A record of captive reproduction in the Red bellied toad *Melanophryniscus klappenbachi* with notes on the use of a short-term brumation period

ADAM W. BLAND

The Manchester Museum, Oxford Road, Manchester M13 9PL
Email: adam.bland@manchester.ac.uk

INTRODUCTION

The South American genus *Melanophryniscus* is represented throughout Argentina, Bolivia, Brazil, Paraguay and Uruguay by 26 species (Frost 2013) of small to medium sized Bufonids. These are collectively and commonly referred to as the red-bellied toads, as many species possess red or orange flash markings upon their ventral surface and the undersides of the hands and feet. Some species are also in possession of a brightly coloured dorsum; much like members of the *Dendrobatidae*, although most remain less conspicuously marked. These toads are mostly diurnal, spending the day foraging for small invertebrates. Their reproduction is seasonal and opportunistic; many species occur in usually dry habitats where explosive breeding takes place after heavy rains, where many individuals emerge to reproduce in temporary water bodies (Aquino et al. 2004, Garcia et al. 2004, Lavilla et al. 2004). The resulting larvae have been known to display rapid development to ensure metamorphosis before the disappearance of these breeding sites in the usually dry environment (Kurth et al. 2013).

Melanophyniscus klappenbachi was described in 2000, following taxonomic separation from *M. stelzneri* (Prigioni & Langone 2000) and occurs in shrubland habitats within the Chaco region of Argentina and Paraguay at elevations of 50 to 100 masl. It is a small diurnal species, reaching an average adult size of 2.5 - 3cm. The dorsal colouration consists of irregular yellow blotches on a black base colour (Fig. 1). This species is considered locally common within its range (Aquino et al. 2004). At the time of writing it is present within the international pet trade, and although this is not currently considered to be a major threat to the species, methods to produce regular captive reproductions should be established as to reduce the collection of wild specimens to be maintained in captivity. This species does not seem to be regularly reproduced within zoological collections (pers. obs.), and developing methodology to reproduce this more common species of *Melanophryniscus* in captivity may also benefit future conservation efforts for more threatened related species.

Kurth et al. (2013) report captive reproduction of this species, although only describe briefly the methods used to induce a successful spawning and subsequent rearing of offspring, focusing on the description of the species larvae and development. This included providing a three week brumation period for all specimens prior to breeding. This paper describes a method used to maintain and successfully reproduce *Melanophryniscus klappenbachi* in captivity using a much shorter brumation period for the female specimen only; the benefits of this method are discussed.

Figure 1: Adult *M. klappenbachi* showing black and yellow aposematic colouration.

METHODS

The adult group of *M. klappenbachi* used in this instance comprised two males and one female maintained within the Manchester Museum Vivarium. They were housed in a naturalistic vivarium measuring L 46cm X H 30cm X W 39cm, which was furnished with *Philodendron scandens*, flat rocks were provided which the toads used as shelters and also served as open feeding areas. A substrate mixture of soil and sphagnum moss was used above a drainage layer of loose clay balls. The top layer of substrate was furnished with leaf litter and sheet moss, which was also frequently utilised as shelter by the toads. A shallow water dish was available at all times and was replaced daily. Temperatures maintained fluctuated between 22–26˚C and UVB was provided through the use of Arcadia 6% T5 lighting with a photoperiod of 12/12. The humidity was maintained between 50-60% through a light daily misting provided by hand and the toads had access to fresh water via their water bowl if needed. Food was provided daily and consisted of hatchling crickets, *Drosophila* sp, and tropical springtails (*Siera* sp). All food offered was supplemented with Repashy Calcium Plus and

Figure 2: Newly metamorphosed *M. klappenbachi*.

Figure 3: Basic rearing containers used to house newly metamorphosed *M. klappenbachi.*

gut loaded with fresh fruit and vegetables when possible.

To attempt inducing reproductive behaviour in the specimens it was decided to follow Kurth et al. (2013) and provide a winter brumation period where the temperature was reduced to 4 - 9˚C. In the description of this period by Kurth et al (2013) the brumation of specimens lasted three weeks and was provided for both males and females. In this instance, it was decided to provide this temperature reduction but only for the female whilst the males remained in normal conditions, and this period was only provided for four days. The female *M. klappenbachi* was housed within a ventilated container containing 10-15cm of sphagnum moss and placed within a refrigerator where temperatures fluctuated between 5-8˚C. Each day the toad was inspected to ensure no negative effects of conditioning were to be observed and as expected activity was reduced almost completely. Upon being removed from the refrigerator after the four day conditioning period was over, the female *M. klappenbachi* was then placed directly back into the regular vivarium where within minutes she became active and even began to feed. All specimens remained within normal conditions for a further four days allowing the female to feed and reacclimatise until all being placed within a semi-aquatic rain chamber.

