



Captive management, reproduction, and comparative larval development of Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachii* Prigioni and Langone, 2000

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Abstract.—In this study, we report on the successful keeping, breeding, and rearing of Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachii* Prigioni and Langone, 2000. Breeding and spawning took place after a relatively dry period without using a brumation period. To initiate mating behavior the toads were introduced into a rain chamber with a raised water level and constant irrigation in accordance with the toad's natural habitat and heavy rainfalls. The fast developing tadpoles started metamorphosis after 19 days at a constant water temperature of 23 °C and pH values between 6.5 and 7.9. A higher pH value led to slightly faster growth irrespective if tadpoles were reared singly or in groups.

Keywords. Amphibians, Anura, captive breeding, conservation breeding, environmental factors, spawning

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Introduction

The genus *Melanophryniscus* Gallardo, 1961 is currently represented by 29 species which have been reported from southern Bolivia and southern Brazil in the north over Paraguay and Uruguay and northern Argentina in the south. They are commonly referred to as South American Redbelly Toads due to the red or orange flash markings upon their ventral bodies, hands, and feet (Frost 2016). Species of this genus have been divided into three phenotypic species groups based on morphological characteristics: the *Melanophryniscus tumifrons*, *M. moreirae*, and *M. stelzneri* groups (Cruz and Caramaschi 2003). Klappenbach's Red-bellied Frog (*M. klappenbachii* Prigioni and Langone, 2000) is part of the *Melanophryniscus stelzneri* group (Cruz and Caramaschi 2003), which currently includes eight more species, i.e., *M. atroluteus* (Miranda-Ribeiro, 1920), *M. cupreuscapularis* (Céspedes and Alvarez, 2000), *M. dorsalis* (Mertens, 1933), *M. fulvoguttatus* (Mertens, 1937), *M. krauczuki* (Baldo and Basso, 2004), *M. montevidensis* (Philippi, 1902), *M. rubriventris* (Vellard, 1947), and *M. stelzneri* (Weyenbergh, 1875).

Klappenbach's Red-bellied Frog is characterized by a yellow stripe between the eyes or two to three large yellow blotches forming a distinct interocular band. Its dor-

sal and ventral surfaces are covered with small and large, irregularly formed yellow blotches on a black base color (see Fig. 1A and B) (Kwet et al. 2005). Adults of this diurnal species reach an average size of 2.5 to 3.0 cm (Prigioni and Langone 2000). *Melanophryniscus klappenbachii* inhabits usually dry environments, such as shrubland habitats, in north-eastern Argentina and Paraguay at 50 to 100 m above sea level (Bland 2015; Frost 2016). After heavy rainfalls explosive breeding takes place in the emerging ephemeral water bodies (Aquino et al. 2004). Fast development of tadpoles increases the probability of completing metamorphosis before water bodies dry out (Kurth et al. 2013).

Currently Klappenbach's Red-bellied Frog is listed as Least Concern by the IUCN Red List of Threatened Species, due to its wide distribution, large and stable populations, and its tolerance for habitat modification (Aquino et al. 2004).

Although it was once a popular species in the pet trade it does not seem to be regularly reproduced in captivity (Bland 2015; Aquino et al. 2004). Amphibians are one of the most threatened animal taxa with more than one third of the currently described species (ca. 7,520) recognized as threatened with extinction (Frost 2016; Hoffmann et al. 2010; Stuart et al. 2004). For threatened species *ex situ* captive-breeding programs are relevant instruments

