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Life History of the Golden Puddle Frog, *Phrynobatrachus auritus* Boulenger 1900 (Anura: Phrynobatrachidae)

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Abstract

Frogs of the genus *Phrynobatrachus* Günther, 1862 are endemic to sub-Saharan Africa. These are increasingly threatened by a number of factors and are believed to be declining. We report on captive breeding experiments involving *Phrynobatrachus auritus* Boulenger, 1900. We provide a comprehensive life history for this frog with emphasize on tadpole development time, as well as a description of both the advertisement call and calling behaviour of the adult.

Keywords: *Phrynobatachus*, captive breeding, life history, call behaviour

1. Introduction

Puddle frogs from the genus *Phrynobatrachus* Günther, 1862 (Phrynobatrachidae) are endemic to sub-Saharan Africa and are one of the most speciose lineages on this continent with 89 species currently known (Frost, 2016). Numerous species have been described in recent years as a result of the inclusion of molecular data and an increase in the general knowledge of this previously overlooked lineage (Zimkus, 2009; Rödel, Sandberger, Doumbia, & Hillers, 2009; Rödel, Ohler, & Hillers, 2010), Rödel et al., 2012a; Rödel, Onadeko, Barej, & Sandberger, 2012b; Rödel, Burger, Zassi-Boulou, Emmrich, Penner, & Barej, 2015; Blackburn, 2010; Blackburn, Gvoždík, & Leaché, 2010; Blackburn & Rödel, 2011; Zimkus & Gvoždík, 2013). Among these, *Phrynobatrachus auritus* is an interesting species, exhibiting extremely high colour and pattern polymorphism that are still leading to speculation regarding whether this is merely intraspecies variation or if there are cryptic species present (Herrman et al., 2005). The species ranges from south-eastern Nigeria and southern Cameroon, east to eastern Democratic Republic of Congo, western Uganda and Rwanda (Amiet, Burger, & Howell, 2004). It is distributed widely across Gabon and has been recorded from the following National Parks: Ivindo, Loango, Lopé, Monts de Cristal and Moukalaba-Doudou (Pauwels & Rödel, 2007, Bell, Garcia, Stuart, & Zamudio, 2011). Due to its wide distribution, the ecology and life history of this species is important to assist in full understanding the basic biology of the genus. This information will ultimately aid in the conservation efforts involving other *Phrynobatrachus* whose populations are rapidly decreasing in the region (Amiet et al., 2004, Doherty-Bone, 2014). In this paper, we provide a comprehensive review of published work involving this species, complemented with findings from our field and laboratory observations to characterize life history traits of this frog. The goal of this work is to provide a comprehensive species profile for *P. auritus* to aid in the conservation of this species, as well as related species and habitats inhabited by these amphibians.

2. Materials and Methods

From May 2014 to March 2016, *P. auritus* were observed and subsequently collected across Cameroon and Equatorial Guinea (Figure 1). Ninety-eight founder individuals were collected then transported and housed at the Laboratory for Biodiversity and Conservation Biology (LBCB) within the University of Buea, Cameroon. Frogs were housed in aquaria 40 cm X 50 cm X 30 cm with moistened paper towels as substrate. See *Tadpole husbandry* for additional information about frog housing. Females were continuously gravid, amplexus and spawning was occasionally observed.