The rain chamber created for this species measured L 60cm X H 45cm X W 45cm and contained 10cm of water maintained at temperatures between 20-25˚C. Floating pieces of cork bark were used as land areas and the water was well planted with *Elodea densa*, densely filling the rain chamber with plants aided the toads when moving through the water as they were not adept swimmers for long periods, they also provided egg deposition sites. An aquarium pump was used to circulate the water as artificial rain over the toads during the daytime hours between 10.00am and 4.00pm.

RESULTS

All three specimens were introduced to the rain chamber simultaneously and reacted to the change in environment immediately. Within 20-30 minutes both males began to call and actively pursued the female. Amplexus was achieved within 2 hours of introduction to the rain chamber, the amplectant pair continued to pace around the enclosure whilst the single male persisted with loud calls and attempts to displace the successful male.

After 24 hours egg deposition began and multiple clumps of spawn each containing in excess of 20 individual eggs were deposited in various areas of the rain chamber, attached to the aquatic plants and submersed areas of cork bark. During spawning and fertilisation the male cradled the eggs with his feet and carefully attached them to the chosen surface. The aquarium pump was turned off at this time to reduce the risk of rainfall causing damage or displacement of the eggs. Once the female finished depositing eggs, approximately two hours after egg deposition began, the male remained firmly in amplexus and they were separated in an effort to avoid the risk of exhausting the female. At this stage all specimens were then placed back into their regular vivarium, where breeding activity almost immediately ceased; short intermittent calls were heard as they began to settle back into their regular environment. The spawn was left to develop within the rain chamber.

Egg and larval development followed the description by Kurth et al (2013) with larvae hatching after 48 hours and developing to metamorphosis in a relatively short amount of time. The larvae remained in the rain chamber for the duration of their development. No water filtration was used, but instead 50% water changes were undertaken every 24-48 hours and an air pump was used to oxygenate the water. The diet for the larvae consisted of finely powdered foods such as sera micron and powdered Spirulina algae dusted onto the surface of the water twice per day. The larvae spent much of their time clinging to the sides of the aquarium or objects in the water using their specialised mouthparts. The water temperature was maintained at 25˚C, which appeared to ensure healthy and consistent growth. The first specimen metamorphosed after 19 days of development, although others took double this length of time to metamorphose. When leaving the water, the toadlets climbed out onto the floating cork without any problem and care was taken to ensure newly emerged young were removed promptly as to avoid the risk of drowning if they were to fall back into the water; during the peak of metamorphosis the aquarium was inspected regularly.

The newly metamorphosed young measured between 5-7mm and lacked the colouration seen in the adults; they were mostly uniformly black in colour (Fig. 2). They were

housed in small partially ventilated plastic containers measuring L 15.5cm X H 6cm X W 7cm with a substrate of damp paper towel and moss for shelter (Fig. 3). Groups of up to 10-11 toadlets were housed in these containers. The containers were lightly misted with water once per day and the damp paper towel was replaced every second day. Feeding began 24 hours after metamorphosis and was provided by supplemented tropical springtails (*Siera* sp.) of varying sizes; young and adult springtails were readily consumed although young toads were unable to consume hatchling crickets. The use of the small rearing containers appeared essential in successful rearing of young, as this enabled toadlets to easily locate their prey items. It was found that feeding opportunities were required constantly for toadlets to maintain and support growth and body condition, large quantities of supplemented springtails were required daily and under this regime initial growth during the first 7-14 days was rapid. Early stages of the development of adult colouration also appeared during this time. Temperatures provided were between 22–26˚C, as with the adults. Size of rearing container was increased according to growth until specimens could be housed in naturalistic vivaria as used with the adults. This was achieved when young toads reached over 1cm in total length, at which time they also began to display their full adult colouration (Fig. 4) and feed upon hatchling crickets.

Figure 4: Adult colouration showing in toadlets measuring over 1cm in total length.