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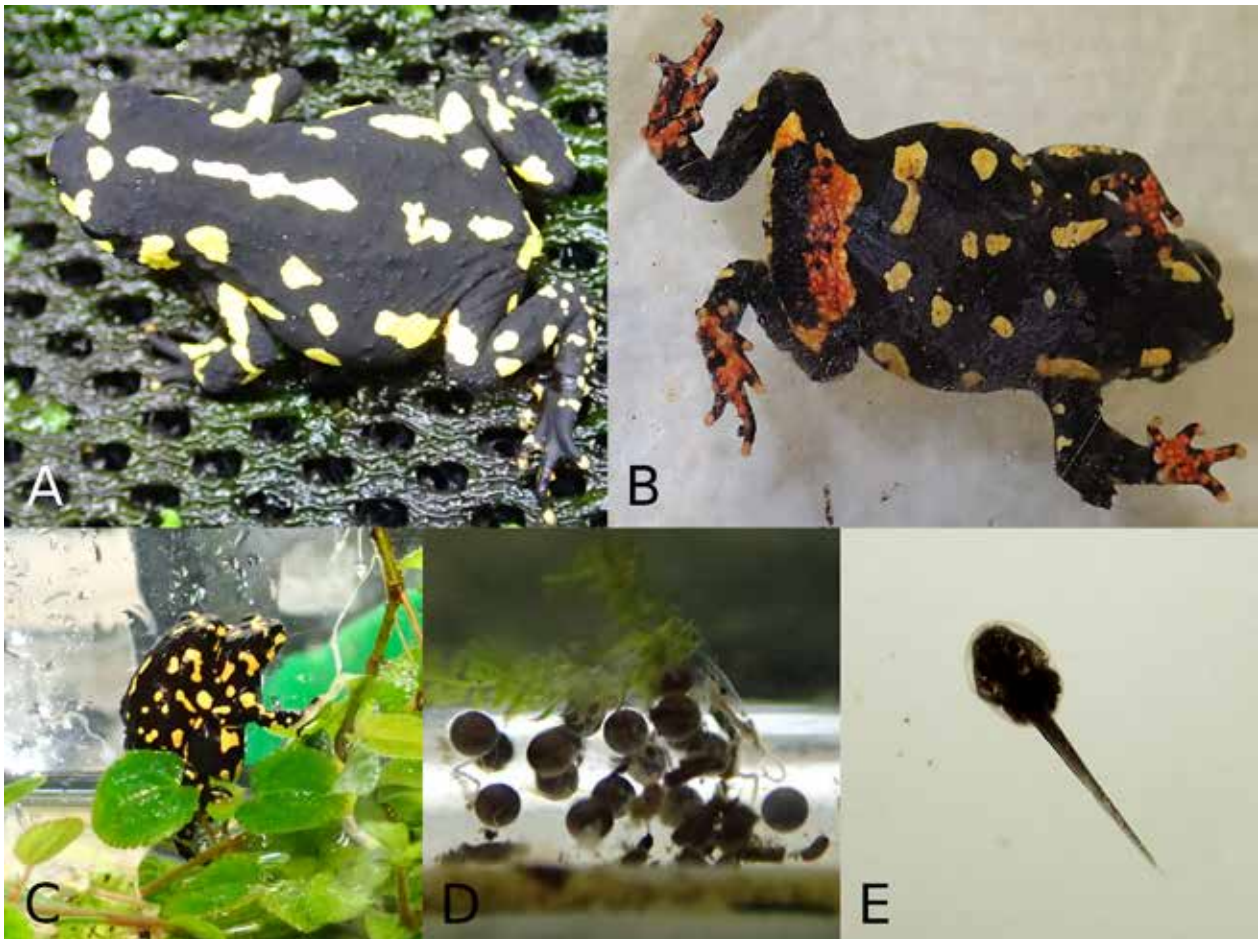


Fig. 1. *Melanophryniscus klappenbachi*. (A) Dorsal and (B) ventral view of an adult female. (C) Amplexus. (D) Egg clump attached to moss. (E) Contrasting photo of a tadpole, used for evaluating the growth.

in learning more about a species and to build up assurance populations against extinction (Gawor et al. 2011).

Kurth et al. (2013) have already reported on reproductive cues and larval development in *M. klappenbachi*, while Bland (2015) has given a short report of rearing captive bred *M. klappenbachi*. However, both papers did not consider influences of differing water parameters or group sizes on the development of young tadpoles.

Herein, we present captive management conditions at the animal keeping facility of the Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany, experiences in breeding without the use of a brumation period, and rearing tadpoles. Further this paper reports on the influence of different pH values and group sizes for growth and mortality rates of early developmental stages of Klappenbach's Red-bellied Frog.

Materials and Methods

Captive management and breeding

The basis for the breeding stock used in this study was built up by a group of eight *M. klappenbachi* purchased from a pet shop in 2011, which were imported from Paraguay according to the vendor. The group of adult toads

were housed in a terrarium measuring $120 \times 50 \times 50$ cm (L \times W \times H) in the animal keeping facility of the ZFMK. The terrarium bottom was covered with a six cm thick filter pad which was diagonally cut in the front, resulting in a water surface of 120×12 cm. There was at least a water level of two cm depth in the tank at all times, including the dry phase. Previous setups have revealed that toads were not able to swim for long periods of time, thus water levels needed to be shallow or a number of aquatic plants provided to prevent drowning. The complete ground and all three side walls were covered with Hygrolon®, a novel synthetic material that is non-decomposable and mimics the features of dead cellulose cells. This material is highly hygroscopic and used to ensure high air humidity.

