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Husbandry and captive reproduction of the giant Mexican leaf frog *Agalychnis dacnicolor*

ADAM W. BLAND*, ELLIE MCLAREN & ADAM TRIMMINGS

Chester Zoo, Moston Road, Upton-by Chester, Chester CH2 1EU, UK
*Corresponding author e-mail: a.bland@chesterzoo.org

ABSTRACT - The giant Mexican leaf frog, *Agalychnis dacnicolor*, is a large arboreal frog endemic to Mexico. This species was previously under-represented in European zoological collections and specific techniques for reproductive management under vivarium conditions little known. A group of four males and one female *A. dacnicolor* were maintained in captivity at Chester Zoo (Great Britain). To bring them into reproductive condition, they were subjected to three simulated environmental phases that differed in temperature, humidity and feeding regime. This proved successful so that two clutches of spawn, each containing 150-300 eggs, were deposited on leaves overhanging water. Tadpoles hatched from 4 days following oviposition with approximately 80 % success rate. They were reared at a water temperature of 27 °C to 29 °C and displayed no negative effects from living in high density. All tadpoles metamorphosed successfully and froglets with resorbing tails left water after about 32 days. Their tails were resorbed in a further 4 to 5 days at which time they began to feed; at least in the case of males, sexual maturity was reached after 10 months. This methodology will enable zoological collections in Europe to breed this species for potential conservation, research and educational purposes.

INTRODUCTION

The giant Mexican leaf frog *Agalychnis dacnicolor* (Cope, 1864) is a large hylid frog endemic to Mexico and on the IUCN red list is shown as of Least Concern (IUCN, 2021). It belongs to the subfamily Phyllomedusinae, previously placed within the monotypic genus *Pachymedusa*. Following a review by Faivovich et. al (2010) this species was consequently included within the genus *Agalychnis*, which currently comprises 14 species distributed widely throughout Mexico, Central America, and northern South America (Frost, 2021).

Agalychnis dacnicolor inhabits semi-arid subtropical Pacific lowland forest in Mexico (Duellman, 1970; Weiwandt, 1971). It is especially adapted to survive in hot environments with prolonged dry periods and in some instances has been observed to use humid rodent burrows to escape the heat (Weiwandt, 1971). The breeding cycle of this species is highly seasonal and has been reported to take place during the summer rains that begin in June and extend into August (Weiwandt, 1971). The seasonal rains bring temporary breeding ponds and a higher relative humidity, although day time ambient temperatures remain high and may reach 40 °C or above (Weiwandt, 1971). As with all species of *Agalychnis*, the spawn is attached to vegetation overhanging water where upon hatching the tadpoles drop into the pools below (Duellman, 1970). *Agalychnis dacnicolor* has also been observed laying eggs on land close to the water's edge; the hatching tadpoles use rapid movements of the tail to make their way over ground to water (Weiwandt, 1971).

As suggested by the common name, this is a large species; females have been recorded with snout to vent lengths of up to 103.6 mm, and males up to 73.1 mm (Duellman, 1970). When compared to other species of *Agalychnis*, it is noticeably heavy bodied, as highlighted in the naming of the previous genus *Pachymedusa*, of which *A. dacnicolor* was the only member; the etymology refers to the thick body of this species (Duellman, 1970). The species is also sexually dimorphic; the snout of the male is more pointed and narrow than that of the female (Duellman, 1970), and in the breeding season males develop dark brown to black triangular nuptial thumb pads (Bagnara & Rastogi, 1992).

Weiwandt (1971) made detailed field observations of the reproductive biology of this species in Sonora, Mexico. The species was also bred in captivity during the 1980s and early 1990s at the University of Arizona during a time when it was more readily exported from Mexico. The physiological processes of both sexes during their reproductive cycles have been described (Bagnara & Rastogi, 1992), as well as pigmentation (Iga & Bagnara, 1975; Bagnara, 1985) and aspects of endocrinology (Bagnara, 1990). However, the specifics of a methodology to bring this species into breeding condition in captivity when maintained within an indoor vivarium are lacking, as the population maintained at Arizona University were maintained and reproduced under semi-natural conditions within a greenhouse, where temperatures and light cycles were similar to that of the natural habitat in Mexico (Bagnara & Rastogi, 1992). The current study provides details of husbandry and environmental conditioning required to induce captive breeding in *A. dacnicolor*.

MATERIALS & METHODS

In 2018, a group of adult *A. dacnicolor* consisting of four males and one female was donated by the Manchester Museum to Chester Zoo. In order to bring them into breeding condition, they were subject to three distinct environmental phases

(summarised in Table 1) from October 2019 to June 2020. Since the frogs were of unknown origin these environmental phases were developed following previous observations of reproduction in this species (Weiwandt, 1971; Bagnara & Rastogi, 1992).

