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REPRODUCTIVE BEHAVIOUR OF *MELANOPHRYNISCUS* SP. FROM SIERRA DE LA VENTANA (BUENOS AIRES, ARGENTINA)

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Abstract. Little is known about the reproductive biology of species of the genus *Melanophryniscus.* This study provides the first description of amplexus behaviour, egg-laying sites, clutch characteristics and larval development of *Melanophryniscus* sp., a species of uncertain taxonomic status that is found in the southernmost range of the genus (Sierra de la Ventana, Argentina), and their relationship with habitat features and selection pressures is discussed. Results are compared with the reproductive biology of other species in the genus.

Key words. Red Bellied Toads, amplexus, egg-laying, larval development, Pampas grasslands.

Introduction

The genus *Melanophryniscus* consists of 20 species clustered in three phenetic groups: *stelzneri, tumifrons,* and *moreirae* (Caramaschi and Cruz, 2002; Cruz and Caramaschi, 2003; Baldo and Basso, 2004; Di-Bernardo *et al.,* 2006). These species are commonly known as South American Red Bellied Toads and are distributed in central and northern Argentina, southern Bolivia, southern Brazil, Paraguay and Uruguay (Baldo and Basso, 2004; Frost, 2004; Kwet *et al.,* 2005).

The southernmost populations of the genus, occurring in Sierra de la Ventana, Monte Hermoso and Tandil, in the south-west of Buenos Aires Province, Argentina, have been cited as *M. stelzneri montevidensis* (Cei, 1980). Klappenbach and Langone (1992) questioned the identity of this taxon and since then it has been reported as *M. montevidensis, M. atroluteus, M. stelzneri* (Lavilla *et al.,* 2000; Cabrera, 2001; Lavilla and Cei, 2001), or just as to "phenotype 2" (Céspedez and Motte, 2001). The taxonomy of the three species belonging to the *stelzneri* group has not yet been adequately resolved (Kwet *et al.,* 2005). Consequently we refer to this population as *Melanophryniscus* sp.

Notes on the life-history of *Melanophryniscus* are scarce (Kwet *et al.,* 2005) and, in particular, little is known about the reproductive biology of species in the *stelzneri* group. Fernández (1927) provided the first data about *Melanophryniscus stelzneri* (as *Atelopus stelzneri*) from Córdoba, Argentina, including reports on explosive breeding events in temporary ponds associated with heavy rainfall. Similar observations were reported for *M. montevidensis* in Uruguay (Prigioni and Garrido, 1989), for *M. stelzneri* from Córdoba Province, Argentina (Bustos Singer and Gutiérrez 1997), for *M. rubriventris* in the Yungas mountain rainforest in northwestern Argentina (Vaira, 2005; Goldberg *et al.* 2006) and for *M. montevidensis* and *M. dorsalis* in Uruguay and southern Brazil (Kwet *et al.* 2005). These papers also include information on mating behavior, egg laying, and embryological and larval development. In contrast, the reproductive biology of *Melanophryniscus* sp. in Sierra de la Ventana is almost unknown. Previously, we reported seasonal reproduction occurring from October to March, with males mostly calling from open and closed sites inside temporary ponds (Fig. 1) immediately after heavy rains amplexus and oviposition occurring during spring and summer (Cairo and Zalba, 2007).

In this study we describe the amplectic behaviour, reproduction and oviposition sites, clutch characteristics and larval development of *Melanophryniscus* sp. from Sierra de la Ventana (Buenos Aires, Argentina).

Material and Methods

The reproductive biology of this toad was studied at Ernesto Tornquist Provincial Park (ETPP, 38°03'S, 62°02'W) during two breeding seasons (October to March) in 2004-2005 and 2005-2006. This park comprises 6718 ha of mountain range landscape of me-

dium height between 450 and 1186 meters above sea level and is considered one of the last relicts of Pampas grasslands with a relatively good conservation status (Bilenca and Miñarro, 2004). The climate is temperate, with a mean annual temperature of 14.6°C. The annual rainfall ranges from 600 to 800 mm, with a dry period between November and April and abundant precipitation from July to November (Frangi and Bottino, 1995).