The terrarium setup for the frogs was automatically misted three times per day for 30 seconds and air humidity varied between 70% and 80%. The setup was equipped with different plants, i.e., *Ficus pumila*, *Begonia* sp., *Neoregelia schultesiana*, *Pilea* sp., an undefined fast-growing Venezuelan tendril, and different mosses. Additionally leaf litter, pieces of cork bark, and roots were added to the terrarium in order to provide hiding and climbing space for the toads. Photoperiod was set to daylight between 8:00 and 20:00 h, as lighting LED light



Fig. 2. Keeping and rearing *M. klappenbachi*. (A) Terrarium of the adult group housing eight specimens. (B) Rearing of the tadpole test groups in a climate chamber. (C) Rearing containers for the young toadlets.

strips (Solar Stinger 1, 100 mm Sunstrip Dimmable Driver). Temperatures varied between 22 °C and 26 °C. Toads were fed flightless fruit flies (*Drosophila melanogaster* and *D. hydei*) and rarely with pinhead crickets (*Acheta domestica* and *Gryllus assimilis*). All prey was dusted with mineral or vitamin powder (i.e., herpetal Amphib, herpetal Mineral + Vitamin D3, and herpetal Complete Terrarium) and gut loaded with fresh vegetables or fruit puree before being fed to the toads.

To artificially initiate breeding season toads were placed into a second terrarium measuring 60 × 50 × 70 cm. The breeding terrarium was filled with water to a depth of six to seven cm and equipped with a few pieces of xaxim, a big root, and some plants (i.e., *Spathiphyllum* sp., some mosses, and *Microsorium pteropus*) to provide small areas above the water. Temperature and lighting were the same as described above. In this tank a permanent rain system was installed that irrigated the back wall of the terrarium all day long. After successful mating and spawning, when the males stopped calling and the pairs did not exhibit amplexus any longer, all toads were transferred into the first terrarium again.

The spawn was divided into four aquariums measuring 30 × 30 × 30 cm filled with osmosis water to a depth of about 20 cm. Water temperature varied between 22 °C and 25 °C. A few aquatic plants (Java Fern, *Microsorium pteropus*) and small aquatic snails (Bladder Snails, *Physella* sp., and Ramshorn Snails, *Planorbella* sp.) were added into each aquarium.

Comparative setup

For rearing tadpoles water with various pH values was used: osmosis water with a measured pH of 6.5–7.0 and pond water with a measured pH of 7.7–8.0. To obtain water with a reduced pH value a small amount of peat was added to the osmosis water, until a pH of 5.5–5.8 was obtained. Afterwards water was filtered to remove the remaining peat. Table 1 lists the additionally measured water parameters.

Once tadpoles had hatched, a total of 108 larvae were randomly chosen and divided into different groups. All tadpoles were transferred into plastic boxes measuring 10 × 10 × 10 cm filled with a water depth of eight cm. Every box was equipped with a single aquatic plant (either a small Java Fern, *Microsorium pteropus*, or a short branch of Hornwort, *Ceratophyllum* sp.) to provide shelter and one aquatic snail (either Bladder Snails, *Physella* sp., or Ramshorn Snails, *Planorbella* sp.) to remove remaining food. For each pH value there were two different group sizes of either one tadpole or five tadpoles per box, to determine if group size influenced individual growth rate. Six samples were set up for each group size, so that a total of twelve boxes and 36 tadpoles were exposed to each pH value.

All tadpoles were kept in a climate chamber (Versatile Environmental Test Chamber MLR-352H-PE) under standardized conditions. The temperature was set to 23 °C and the photoperiod was set to daylight between 6:00 and 18:00 h each day. Inside the climate chamber boxes of different group sizes and pH values were placed randomly (see Fig. 2 B). The larvae were fed every second day with a mixture of pulverized fish food dissolved in water. Additionally one object slide overgrown with a thin layer of algae was placed in every box and renewed every three days. Two thirds of entire water content was exchanged every second day. All boxes were checked daily to remove deceased tadpoles and later to transfer metamorphosed froglets to a terrestrial setup.