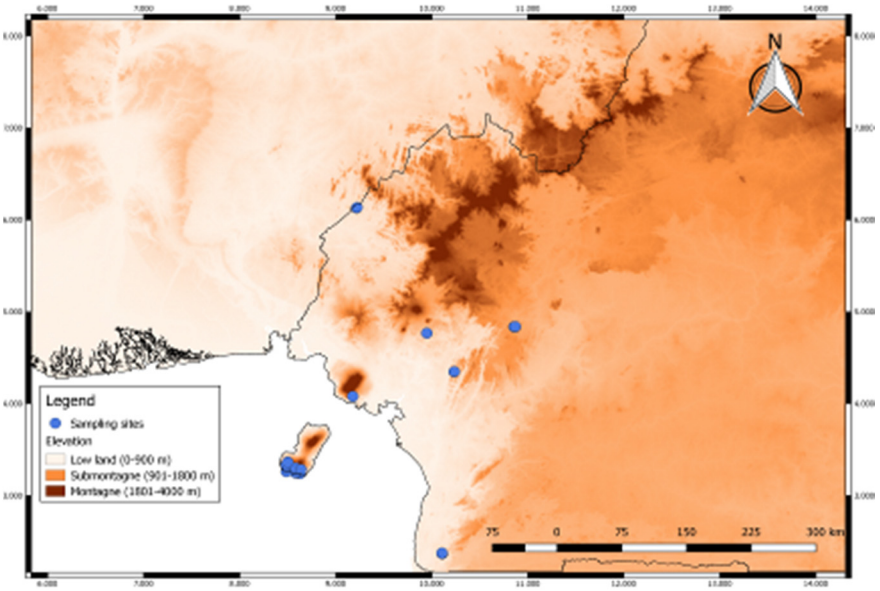

Figure 1. Map showing the sampling sites for *Phrynobatrachus auritus*

2.1 Behaviour

Frogs were housed in 40 cm X 50 cm X 30 cm plastic terrariums at a maximum density of 4–5 individuals. To assess the behaviour of *P. auritus*, the frogs were quarantined for a period of three weeks and monitored daily to observe potential interactions, such as mounting, fighting, emission of release-calls, stalking, and waving. A total of 52 captive frogs survived the quarantine period and were subsequently monitored over a complete year. A single observer tallied behaviour for individuals within each tank for 5 minutes twice a day, in the morning between 07:00–08:30 and in the afternoon between 14:00–15:30. The order of sampling was randomized to prevent any sequential bias due to time of day. All observations in a single week were summed and divided by the number of frogs in each tank to obtain a total number of interactions observed per frog per week.

2.2 Adult Advertisement Call

Calling males were identified in the field by sight, and advertisement calls were recorded with a Marantz digital recorder (PDM660) coupled to a Sennheiser ME66 shotgun microphone. Calls were recorded at approximately 1–3 m from the calling male at air temperatures of 22.5ºC. Calls were digitized at a sampling rate of 44100 Hz and analyzed using Raven v1.5 software (Bioacoustics Research Program, 2014). Oscillograms (waveforms) and audiospectrograms (sonograms) were studied to characterize spectral and temporal features of the calls. Although our call description is based on wild males, calling behaviour in captive animals was also observed.

2.3 Spawning

Spawning was either natural or induced using injections of gonadotrophin-releasing hormone within the laboratory environment. Eggs were observed macroscopically, and time until hatching was recorded.

2.4 Tadpole Husbandry

Phrynobatrachus auritus tadpoles were successfully reared to froglets (neometarmorph size). Tadpole enclosures were 40 cm X 50 cm X 30 cm plastic aquaria maintained at room temperature (18-22°C). Air in the system was provided by an ActiveAQUA AAPA25L aerator. Between 20 and 40 tadpoles were reared per tank with the maximum density of one tadpole per 3.6 litres. Tadpoles were fed once daily (between 08.00-09.00) on a suspension of commercially available fish food and Spirulina alga. Aquaria were cleaned every other day. Cleaning consisted on the replacement of ¾ of used water in the system by aspiration using a flexible water pipe, taking care of discarding uneaten food.

Tadpoles were observed regularly to assess development. Measurements were performed using the software imageJ (imagej.nih.gov). This report on the development is based on Gosner (1960) stages.

3. Results

3.1 Description

Phrynobatrachus auritus was described by Boulenger (1900). This species is easily recognizable in the field due to its larger body size when compared to many miniature and small puddle frog species. It is a medium to large sized species (snout-vent length < 35 mm) of puddle frog (Anura: Phrynobatrachidae). Members of this genus are identified by the presence of a midtarsal tubercle, elongate inner metatarsal tubercle, and outer metatarsal tubercle. A distinct tympanum, moderate to extensive pedal webbing, and large discs on the fingers and toes characterize this species. A pair of narrow glandular folds is normally present that begin behind the eyes, converging in the scapular region and continuing down the back to the sacral region.

Phrynobatrachus auritus tadpoles have been thoroughly described by Pfalzgraff et al. (2015). Tadpoles have an ovoid body in dorsal view, snout rounded; body oval to slightly compressed in lateral view. The nostrils are situated dorso-laterally, closer to snout-tip than to eyes. The tail has well-developed fins. The tail axis height at its base is almost equal to maximum height of dorsal fin. The vent tube is dextral whereas the spiracle is sinistral and are visible in dorsal view. The mouth opens antero-ventrally with small oral disc and width less than a quarter of body width.

3.2 Longevity, Behaviour and Reproduction

Field-collected adult *P. auritus* lived over a year in plastic enclosures 40 cm X 50 cm X 30 cm with moistened paper towels used as a substrate. The system was provided a constant small pool of water for the frogs, while the top was covered with net to allow airflow and prevent frogs from escaping. Although the husbandry of adults is largely similar to that established for other frog species (Poole & Grow, 2012), mortality was initially an issue. Keeping frogs at a low density (4-5 frogs per terrarium) significantly reduced mortality from 80% to 12%.

