^{++}=19–20mg/L . However, low calcium content in water can lead to Spindly Leg Syndrome, a musculoskeletal abnormality characterized by underdevelopment of limbs (**Figure 6**) [26]. Because water quality can vary greatly depending on the source, it is often difficult to obtain the correct chemistry. For example, underground water tends to have high levels of iron and pH tends to rise rapidly upon contact with air. Despite the extra cost involved, we opted for reconstituted reverse osmosis water according to the recipe by Kevin Zippel [3] and modified by PARC to reduce the incidence of Spindly Leg Syndrome in metamorphs [26] (**Box 1**).

BOX 1: Water reconstitution recipe

$MgSO_4$ Anhydrous	11.6	mg/L
$KHCO_3$	35.8	mg/L
$NaHCO_3$	29.8	mg/L
$CaCl_2$	79.0	mg/L

Final readings: Ca^{++}=24–30mg/L; Mg^{++}=19–22mg/L; pH=6.85–7.2.0; TDS=140–150. (YSI 9300 Photometer)

Figure 7: **Spindly Leg Syndrome.** Low calcium content in water can lead to Spindly Leg, a musculoskeletal abnormality associated with captive-rearing characterized by underdevelopment of limbs.

7.2.5 Automation

Automation and remote control systems allow for a 24x7 monitoring of key processes with reduced personnel. We use wifi–operated relays (Shelly™) for programming light and mist switches, for real-time observation of the correct operation of mist, lights and air diffusion systems through power consumption data and for monitoring and controlling temperature remotely (**Figure 7**). Temperature is plotted on real-time and alerts are sent to phones if user-defined thresholds are reached or system fails.

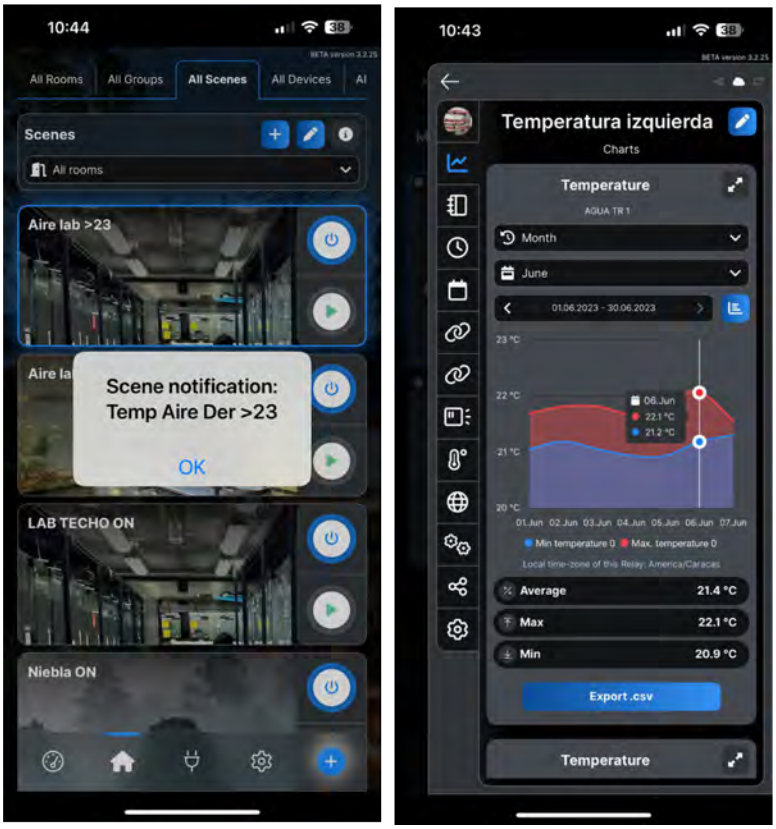

Figure 8: **Automation and remote control system.** Phone application for on-line control and monitoring of lights, mist, temperature and aeriation systems.

7.3 Diet

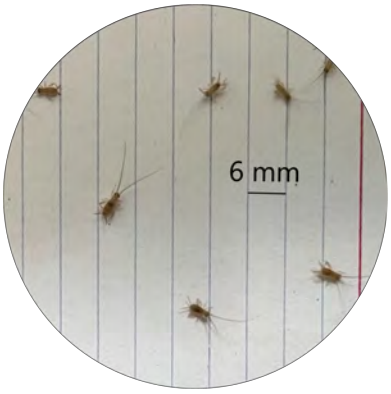

Figure 9: **Preferred size of prey items for adults of Rancho Grande harlequin toads (*Atelopus cruciger*).** Two-week old crickets (*Acheta domesticus*).