Newly morphed toads were relocated into plastic containers measuring 33 × 21 × 28 cm. Two layers of HygroLON® were used as ground layer to ensure high air humidity in rearing containers. One side of the boxes was placed on a heightened surface, so a height difference of 10 cm was formed from one end of the box to the other and a water part with a depth of 1–2 cm was created (see Fig. 2 C). As a result, a humidity gradient was created in the box, letting toads choose their preferred humidity. Containers were equipped with a small plant (undefined Venezuelan tendril) and some oak leaf litter.

Table 1. Water parameters of the different pH value groups.

	NO ₂ [mg/L]	NO ₃ [mg/L]	NH ₄ [mg/L]	Cu [mg/L]	KH [°dH]	GH [°dH]	pH
Osmosis water	<0.05	7.5	<0.05	<0.1	3	5	6.5–7.0
Pond water	<0.05	<0.5	<0.05	<0.1	9	8	7.7–8.0
Peat water	<0.05	7.5	<0.05	<0.1	3	5	5.5–5.8



Fig. 3. Developing coloration in young toadlets of different ages. **(A)** Recently metamorphosed toadlet. **(B)** Ten days after metamorphosis. **(C)** Twenty-three days after metamorphosis. **(D)** Two months after metamorphosis.

Data collection and evaluation

Photos of each single tadpole were taken three times: first, on the day they were transferred into the boxes, second, five days later and third, one week later when the first tadpoles had already grown hind legs. Photos were taken with a digital camera (Olympus TG-2). Tadpoles were individually transferred into a petri dish placed on a transparent glass plate lightened from below thus increasing the contrast between tadpole and its surroundings (see Fig. 1 E).

To evaluate the growth of tadpoles the digital image analysis tool SAISAQ (Kurth et al. 2014) was used. This tool is programmed on the open source statistics platform R (R Developmental Core Team 2016) and facilitates the semiautomatic processing of standardized image files computing the surface area of a tadpole, which is highly correlated with its body mass (Kurth et al. 2014). As this method is non-invasive it is ideally suited for repeated measurements on live animals.

Results

Breeding

Within one hour after relocating adult toads into the breeding terrarium males began to call. The first amplexus could be observed only a few hours after relocating toads. Klappenbach's Red-bellied Frogs show an axillary amplexus. Eggs were found on the second day in the breeding tank. Spawn was mainly deposited in clumps of 10–30 eggs, some clumps were attached to the plants or xaxim pieces under water and some were just deposited in the water without any such attachment. Often next to egg clutches were a few single eggs. Eggs were greyish and had a diameter of 1.5–2.0 mm surrounded by a gelatinous capsule. The last recorded spawn was produced on the ninth day after transfer to the breeding terrarium. No amplexus was observed after egg laying and females appeared slimmer. After two more days in which no amplexus was achieved and no more spawn was deposit-

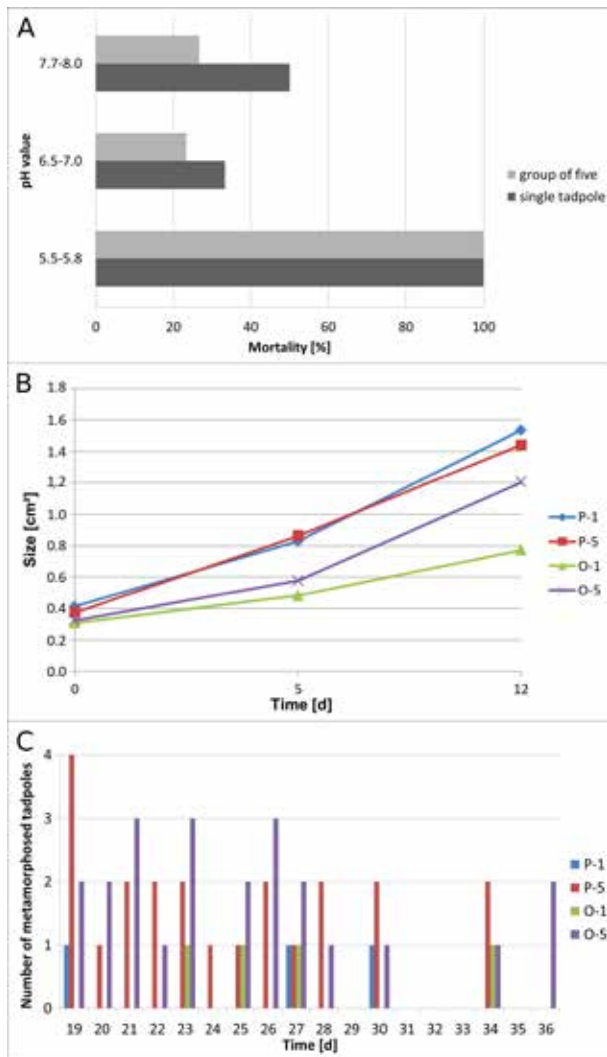


Fig. 4. (A) Mortality rate of different test groups until metamorphosis. (B) Average growth rate of the different test groups. (C) Number of tadpoles metamorphosed per day after hatching (O = osmosis water, P = pond water, number indicates individuals per container).

ed all frogs were relocated into their regular terrarium. Males continued calling infrequently, females showed no further reproductive behavior.