Phrynobatrachus auritus is both diurnal and nocturnal. In the field, *P. auritus* stands mostly on substrate matching its colouration for camouflage and/or hides in crack and crevices during the day. In comparison, this frog climbs on leaves at knee level to hunt for insect meals at night. *Phrynobatrachus auritus* preys on all type of live insects or larvae that its size could accommodate. These ranged in the field from termites and moving larvae to small cockroaches. Interestingly, frogs exhibited a paler coloration in response to stress. During our night surveys, a stressed individual could almost instantly switch to a paler colouration. No mounting, fighting, release-call, stalking, and waving nor territoriality were observed in this frog. Amplexus and oviposition were both diurnal and nocturnal. Males called intermittently throughout day and night. Neither the ambient noise of wind and flowing water, nor the call of congeners disrupted the activity of calling males. Males called from cracks and crevices while sitting partially submerged in water or above water standing on a substrate (Figure 3). Calling begins when a previously resting male takes a more erect posture, raises the head, and takes several inhalations, buccal pumping while filling the lungs. The single subgular vocal sac is extended during the calls, and could remain partly inflated between calls. The male produces a largely invariant and unique advertisement call, sounding to the human ear like a long continuous series of low-frequency one-syllable barking notes known as: “criikk criikk criikk criikk” (described below) on expiration by contraction of body wall musculature, which fills the vocal sac. The elastic vocal sac then recoils, forcing air back into the lungs, with the process repeating over a series of up to several seconds, with air being shuttled back and forth between the vocal sac and the lungs. Generally, the call lasts between 20-60 second or more. Single calling males were often joined by a chorus of other males calling. Calling continues for some minutes of uninterrupted, steadily paced vocalizations. Call consisted of 44–74 pulses (53±4 pulses, n=2 calls). Call duration was 3073-4240 (3557±497ms, n=3 calls). Pulse duration was 11–22ms (18.03±2.8ms, n=34 pulses), duration of intervals between pulses was 6–16ms (8.9±2.6ms, n=69 intervals). The dominant frequency was 22050–36500 (2526±526 Hz, n=3 calls) with calls per hour ranging between 0–6. Calling males would immediately cease if congeners jumped closer and attempted amplexus with any female.

3.3 Egg and Tadpole Descriptions

We observed batches of 122–276 eggs scattered singly from 4 spawning events in lab conditions. Eggs measured 0.8–1.2 mm (N=30) and exhibited a dark whitish colouration. *P. auritus* adults did not exhibit parental care. Eggs hatched between 20–36 hours before having free moving tadpoles.

The tadpoles are exotrophic, lenthic and benthic (Altig & McDiarmid, 1999; Rödel, 2000a; de Sa & Channing, 2003). Tadpole development ranged between 38.33±9.18–70.33±10.14 days. Neometamorphs measured 10.48±2.60 (N=32) mm in snout-vent length and weighed 46.96±17.04 (N=32) mg. We observed a pronounced disparity between developing tadpoles within and between batches.