Environmental phases to induce breeding

Phase 1

From October 2019 to April 2020, the group were maintained in a 90 cm (H) x 60 cm (W) x 45 cm (D) Exo Terra vivarium (Rolf C. Hagen Inc.). Thick branching was provided for perching and climbing areas, no substrate was used and a potted *Dracaena fragrans* was used to provide cover and additional arboreal perching. The floor of the enclosure contained a large water tray measuring 24 cm x 18 cm x 8 cm which was replaced daily along with the removal of waste from any glass surfaces within the enclosure. The vivarium was illuminated with a 6 % T5 Arcadia fluorescent lamp (Arcadia Products, Monkfield Nutrition Ltd) providing an ambient UVB index gradient of 0 – 3 (Readings taken using a Solar Meter® 6.5). The ambient daytime temperature fluctuated between 24 °C – 28 °C with a night time drop in temperature reaching 18 °C – 20 °C. In addition to this, a basking site was provided consisting of a Solar Raptor 50 W high output lamp (©ECONLUX GmbH) combined with a 50 W Arcadia deep heat projector positioned over branching. This provided a localised area (approx. 25 cm^2) of increased heat and UVB during daytime hours. The temperature range in this area was between 29 °C – 35 °C and UVB index range accessible at the level of the dorsum of a basking frog was 2.0 (outer basking zone) to 4.0 (central basking zone). The frogs were provided with a 12:12 photoperiod and the enclosure was lightly misted with water every second day; ambient relative humidity of 40 % – 50 % was maintained during this period. Food was offered three times per week and consisted of Dubia cockroaches (*Blaptica dubia*), black field crickets (*Gryllus bimaculatus*), banded crickets (*Gryllodes sigillatus*) and locusts (*Schistocerca gregaria*). All live foods were gut loaded with fresh vegetables and Repashy Super Load gel (Repashy Specialty Pet Products ©) 24 h prior to feeding, and supplemented directly by dusting with Repashy Calcium Plus.

Phase 2

For the month of May 2020, the group were subjected to an increase in temperature and ambient humidity. They were moved into a larger custom made glass vivarium (dimensions 120 cm H x 120 cm W x 45 cm D) with one third mesh ventilation on the roof. The vivarium was furnished with thick branching to provide perching and with live potted plants (*Ficus elastica* and *Philodendron* sp.) for cover, additional perching, and potential oviposition sites. The ambient temperature was increased to 28 °C – 34 °C during the daytime, with a night time drop in temperature to 20 °C – 22 °C. To raise the temperature in the basking zone the original basking lamp was replaced with a 160 W Arcadia D3 lamp as before positioned over the branching to give a basking zone (now approx. 30 cm^2) with temperatures of 38 °C – 42 °C and a UVB index of 3.0 – 4.0. The general enclosure lighting was provided as detailed in phase one. The ambient humidity was increased to 60 % - 70 % by providing a daily misting with water in the afternoon, and fresh water was provided in a large water tray which was replaced daily. This enclosure did not contain substrate and surfaces were cleaned on a daily basis. During this phase, the frogs were fed more frequently (4 – 5 times per week) using the same food items as detailed previously. The photoperiod also remained the same.

Phase 3

The final environmental phase simulated the rainy season and commenced on 1st June 2020 for two weeks. In this phase, the frogs were not fed and the base of the enclosure was filled with 10 cm of water at room temperature (20 °C – 21 °C). All water was filtered (HMA - Heavy Metal Axe) tap water with a pH of 7.4 at source (tested with API® Freshwater Master test kit and colour chart). The enclosure was not misted during this time and after the first 48 h the water was heated to 27 °C – 29 °C using a 100 W submersible aquarium water heater. The increase in water temperature resulted in an increase in ambient humidity to above 80 %, causing condensation to form inside the enclosure. Further 'rain' sessions over the frogs were created manually using a hosepipe. This increased the water depth of the enclosure to 20 cm. The water was allowed to drain away completely and was then re-filled using the hose rain method up to four times in one rain session. Rain sessions lasted approximately 30 minutes and were provided twice daily for five days, and subsequently every second day for the following nine days. Upon successful oviposition this phase was terminated.