About three weeks after relocating the toads into their regular terrarium at least one female toad spawned again. Several egg clumps were found in the water, the spawn was attached to moss and leaves. As before, these eggs were relocated into an aquarium measuring 30 × 30 × 30 cm and raised in this tank at a temperature of 22–25 °C and a water depth of about 20 cm.

Development

Development of eggs and *M. klappenbachi* tadpoles have not been documented in the wild and development mostly correlated the records of Kurth et al. (2013) and Bland (2015). Tadpoles hatched within two to four days after spawning. There was no difference in hatching rate

between eggs deposited in clumps and those deposited individually. After giving a few drops of the food mixture into water tadpoles seemed to actively seek for food on the ground. Larvae did also feed by rasping algae from the object slide. The first tadpoles metamorphosed after 19 days, whereas the last tadpoles of the test groups needed much more time to complete their development and left the water after 36 days. However, there were still a few tadpoles left in the bigger aquarium (30 × 30 × 30 cm), which did not metamorphose by this time. They were still fully aquatic and did only grow hind legs or no legs at all. After 83 days the last two of these remaining larvae metamorphosed, being the same size as the earlier metamorphosed toadlets.

Just after reabsorbing the tail the small toadlets measured 6–7 mm (snout-vent-length). At this stage their coloration was dark grey to black without the conspicuous yellow markings (see Fig. 3 A). Typical patterns developed after one to two weeks (see Fig. 3 B). However, all bred toadlets did not develop any red coloration on their ventral surface, unlike the wild caught adults which showed ventral flash markings colored yellow. The first three weeks young toads were fed tropical springtails (*Collembola* sp.) once a day, so that food was always available. Afterwards they were fed every second day.

Mortality

All larvae kept in water with the lowest pH value (pH 5.5–5.8) survived for at least two days, but then died within the following four days. Tadpoles raised in water with the highest pH value (pH 7.7–8.0) had a total mortality rate of 30.56% (11 out of 36); the singly kept larvae had a mortality of 50.00% (3 out of 6). Those kept in groups of five had a mortality of 26.67% (8 out of 30). Those larvae which were kept in osmosis water with a pH value of 6.5–7.0 had a total mortality rate of 25.00% (9 out of 36). Single tadpoles had a mortality of 33.33% (2 out of 6) and larvae raised in groups of five showed a mortality of 23.33% (7 out of 30) (Fig. 4 A).

In the first eight days after hatching the total number of deceased tadpoles was at the highest level. After this period there were only occasional losses in the different test groups. In the test group of single tadpoles kept in pond water (P–1) there were no losses of larvae after day six.

Growth rate

Tadpoles in water with the lowest pH value of 5.5–5.8 did not show any sign of growth before they died. The group size did not show an influence on the growth of the tadpoles in the more alkaline pond water (P–1, single tadpole, and P–5, five tadpoles per box). In osmosis water the single-kept tadpoles (O–1) grew slower than tadpoles in groups of five larvae per box (O–5). Aside from these results, tadpoles grew faster in pond water than in

