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 been sighted in almost two decades. 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 Intenational Union for the Conservation of Nature (IUCN). Here, we described guidelines for the husbandry and breeding of the Rancho Grande Harlequin Toad, based on preliminary 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.

Contents

List of Figures

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 been sighted in the past 17 years. 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] for *Atelopus varius/zeteki*, 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 our program is in its initial phase and new knowledge is expected to emerge, this first version will be adjusted and updated.

2 The species

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 ~20 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 30 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.

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.

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 [9]. 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 found 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 [10].

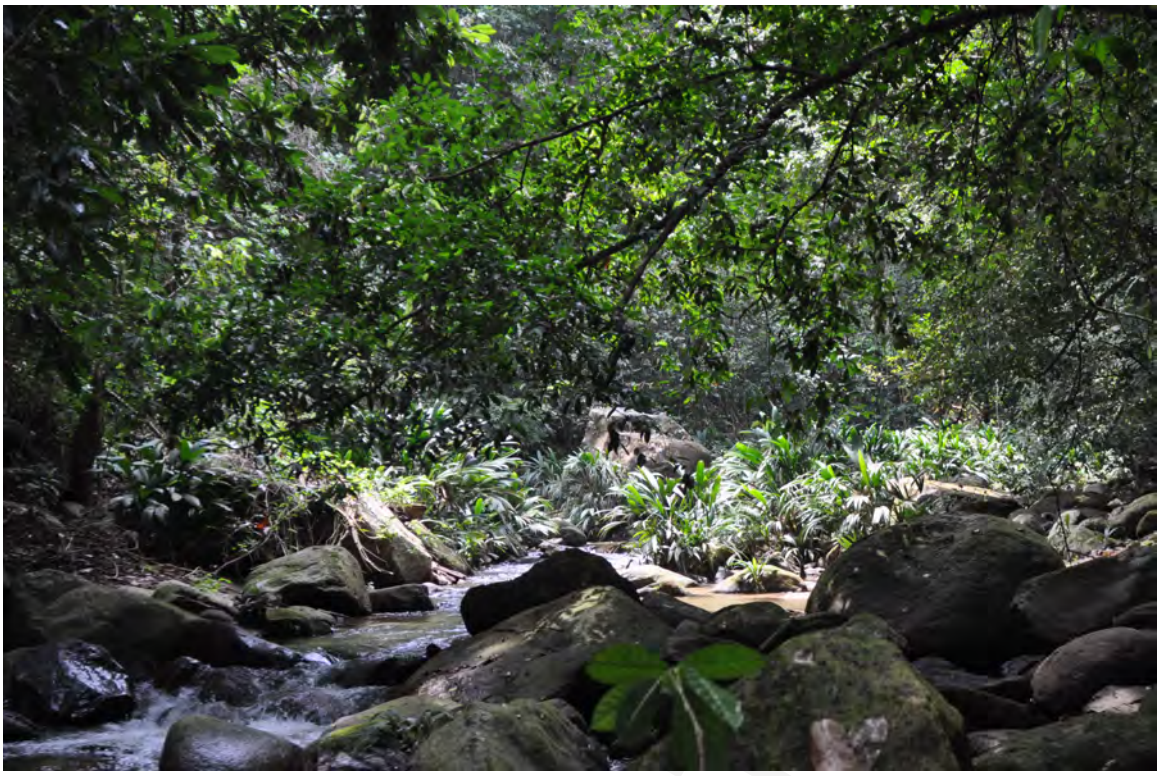

Figure 2: **Habitat of the Rancho Grande Harlequin Toad (*Atelopus cruciger*).** Lower section of Cata river, Aragua State, Venezuela.