7.3.1 Adults and juveniles

In its natural habitat, Rancho Grande harlequin toads prey on a great variety of insects and arthropods. Twenty one types of items have been identified in stomach contents with the predominance of ants (Formicidae) and beetles (Coleoptera) [11]. In captivity, adults will eat ants, termites (*Isoptera*), crickets (*Acheta domesticus*), fruit flies (*Drosophila heydi*) or humpbacked flies (Phoridae). The preferred prey size for adults is 3–5 mm (**Figure 8**).

Adults are fed every other day. We offered between 8—12 items per adult, depending on the prey size. Females with visible eggs are also complemented twice a week with humpbacked fly larvae. The diet is supplemented by dusting or gut-loading with vitamins: vitamin A (Vitamin A Plus by Repashy™) every 2 weeks, calcium with D_3 (Calcium + D_3 by Exo-Terra™or Repashy™) in every meal and a micro-fine vitamin supplement (Supervite by Repashy™) once a week.

7.3.2 Tadpoles

Tadpoles are fed a 50/50 combination of Soilent Green™and dandelion. Despite using high levels of calcium in reconstituted water [26] (see BOX 1 readings), we continue to observe a low but persistent incidence of Spindly Leg Syndrome (SLS). Natural streams inhabited by Rancho Grande Harlequin Toad tadpoles have very low calcium levels ($CaCO_3$ <<1, YSI 9300 Photometer). Imbalanced diets have been suggested as potential contributors to increased SLS, but the specific relationship between SLS and individual nutrients remains unclear. We are still testing different diets to further reduce the incidence of this syndrome.

7.4 Breeding

The Rancho Grande Harlequin Toad tends to breed at the beginning of the dry season. Amplectant pairs are frequently seen close to the stream between mid-December and February. We do not fully understand which variables trigger breeding in wild populations. Although the average daily temperature and humidity changes little during the year, daily variations in temperature and humidity tend to be larger during the dry season compared to the wet season. For example, temperature can vary 5–7°C and humidity can drop from 100 to 50% between night and day (**Figure 3**). Thus, it is possible that day-night variations play an important role in triggering spawning. In captivity, toads will breed all year round.

Only mature males and gravid females are paired in breeding tanks. Mature males have nuptial pads on their hands and frequently vocalize. Gravid females can be recognized by a light-coloured abdominal mass that can be seen through the ventral skin (**Figure 9**). When paired in breeding tanks, males usually engage in amplexus with females in less than 24 hours. However, the first amplexus often fails, lasting less than two days. Males attempt a second amplexus after a few days and it is usually the definitive amplexus. Amplexus can last from days to months. Amplexus over 20 days are separated to avoid deterioration of male's body condition. Males can loose 60% of their body mass in 40 days. If females have reached phase 4–5 and suitable spawning sites are available, amplexus will usually last 3–5 days.

Figure 10: **Gravid female of Rancho Grande harlequin toad (*Atelopus cruciger*).** This female was non-gravid when collected in the field. After eight months in captivity, the oocyte mass occupied 2/3 of her abdomen.

7.5 Development and care of eggs and tadpoles

Breeding tanks with amplectant pairs should be checked daily for eggs. Females can develop between 400–2,000 oocytes within ovisacs, but we do not have data on how many of these are released in a single spawning. In captivity females have laid 60–564 eggs (5 spawning events). When eggs are detected, both parents should be removed from the breeding tank and placed

Figure 11: **Aquatic stages of Rancho Grande Harlequin Toad.** A. Embryos, B. Early stage tadpoles C. Stage 25–27 tadpoles. Photos: Margarita Lampo (A and B) and Jaime Culebras (C)

individually in small cages to recover. If the eggs are laid out in the open under direct light, then steps should be taken to shelter them from the light [2]; we cover the breeding tank with a black plastic bag. The water level of the breeding tank is raised to the maximum level. To prevent accumulation of nitrates of phosphates from waste material, 1/3 of the water is changed every other day.