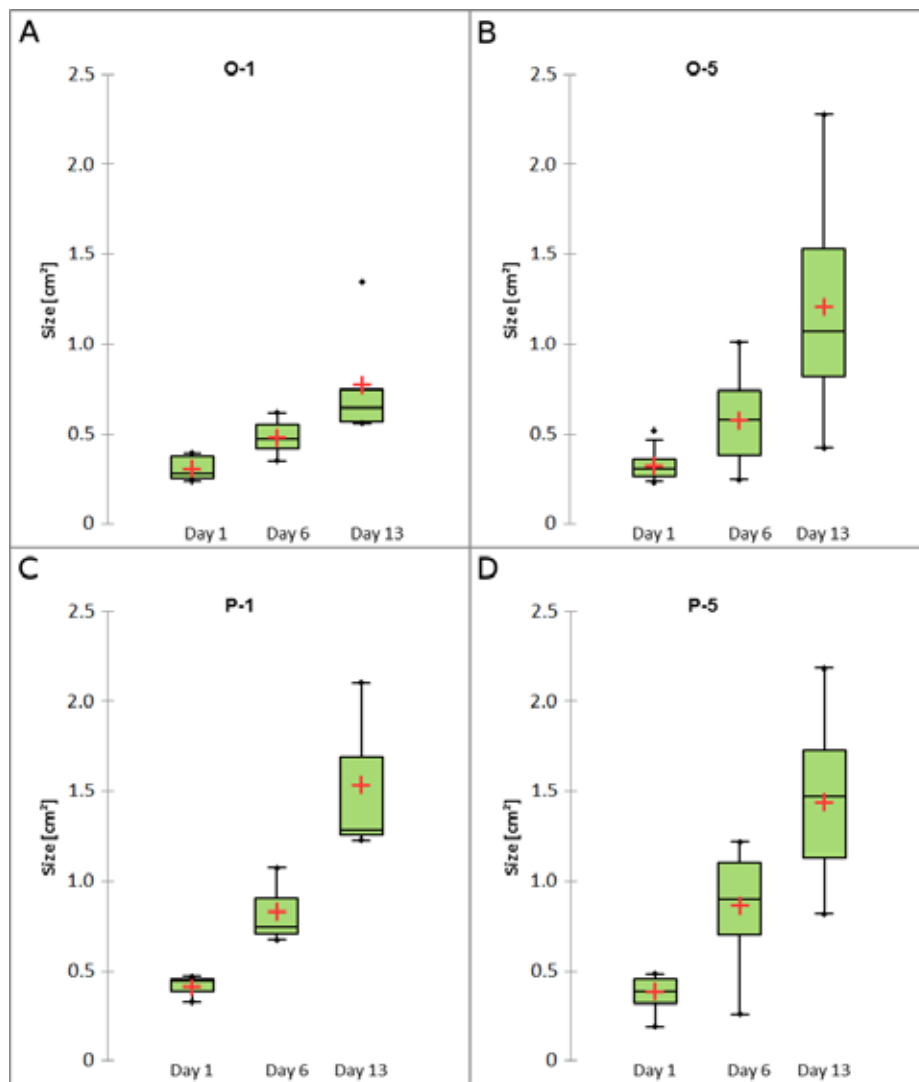


Fig. 5. Body size of different test groups. (A) Single tadpole, O–1, and (B) five tadpoles per box, O–5, in osmosis water. (C) Single tadpole, P–1, and (D) five tadpoles per box, P–5, in pond water.

osmosis water (compare Fig. 4 B).

Single kept tadpoles raised in osmosis water (O–1) showed the slowest growth of the test groups, with a mean body size of 0.773 cm² on day 13 after hatching and the biggest larva measuring 1.347 cm² (Fig. 5 A). Tadpoles in groups of five (O–5) grew faster and had a mean body size of 1.206 cm² on day 13. The largest tadpole of the test groups was in the prior group and measured 2.281 cm² on the third measurement (Fig. 5 B). The growth rate of tadpoles raised in pond water was quite similar in both test groups. On day 13, single kept larvae (P–1) had a mean body size of 1.537 cm² and a maximum size of 2.102 cm² (Fig. 5 C), while tadpoles reared in groups (P–5) showed a mean body size of 1.441 cm² with the largest tadpole measuring 2.183 cm² (Fig. 5 D).

Metamorphosis

On day 19 after hatching the first tadpoles of three test groups (P–1, P–5, and O–5) metamorphosed. The number of tadpoles metamorphosing per day reached its highest

level on this day: in total seven larvae metamorphosed, four out of these seven tadpoles were in group P–5. The first tadpoles of the fourth group (O–1) metamorphosed on day 23. The last two larvae metamorphosed on day 36 (Fig. 4 C).

Discussion

Based on our husbandry experiences, the keeping, breeding, and rearing of *Melanophryniscus klappenbachi* in captivity is rather easily achieved. Most noticeable in the lifecycle of Klappenbach's Red-bellied Frog is of course the rapid larval development with the first tadpoles completing metamorphosis after 19 days at pH values between 6.5 and 7.9. This fast development represents an adaptation to the climate in their natural habitat. After heavy rainfalls adults start breeding in the emerging small temporal water bodies, which have a high desiccation risk due to drying up of these small puddles. Thus, the fact that a few tadpoles did not metamorphose after 80 days is rather surprising and has never been reported.

Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachii*

Table 2. Husbandry parameters for adult breeding groups of *Melanophryniscus klappenbachii* according to Kurth et al. (2013), Bland (2015), and data from this study.

	Kurth et al. (2013)	Bland (2015)	own data
Breeding group	12 adults	3 adults (2 males + 1 female)	8 adults
Terrarium size (L × W × H)	80 × 40 × 40 cm	46 × 39 × 30 cm	120 × 50 × 50 cm
Water part (L × W)	30 × 10 cm	shallow water dish	120 × 12 cm
Water depth	10 cm	—	2 cm
Temperature	—	22–26 °C	22–26 °C
Hibernation: duration; temperature	3 weeks; 8 °C	4 days; 5–8 °C (only the female)	not applied
Rainy season: duration; temperatures	—	—; 20–25 °C	11 days; 22–26 °C
Rain chamber (L × W × H)	40 × 50 × 40 cm	60 × 45 × 45 cm	60 × 50 × 70 cm
Water part (L × W)	1/4 of the terrarium (500 cm ²)	60 × 45 cm (only floating cork bark as land areas)	60 × 50 cm (only xaxim, a root and plants as land areas)
Water depth	5 cm	10 cm	6 cm
Nourishment	—	Hatchling crickets, <i>Drosophila</i> sp., <i>Siera</i> sp.; supplemented with Repashy Calcium Plus	Hatchling crickets, <i>Drosophila melanogaster</i> , <i>D. hydei</i> ; supplemented with herpetal powder (Amphib, Mineral + Vitamin D3, Complete Terrarium)

It was not known that tadpoles of *M. klappenbachii* might stay in the larval stage for a longer time before metamorphosing. This behavior was only observed in the tadpoles which were kept in the bigger aquarium with a water level of at least 20 cm, whereas none of the test animals in the small boxes (10 × 10 × 10 cm) showed this long-time larval stage. A possible reason for this might be intraspecific competition amongst tadpoles. The availability of food and other resources could directly influence length of the larval stage as well. It could also be possible that tadpoles might be able to sense water levels of their surrounding environment so larvae could metamorphose before water levels decrease. Perhaps it is an adaptation, which allows a few tadpoles to survive in deeper water pools increasing overall species survivorship as a “backup.” If the majority of the first, fast metamorphosing froglets die due to unstable environmental conditions, these “backup” tadpoles could increase the persistence of the species. Richter-Boix et al. (2011) investigated the influence of drying conditions on larval development in anurans and found plasticity of development across different taxa. Therefore, it would be an interesting approach for future studies on *M. klappenbachii* to investigate the influence of desiccation stress on the duration of tadpole development and growth rates.

For the last steps of metamorphosing, growing legs, and reabsorbing the tail, tadpoles did not require more time than larvae metamorphosing earlier, these steps were accomplished in only two to three days. The presence of long-term tadpoles under natural conditions might provide a steady supply of metamorphosed toads to their environment. However, this is currently not

known from wild populations of *M. klappenbachii* and further field studies are suggested.

Water with a low pH value of 5.5–5.8 had a lethal effect on tadpoles within a few days and tadpoles which survived for five or six days in this water did not show any sign of growth. As *M. klappenbachii* deposits its eggs into small ephemeral ponds produced by rainwater, the pH value of these breeding sites is directly dependent on the characteristics of rainwater and soil. Therefore, the ground in Klappenbach's Red-bellied Frog's native environment has most likely neutral or even alkaline characteristics. Hence acid rain, which for example could occur due to air pollution, might be a possible future threat to tadpoles of *M. klappenbachii*. The burning of woodland is a common procedure in slash-and-burn agriculture to establish land for agricultural cultivation and can lead to a higher amount of acidity in regional rainfall (Tinker et al. 1996).

In the groups of five tadpoles it was often the case that there were three or four big tadpoles which grew faster than the remaining one or two. These remaining tadpoles showed slower growth, stayed smaller for a longer time, and metamorphosed a few days later than the larger tadpoles in the group. Additionally, these smaller specimens showed a higher mortality rate. Though live cannibalism was not observed, the deceased tadpoles were often partly or even completely eaten by their kin. Intraspecific competition for resources, mostly food and space, is a likely explanation for this observation. However in the single kept tadpoles there were a few slow-growing specimens (compare growth rate, Fig. 4 B). Since these tadpoles were not influenced by conspecifics and intra-

specific competition for food, the difference in growth might be at least partly genetically controlled. Small and weaker tadpoles might be predestined to be cannibalized by conspecifics and thus make up an important source of food, as there could be a lack of other food options in ephemeral breeding sites. A similar case is the cannibal morph of some *Ambystoma tigrinum* larvae, which have the genetic capacity to develop distinct characters adapted to feeding on conspecifics and other salamanders (Rose and Armentrout 1976).