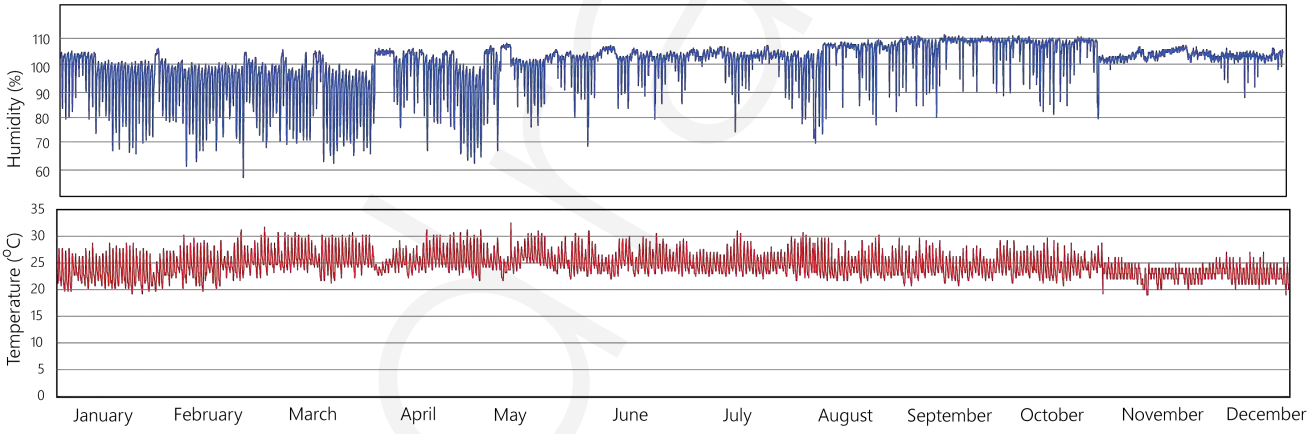

Figure 3: **Temperature and humidity in Cata river, Aragua state, Venezuela** Hourly measures between January and December of 2010.

4 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].

4.1 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.

4.2 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. After females lay their eggs. Partial water changes can be performed without disturbing the animals, by replacing the water in the plastic container.

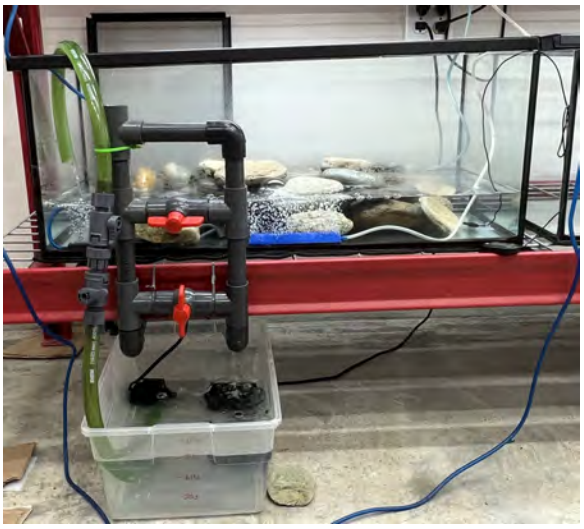

Figure 5: **Breeding tank.** Breeding couples and tadpoles are housed in these tanks. Each breeding tank has three air diffusion stones, an external water storage container with a filter and a pump, and a tubing system that allows for easy changing of water level and replenishing of tanks.

5 The environment

5.1 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.

5.2 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. 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) (**Figure 3**).

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 22°C as they descend from high mountain peaks. This means that, unless water chillers are used to control water independently of air temperatute ($$$), ambient temperature should not exceed 22 °C to keep water temperature close to 23 °C (1° above 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.

5.3 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 3, one minute cycles each day to keep relative humidty high. In the breeding tanks where the adults are introduced to dry season conditions to trigger breeding, the rate of misting is dropped in coordination with the air temperature with the air conditioning kept below 23°C.