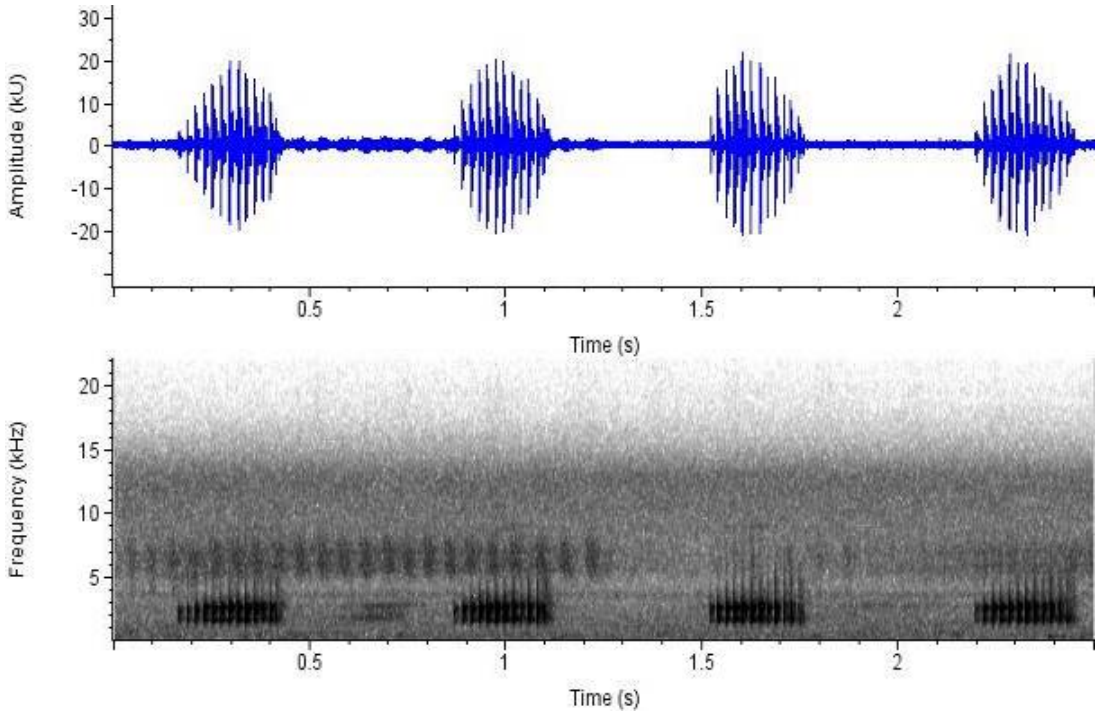

Figure 2. Spectograms (above) and oscillograms (below) of *Phrynobatrachus auritus* call

Figure 3. Calling male photographed during day time from Moka Mid-point, Equatorial Guinea on 26 November 2015

3.4 Conservation Assessment

In the highly diverse regions of Cameroon, several amphibian species are critically endangered out of a total of 295 species presently known (Frost, 2016). *Phrynobatrachus auritus* is listed as least concern in the IUCN Red list (Amiet et al., 2004) in view of its wide distribution, tolerance of a degree of habitat modifications as agroforestry system. We collected it in a cocoa farm in Ndikinimeki, Cameroon (N4°50’47” and W10°52’78”). It is presumed to have a large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category in the near future (Amiet et al., 2004). It is listed neither in the Convention on International Trade in Endangered Species (CITES) nor the Evolutionarily Distinct and Globally Endangered (EDGE) list of the Zoological Society of London. However, it occurs in several protected area across its large distribution range.

4. Discussion

The egg masses in *Phrynobatrachus auritus* are laid floating on a single layer, which is similar to most *Phrynobatrachus* depositing clutches of several hundred to a few thousand that float on a single layer at the surface or slow running water (Steward, 1967; Rödel, 2000). The three main exceptions to this reproductive mode are species depositing eggs terrestrially, including *P. alticola*, which oviposits on leave of the forest floor and exhibit direct development (Rödel & Ernst, 2002a), *P. phyllophillus* which deposits its eggs in leaves close to extremely small puddles on the forest floor (Rödel & Ernst, 2002b) and *P. guineensis* depositing its eggs in tree holes and abandoned shells (Rödel, Rudolf, Frohschammer, & Linsenmair, 2004). Clutch size was greater that in *P. guineesis* (2–35 eggs, Rödel et al., 2004). This may partly reflect the larger body size of *P. auritus*.

To our knowledge, there is no previous record on longevity in *Phrynobatrachus*. Our observation on *Phrynobatrachus auritus* for more than a year shows that this species lives more than a single season.

In rearing tadpoles, although large amount of food was provided, exploitative competition, individual recognition and the formation of hierarchies is believed to explain the observed disparity. It has already been reported that densities of conspecific might actually give supplementary information, which individuals can use to 'fine-tune' the timing of their phenotypic transformations (Goldberg et al., 2012). Some authors have proposed changes in habitat, such as pond desiccation, limited food resources, and the presence of predators or competition as mean to affect the duration of the larval stage and the timing of metamorphosis (Newman, 1998). However, we do not throw away the potential of synergetic influence in the setting as a function of relatively stable habitat, where very low seasonal variation in environmental parameters, and no risk of the water body drying up.

In the wild, *Phrynobatrachus auritus* tolerates some forest disturbance and can even occur in agroforestry settings. Generally, this species lives on the floor of secondary forest and assembles in riparian for reproduction. Although most *Phrynobatrachus* species seem to be habitat-specific, seasonal habitat change was also assumed for some West African species, living at forest-savannah ecotone during the rainy season, but in forest during the dry season (Rödel, 2000, 2003).

The role of synchronized calling has already been proposed in some amphibians as a protective clue for calling males (Tuttle & Ryan, 1982). The presence of chorus in the genus is also known from *P. guineensis* (Rödel & Ernst, 2002b) with similar calling activity and call structure in *P. auritus*. This might potentially be altered by the presence of a female with shorter notes.

5. Conclusion

The present report forms the first comprehensive compilation of life history traits, including the reproductive behaviour of *P. auritus*. This species breeds next to ponds in open forests where the males assemble next to puddles and initiate choruses to attract potential partners. The large distribution of this species might be attributed to its potential resilience toward some human activities precipitating the degradation of its habitat and females laying eggs several times in the year with breeding peaking during the raining season.

The question of this species being a complex of cryptic species or subspecies occurring across its large range remains open. It has been suggested that eastern populations of this species be separated as *P. discodactylus* (Amiet et al. 2004). Sampling and molecular phylogenetic analysis is needed across the Congo Basin to determine the taxonomic future of this species.

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