Breeding of Klappenbach's Red-bellied Frog is often stimulated by a brumation period followed by the simulation of a rainy season (Kurth et al. 2013; Bland 2015; see also Table 2). However, our data indicates that a brumation is not a crucial factor for the successful mating and breeding of *M. klappenbachi*. After a long dry season of five to six months we relocated toads into the rain chamber (without hibernating at low temperatures) and males began to call almost immediately. This method is less stressful for the toads, thus it is recommended to use a mild brumation period as described by Bland (2015), if females do not deposit eggs after one to two weeks in the rain chamber.

Klappenbach's Red-bellied Frogs rely on the availability of very small prey items. Anything larger than a large *Drosophila* sp. was observed to not be eaten. Fruit flies, pinhead crickets, small isopods (e.g., *Trichorhina tomentosa*), and springtails are easily accepted food items, which can be purchased in pet stores and online shops, and are consumed willingly by adult toads. One interesting observation made while feeding breeding groups was that fully grown adult toads preferred smaller prey items to those larger in size. It was noticed that while feeding fruit flies and springtails at the same time springtails were favored over fruit flies.

One problem in rearing young metamorphosed toadlets was the need to have the smallest food items available. We fed these toadlets only springtails, as these are the smallest available food insects which can be purchased in most pet stores. It is most probable as well that small toads would feed on mites, tiny ground-dwelling insects, and other invertebrates. *Drosophila hydei* were fed to toadlets after two to three months though only the larger toads managed to catch these fruit flies successfully. Smaller toads tried to eat these flies, too, but showed problems swallowing them. None-the-less, larger toadlets preferred to feed on springtails.

To prevent high mortality, toadlets need to be kept on humid ground with high air humidity. For that purpose a few layers of the artificial material HygroLON® were used and worked well keeping humidity high in containers. The HygroLON® layers soaked up water, thus ensuring a high humidity in rearing boxes. However, with advancing age the froglets prefer dryer areas in their rearing containers and seem to suffer from ground humidity that was too high, especially in combination with insufficient air ventilation. Thus, toads must be observed carefully

in the first few weeks and months to recognize the right time to decrease humidity or to relocate toadlets into a dryer box. The right humidity turned out to be a crucial factor in the husbandry of Klappenbach's Red-bellied Frogs, especially in rearing young toads.

Introducing captive breeding programs and maintaining reserve populations in captivity might be one basic requirement for adequate *ex situ* conservation arrangements. Together with natural history studies the husbandry and captive breeding of endangered species give an insight into behavior and leads to a better understanding of amphibians, the most endangered group of vertebrates. Currently, *M. klappenbachi* is listed as Least Concern by the IUCN Red List of Threatened Species due to its relatively wide distribution, large and stable populations, and its occurrence in several protected areas in both Paraguay and Argentina (Aquino et al. 2004). Nevertheless Aquino et al. (2004) have stated that more research on the species' distribution and the effects of the pet trade are necessary. Furthermore the explosive breeding behavior of this species makes it more vulnerable to diseases, as many adults gather in small vernal pools for mating and spawning. As Bland (2015) has noted, *Batrachochytrium dendrobatidis* or other infectious diseases could become a serious threat for Klappenbach's Red-bellied Frog and could lead to severe population declines or extinction. According to the IUCN Red List many other species of the genus *Melanophryniscus* are listed as Near Threatened or Threatened, with three species listed as Critically Endangered (i.e., *M. admirabilis*, *M. langonei*, and *M. peritus*) and for some species sufficient data is missing suggesting further field studies are necessary (Acquino et al. 2004). These endangered species might benefit from detailed knowledge about the husbandry and reproduction in captivity of a closely related species like *M. klappenbachi*, as the methods described herein may be applicable to them as well. This knowledge can be used to build up reserve populations and to set up breeding programs to returning captive produced specimens to the wild, if necessary.

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Klappenbach's Red-bellied Frog, *Melanophryniscus klappenbachii*

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