5.4 Water

Maintaining water quality is key for tadpole survival and the emergence of healthy toadlets. The mountain streams inhabited by the Rancho Grande Harlequin Toad have soft waters, with temperatures of 18–20°C and pH 6.5–7. However, low calcium content in water can lead to Spindly Leg Syndrome, a musculoskeletal abnormality characterized by underdevelopment of limbs (**Figure 6**) [11]. 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 [11] (**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^{++}~24mg/L; Ca:Mg ~3:2; pH~7.0 TDS~150

Figure 6: **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.

5.5 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.

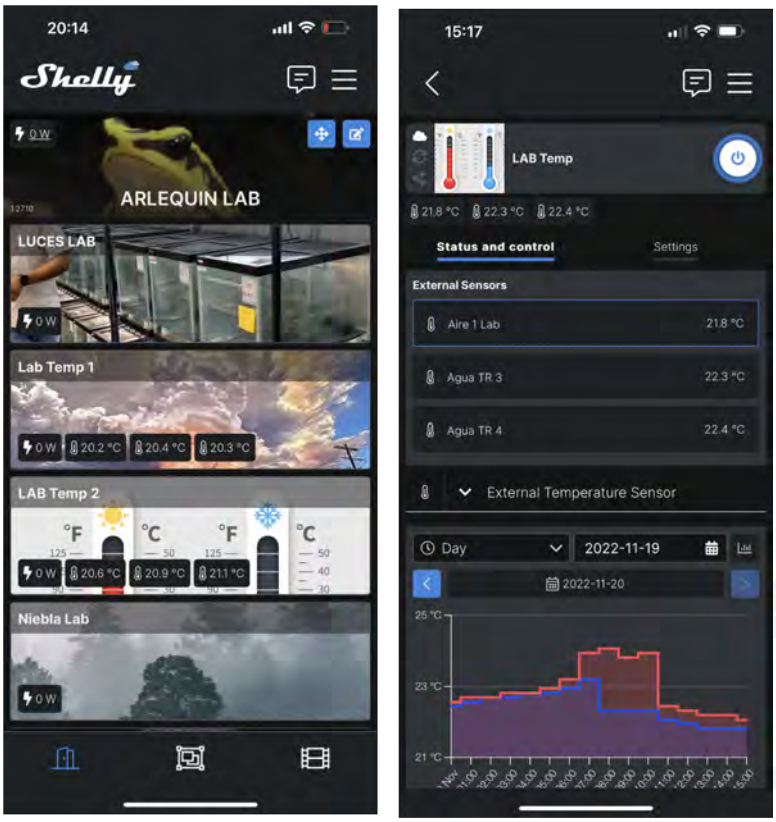

Figure 7: **Automation and remote control system.** Phone application for on-line control and monitoring of lights, mist, temperature and aeriation systems.

6 Diet

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) [10]. In captivity, adults will eat ants, termites (*Isoptera*), crickets (*Gryllodes sigillatus*), fruit flies (*Drosophila heydi*) or humpbacked flies (Phoridae). The preferred prey size for adults is 3–5 mm (**Figure 8**).

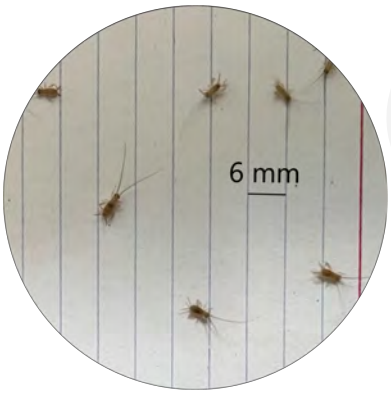

Figure 8: **Preferred size of prey items for adults of Rancho Grande harlequin toads (*Atelopus cruciger*).** Two-week old crickets (*Acheta domesticus*).

All adult and juvenile toads are fed daily. 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™) once a week, calcium with D_3 (Calcium + D_3 by Exo-Terra™) twice a micro-fine vitamin supplement (Supervite by Repashy™) twice a week.