Table 1. A summary of the parameters used for three sequential environmental phases leading to breeding in captive *Agalychnis dacnicolor*

	Phase 1 (Oct 2019 - April 2020)	Phase 2 (May 2020)	Phase 3 (June 2020 for 2 weeks)
Ambient temperature	Day: 24 – 28 °C Night: 18 – 20 °C	Day: 28 – 34 °C Night: 20 – 22 °C	Day: 28 – 34 °C Night: 20 – 22 °C
Feeding	3 times weekly	4-5 times weekly	Not fed
Basking temperature	29 – 35 °C	38 – 42 °C	38 – 42 °C
Relative humidity	40 – 50 %	60 – 70 %	> 80 %
UVI range	0.0 - 4.0	0.0 - 4.0	0.0 - 4.0
Water provision	Localised water bowl, light misting on alternate days	Localised water bowl, light misting daily	Flooded enclosure base, hose rain method 30 minutes twice daily
Photoperiod	12:12	12:12	12:12

Care of eggs, tadpoles and metamorphs

Following oviposition the adult frogs were removed from the enclosure separated by sex, and then housed as detailed in Phase 1. The spawn was left in situ to develop in the breeding enclosure. The first developmental stages of the eggs could be observed within 12 h of oviposition. Development was completed by the fourth day when tadpoles began to hatch. The hatching was aided by lightly misting the spawn with water which promoted tadpole emergence from the egg capsule. This process took place over a 24 h period after which all tadpoles were free swimming and had begun to feed.

Tadpoles remained within the flooded breeding enclosure for rearing in 110 L of filtered (HMA) tap water, giving an estimated density of three tadpoles per litre.

Tadpoles were fed twice daily on a mixed diet of Repashy Soilent Green gel, tropical fish flakes, tinned spinach leaves, Arcadia Amphibigold Pellets and frozen thawed bloodworm (Chironomidae). Tadpoles also grazed on algae growing on the glass walls of the aquarium. Daily water changes of up to 50 % were undertaken to manage the waste produced by tadpoles and the uneaten food. Water temperature was maintained between 27 °C – 29 °C and the pH between 7.4 – 7.6. Due to the density and metabolic rate of the tadpoles a large amount of nitrogenous waste was produced consistently, with levels of nitrates routinely recorded at 80 – 160 ppm (tested using API® Freshwater Master test kit and colour chart).

Metamorphosed young were housed in 45 cm (H) x 45 cm (W) x 45 cm (D) glass Exo Terra vivariums each with a small water bowl, branching, and potted *Spathiphyllum* sp. and *Epipremnum aureum*. Enclosure lighting, UVB and ambient temperature range was provided as detailed in Phase 1, a high temperature basking area of approximately 35 °C was provided via a 35 W halogen spot lamp. For food, 2nd and 3rd instar brown crickets, dusted with Repashy Calcium Plus, were offered daily.

RESULTS

During environmental Phase 1 the group maintained good health and body condition but showed no signs of reproductive readiness or activity. Males did not develop dark nuptial thumb pads or attempt amplexus with the female. It was presumed that nocturnal activity was minimal due to the lack of signs of activity within the enclosure, such as prints and waste on the glass of the enclosure. Individuals were also often found positioned in the same refugia for consecutive days. Despite low activity during this phase, food was readily consumed after each feeding event.

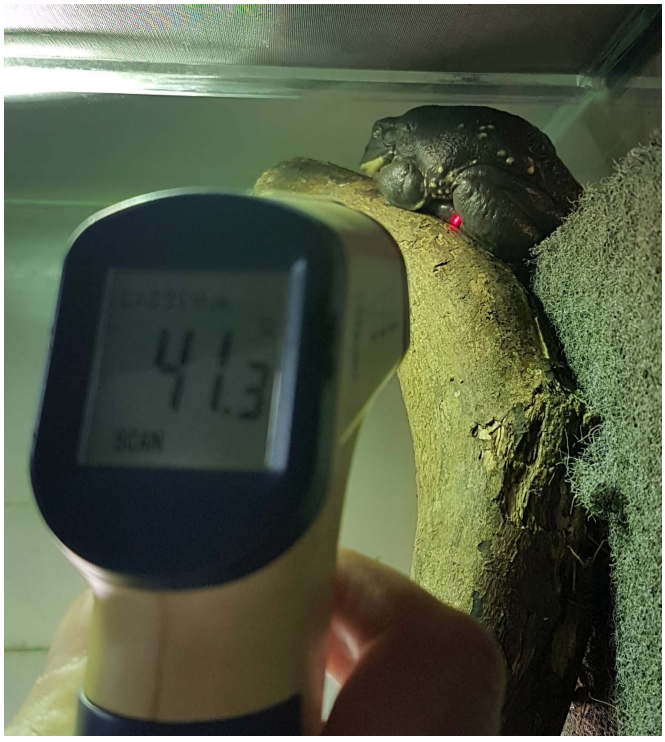

Figure 1. Adult male *Agalychnis dacnicolor* actively basking during the daytime at 41.3 °C