DISCUSSION

There was little sexual dimorphism between the specimens and males were not often observed calling outside of breeding conditions; only infrequent short calls were observed occasionally when in normal conditions. The most useful characteristic to identify sex was size; females are slightly larger than males, the female measured only 4-5mm larger in SVL than the males of this group. Compared directly with one another the female was easily recognisable. It was also found that this species is one that prefers to feed on extremely small food items, any food item offered larger that a 1st instar cricket or even the larger *Drosophila hydei* was a struggle for the adult *M. klappenbachi* to easily swallow and usually refused. It was also evident that to successfully raise toadlets it was essential to provide large quantities of small supplemented invertebrates to satisfy their voracious appetites and high metabolism during this age. Failure to do this will result in inconsistent weights during growth, metabolic problems and loss of specimens. Establishing cultures of tropical springtails in advance to maintain a constant supply was of high importance.

This species does not seem to fare well when maintained within a too humid or wet environment for long periods; as previously mentioned they are inhabitants of sub-tropical shrublands Once settled within the vivarium these mostly diurnal toads were observed to utilise hide areas within the enclosure underneath rocks, foliage and even small burrows in the substrate seemingly made by the toads themselves; although this behaviour was not observed. Activity at times was quite low and localised around their chosen hides where they would be observed feeding; although they would occasionally make use of the floor space of the vivarium during their periods of activity, deciding to rest and feed out in the open. During reproduction presumed multiple paternity was also observed as the competing male amplexed with the pair whilst the female began to spawn.

The decision to induce brumation and attempt breeding was only made when the toads maintained seemed in peak condition and of excellent health. Providing a short brumation period for only the female was a decision made after a previous attempt at reproduction using the same method, although without providing any brumation beforehand, was unsuccessful. This resulted in excellent reproductive behaviour in the male specimens although the female produced no spawn. It was clear that the males did not seem to require a temperature reduction to induce reproductive behaviour and it was shown that they were capable of successful egg fertilization without brumation; the change to a semi aquatic environment was a sufficient stimulus. However, a short brumation period seemed important to stimulate egg production in the female. It was noted that during this period the female began to swell and became noticeably rounder in appearance within 48 hours of being inside the refrigerator.

The length of time the female was maintained at low temperatures in this instance was also significantly shorter than provided by Kurth et.al (2013), showing that providing low temperatures for multiple weeks is not necessary for a successful reproduction and is also not required for both sexes but for females only. Species such as this that occur within a relatively dry environment depending on temporary breeding sites and which also show large seasonal and regular temperature fluctuations must become opportunistic in their reproductive habits. This species seems to remain opportunistic in captivity in its willingness to reproduce, and providing that temperature fluctuations are within the parameters that they would naturally experience in the wild, it may not be necessary for the cold brumation period to last quite as long as it does in situ. As the short term temperature reduction seems to be a sufficient stimulus, and the length of time it persists for in the wild is a constraint of nature, as the toads will explosively breed immediately once conditions are favourable. Therefore, in the captive environment it would seem that providing that the temperature reduction they

would naturally experience in the wild is given, it would not be required to last as long.

Using this short term brumation period method for females may be beneficial for the specimens in the long term, as it provides successful reproductions without the potential health risks of wintering all adult specimens for extended periods of time. This method has been used multiple times with the same success in the Manchester Museum during 2014. Further investigation would be required to study the use of this method on other species inhabiting similar environments that also breed opportunistically and explosively, as it may prove beneficial in the success of their captive breeding, particularly those of conservation concern where ex-situ conservation breeding may be necessary and have little margin for error. This method could produce healthy captive reproductions without unnecessarily risking the health of important specimens by inducing a long term brumation period. It may also be used to quickly induce reproduction as and when the specimens are healthy and in good condition to breed, as creating a prolonged season that would lengthen the process would not be necessary. This method has shown to yield healthy larvae as early as twelve days from the beginning of the brumation period, as apposed to the process extending over one month, and has also shown no negative effects or health issues in the breeding adults which remain much of their time within stable conditions.

M. klappenbachi is currently listed as Least Concern by the IUCN and considered to have a stable population within its natural range (Aquino et al. 2004). However, the explosive breeding habits of this species, and other members of the genus, lend themselves to rapid population declines should an infectious disease such as *Batrachochytrium dendrobatidis* (Bd) become prevalent within their natural environment. As large aggregations of breeding adults coming together in water bodies could consequently spread infection and potentially lead to die offs of adult individuals, causing a sudden population decline. It is reasons such as this, which make it important to establish proven methods of captive reproduction should ex-situ conservation programmes become required for these species in the future. It is also possible that methods established using a more common species of a genus may be of use for these purposes for the more threatened related species. Of the 23 assessed species of *Melanophryniscus*, three species are considered to be critically endangered; *M. admirabilis*, *M. langonei* & *M. peritus* (IUCN 2015) and it is possible that knowledge of the requirements of one species may prove transferrable to another with regards to conservation breeding.

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