Tadpoles are fed with Sera-Micron™, Sera-Spirulina™and, occasionally, with naturally cultivated algae. With powder or flaked Omicron or Spirulina, we prepare a paste, cover a plexiglass

plate with a thin layer, allow it to dry and place the plate within tanks. Plates are replaced as the food is consumed. Rocks covered with green algae are also occasionally placed in tanks. Algae will rapidly grow on rocks submersed in water and exposed to sunlight.

7 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. 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. We are currently varying room temperature between 19 and 23 °C.

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 usually fails, lasting less than two days. Males attempt a second amplexus after a few days and it is usually the definitive amplexus. We have had pairs amplexed for 40 days that we separated after males began to show poor body condition.

So far, only one female has laid eggs in captivity. This event occurred in January, in a enclosure at room temperature with a water chiller set at 20°C. The amplexus lasted 22 days and temperature during the days preceding spawning varied from 24 to 19°C between day and night .

Figure 9: **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.

8 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. During the only spawning event that we have seen in captivity, the female laid 40–60 eggs in strings. When eggs are detected, both parents should be removed from the breeding tank and placed 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].

The water level of the breeding tank is raised to the maximum level. To prevent accumulation of nitrates and phosphates from waste material, 1/3 to 1/2 of the water should be changed twice a week.

Eggs are cream colored, about 1-1.5mm in diameter, and deposited in strings held together by the extremely sticky vitelline jelly. Because fungi may develop in eggs presumably due to low exposure to water flow, air diffusers can be positioned to create current around eggs but taking care not to wash them off. Seven to 10 days after spawning, heads, tails and eyes may be recognized in some developing eggs (**Figure 10**). Free-swimming non-pigmented tadpoles were observed at day 13. On day 19, some tadpoles showed signs of pigmentation (**Figure 11**).

Figure 10: **Egg development in Rancho Grande harlequin toad (*Atelopus cruciger*).** Ten days after spawning, heads and tails are visible.

Figure 11: **Tadpole of Rancho Grande harlequin toad (*Atelopus cruciger*).** Nineteen-day old tadpole shows signs of pigmentation .

9 Toadlets

Toadlets start emerging from water after 15-16 weeks. Prior to week 15, 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: **Toadlet of Rancho Grande harlequin toad (*Atelopus cruciger*).** A two week-old toadlet feeding on springtails.

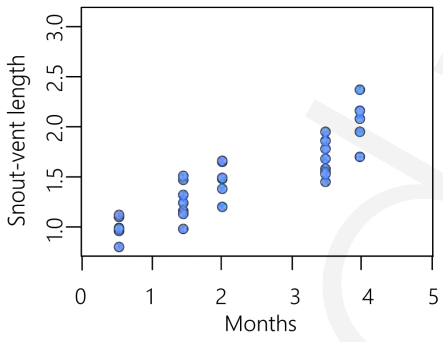

Figure 13: **Growth after metamorphosis (*Atelopus cruciger*).** Growth rates of toadltes of the Rancho Grande harlequin.

Toadlets below 10 mm are fed exclusively on springtails (Collembola) (**Figure 12**). Because toadlets grow from 7–20 mm during the first four months (**Figure 13**), 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 through skin absorption by placing one drop of a solution of 10% calcium gluconate on their backs twice a week.

10 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 14**). Because *Bd* is present in source populations,

all toads should undergo *Bd* testing and preventive treatment with Itraconazole during quarantine to prevent transferring the pathogen to established animals. Because some species appear to be more sensitive than others, dosification should be done carefully. The Rancho Grande harlequin toad are capable of withstanding 10 minute bath treatments for 10 days with 0.1mg/L concentrations, a concentration that has been proved effective to eliminate *Bd* in other species. However, we have not tested its effectiveness on infected individuals of *A. cruciger* yet. 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/L solution of Itraconazole in individual cages.

11 Acknowledgements

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