Figure 2. *Agalychnis dacnicolor* spawn attached to a *Ficus elastica* leaf hanging above water

Figure 3. Metamorphosing *Agalychnis dacnicolor* at Gosner stage 44 (Gosner, 1960) following 32 days aquatic larval development

After being transferred into Phase 2, activity appeared to increase within the group. Individuals were positioned in different resting areas on a daily basis and there was an increase in the amount of waste produced within the enclosure. During day time hours individuals were regularly observed resting in the basking area for prolonged periods at 40 °C – 42 °C (Fig. 1). Although nuptial thumb pads were not evident at the start of this phase, by one week these pads were observed, initially as light brown patches on the thumb of each male and by the third week as dark brown/black swollen nuptial thumb pads.

During Phase 3, males in the group were observed making advertisement calls during the daytime, and amplexus was initiated with the female intermittently during the initial rain events. After five days a male initiated a prolonged amplexus, during this time the female appeared noticeably swollen and began to display restless behaviour, pacing the enclosure carrying the male in amplexus during daytime hours. On the fourteenth day two clutches of spawn were found attached to the leaves of *F. elastica* 10 –15 cm above the water level. Each clutch contained approximately 150 – 300 eggs (Fig. 2) and about 80 % of the eggs gave rise to tadpoles.

Development of the tadpoles was rapid and froglets with resorbing tails emerged from the water from as early as 32 days post hatching (Fig. 3) and in the following 4-5 days tails were fully resorbed. All tadpoles metamorphosed successfully. Once tails had been resorbed, the froglets started to feed readily, began congregating in the high temperature basking area during daytime hours, and grew rapidly. After ten months, individuals reached adult size and the males developed dark nuptial thumb pads, indicating sexual maturity, however these young adults were not exposed to Phase 3 conditions as we did not wish them to spawn at this time.

DISCUSSION

In nature, *A. dacnicolor* requires extreme seasonal changes before it will reproduce. Until males are maintained at above 33 ∞C the hormonal processes that enable spermatogenesis are not triggered (Bagnara & Rastogi, 1992) and in the current study it was not until during Phase 2, when males were maintained consistently above this temperature, that nuptial thumb pads developed. The presence of these nuptial pads was used as an indicator of readiness to begin Phase 3, the 'rainy season'. Similarly, the maturation of ova in females is also linked to seasonal temperature cycles (Bagnara & Rastogi, 1992).

This prolonged period of reproductive development is unlike other members of *Agalychnis*, which are often opportunistic and explosive in their breeding habits (Scott & Starret, 1974; Roberts, 1994), with females in captivity capable of producing spawn several times annually (Bland, 2013). The dependence on a specific and quite extreme seasonal cycle illustrates the environmental pressure upon this species, which is likely only able to reproduce once per annum. This appears to be mitigated by producing a large amount of spawn and subsequently many offspring before seasonal ponds dry out. When compared to congeneric species of *Agalychnis*, *A. dacnicolor* produces on average larger clutches of eggs, with the mean recorded clutch size being 467 (Duellman, 1970). This dependence on seasonality is also a limitation to the frequency with which the species can be bred in captivity, as it would appear that a full seasonal cycle is required for successful breeding.

Experience based on this group of frogs suggests that this species is relatively hardy when maintained in captivity providing that its environmental needs are met. In the case of the tadpoles, regular partial water changes were sufficient for managing the biological load of the rearing aquarium. In nature, tadpoles would become more crowded as temporary breeding ponds start to dry out (Weiwandt, 1971). This predisposes them for situations in captivity where tadpoles may be maintained in high densities, in water containing a significant nitrate load, as this appeared not to have negative impacts. Indeed, the tadpoles of this species display adaptations such as unusually long gills, likely in response to the environmental constraints of the natural breeding ponds (Morrisett, 1986).

Following an analysis of record data from the Zoological Information Management System (ZIMS), which is used by zoological collections worldwide, it appears that this breeding success represents the first captive reproduction of this species in a European zoo. It is also the only reproduction amongst all current ZIMS users within the last eighteen years. The methodology detailed herein will be of use to zoological collections wishing to maintain populations of this species, and perhaps even to collections wishing to work with *A. dacnicolor* as a model species for research (Bagnara, 1990).

Given the specific environmental needs of *A. dacnicolor*, this species will become vulnerable in the future if climate change results in prolonged droughts in Mexico (Seagar et. al., 2008; Sodhi et. al., 2008). This research to gather knowledge about captive maintenance of *A. dacnicolor* may benefit future conservation efforts and will enable zoos to maintain educational exhibits that raise awareness of the global amphibian crisis.

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