Eggs are white coloured, about 1–1.5mm in diameter, and deposited in strings held together by the extremely sticky vitelline jelly. Non-fertilized eggs are frequently infected by fungi (*Saprolegnia*). If the percent of fertilized eggs is low, fungi (*Saprolegnia*) may also take over the few developing embryos. We do not know how to minimize the incidence of fungi in egg batches with many unfertilized eggs. At day 7, embryos from stages 14–19 are recognized (**Figure 10A**).Seven to 10 days after spawning, heads, tails and eyes may be recognized in some developing eggs (**Figure 10B**). Free-swimming non-pigmented tadpoles were observed at day 13. At stage 25,

tadpoles are fully pigmented (**Figure 10C**). Tadpole development can take from 98 to 135 days. Within two days of completing the development of all four limbs, metamorphs leave the water (**Figure 13**).

7.6 Development and care of toadlets

Toadlets start emerging from water after 98 days (week 14). Prior to week 14, water level should be dropped to expose rocks for toadlets can climb out of the water. Their sizes range between 0.5–0.7 mm. They are collected and transferred to plastic cages ($\sim$ 500 cm^2) in groups of 5-10. The greatest mortality risk at this stage is dessication. Cages should be lined with wet paper towels and/or leaf litter to provide humid conditions and hiding places. Because faecal pellets accumulate rapidly in cages with toadlets, substrate need to be changed every 2–3 days.

Figure 12: **Juveniles of Rancho Grande harlequin toad (*Atelopus cruciger*).** A two months-old juveniles born at CRIA. Photo: Jaime Culebras

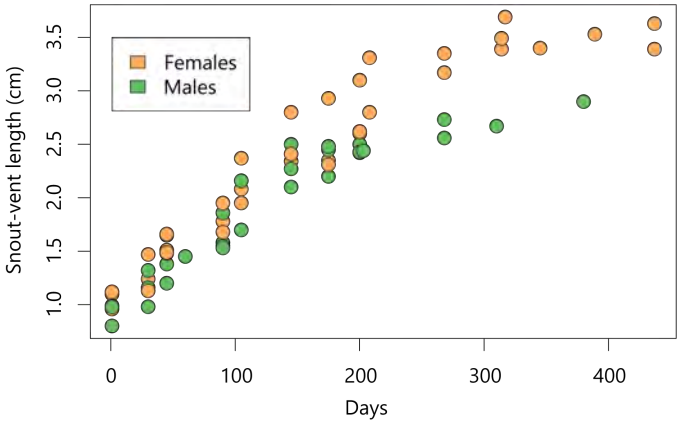

Figure 13: **Growth after metamorphosis (*Atelopus cruciger*).** Females grow faster and larger than males.

Toadlets below 10 mm are fed exclusively on springtails (Collembola) (**Figure 14**). Because toadlets grow from 7–20 mm during the first four months (**Figure 15**), they need to be fed daily with plenty of prey items. Pinheads crickets can be added to their diet after a month and fruit flies after 1 1/2 month. Calcium, which is essential for healthy growth in toadlets, is supplemented by dusting pinhead crickets and fruitflies and through skin absorption by placing one drop of a solution of 10% calcium gluconate on their backs twice a week.

7.7 Handling and transport

Strict biosecurity protocols are followed when handling or transporting toads. Adults and juveniles are always handled with disposable gloves to avoid cross contamination. In the field, toads

are placed individually in plastic bags with wet leaves. Tadpoles are placed in bags filled with water. Adults are transported in individual cages lined with wet paper towel and tadpoles are placed in groups in large plastic containers filled with water, with air supply through an aeration pump. Temperature during travelling is kept between 20–22°C. All animals arriving from the field enter directly to quarantine.

7.8 Quarantine

To avoid pathogen contamination, all animals collected in the field are kept individually in small cages in an isolated room for 30 days (**Figure 16**). Because *Bd* is present in source populations, all toads should undergo preventive treatment with Itraconazole during quarantine to prevent the development of chytridiomycose and transferring *Bd* to established animals. Because some species appear to be more sensitive than others, dosification should be done carefully. Adults Rancho Grande harlequin Toad are capable of withstanding 10 minute bath treatments for 10 days with 0.1 mg/ml, a concentration that has been proved effective to eliminate *Bd* in other species. We also tested 5 minute baths with 0.03 mg/ml for 10 days in infected small juveniles (7 mm SVL). Although they survived for two weeks after treatment was completed, infection reappeared in some juveniles. Liquid Itraconazole is not available in some countries, but tablets can be dissolved in saline solution if pulverized with a mortar.

Figure 14: **Itraconazole treatment during quarantine.** All toads are exposed to baths with 0.1 mg/ml solution of Itraconazole in individual lab mouse cages.

8 Acknowledgements

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