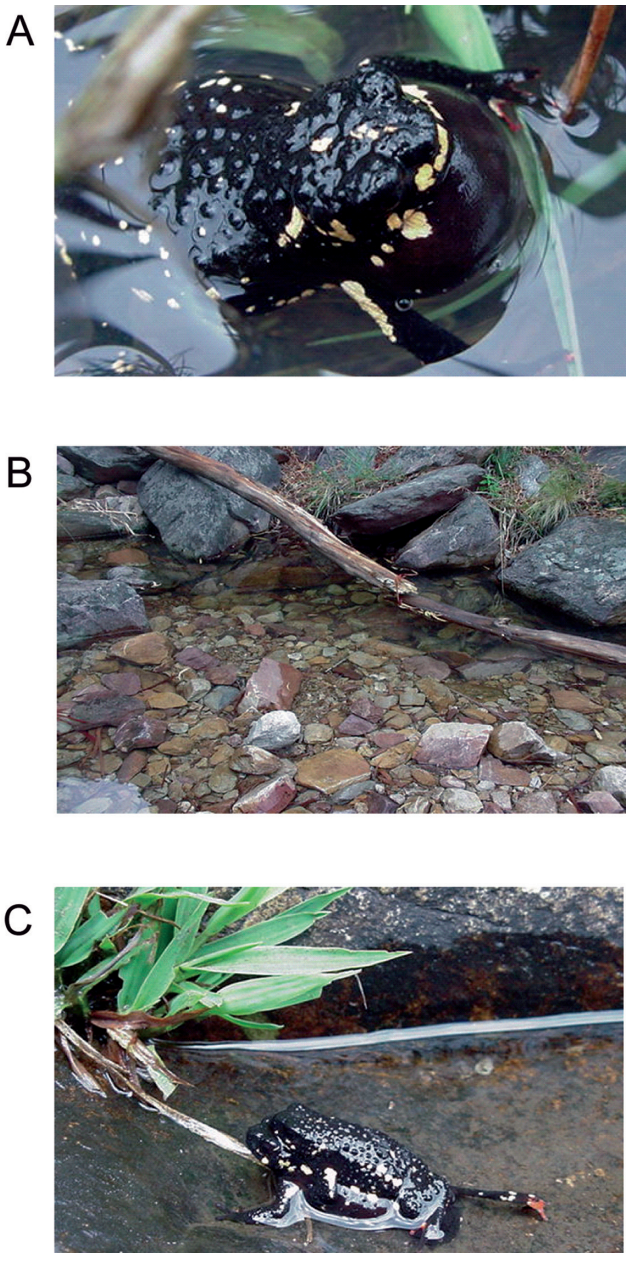

FIGURE 1. A: Male calling in a temporary pond; B: Reproductive habitat (temporary pond) in ETPP (Ernesto Tornquist Provincial Park, Buenos Aires, Argentina); C: Pair in amplexus at the edge of a temporary pond.

The most common sympatric species in the area are *Rhinella arenarum, Hypsiboas pulchellus,* and *Odontophrynus occidentalis,* being all them occasionally seen sharing their breeding habitat with *Melanophryniscus* sp.

Searches for amplectic pairs were conducted after rainfall exceeding 35 mm at sites where the species was known to breed, based on previous fieldwork. Reproductive sites were characterized in terms of size, depth and water flow speed. The number of pairs in amplexus at each pond and their specific location within the pond were recorded. Some pairs in amplexus were captured and placed in individual recipients maintained at room temperature ranging from 16 to 22°C. After spontaneous separation of the breeding pairs, individuals were measured (snout-vent length) with a caliper to the nearest 0.1 mm and -afterwards released at the capture sites. Egg clutches were characterized and the number of eggs and hatched larvae were recorded. The association of these last two variables with female size was analyzed by means of correlation analysis and the same analysis was performed to search for relationships between the number of hatched larvae and male size (Sokal and Rohlf, 1981). Eggs and larvae were measured using a stereoscopic microscope with an ocular micrometer (to the nearest 0.01 mm), and larval developmental stage was assigned according to Gosner (1960). Most of the larvae were released immediately after hatching, but some were kept in captivity in order to study their development. The water temperature during captive breeding ranged from 16 to 22°C.

RESULTS

The average size of ponds where breeding pairs were found (Fig. 1B) ranged between 0.1 and 22 m^2 (mean = 7.1 m^2 ± 7.4 SD, n = 14) and the depth ranged from 9 to 92 cm (mean = 46.8 cm ± 25.2 SD, n = 14). Waterflow was strong during and immediately after rain, slowing down in the following days and eventually stopping.

A maximum of 23 pairs in axilar amplexus were observed simultaneously in a 10 m^2 pond. Amplexus usually started at the inner edge of temporary ponds (Fig. 1C) and oviposition took place in the water, also at the edges of the pond in sites with calm or slow-moving waters, where the eggs could not be washed away. Amplexus lasted from 10 to 120 hours in captivity, but rarely for more than 24 hours under natural conditions. Each amplectic pair deposits multiple

clutches of eggs per amplexus period. Average egg diameter without egg capsules is 1.57 mm ± 0.10 SD (n = 73). The eggs, which have a heavily pigmented animal hemisphere, are laid in masses of variable number and are pushed away from the female cloaca by the male using his hind limbs and stuck to rocks of different sizes on the bottom of the pond or to vegetation, while the pair is still in amplexus.

In captivity 21 out of 26 pairs deposited eggs being the clutches deposited in a similar manner as observed in natural habitats. Mean clutch size was 129 eggs (n = 20, range = 56-223). The clutch size and number of hatched larvae were not significantly correlated to female body size (n = 20, P > 0.098) whereasthe number of hatched larvae was significantly correlated to male size (n = 19, P = 0.002). The mean percentage of larvae that hatched per clutch was 40 %, ranging from 0 to 88 %. Embryonic development was completed in 72 hours at 20-22°C.

According to its external morphology, the *Melanophryniscus* sp. tadpole corresponds to the benthic ecomorphological guild within exotrophic tadpoles (McDiarmid and Altig, 1999). In the lab, larvae hatched at Gosner stages 19-20 with an average length of 5.03 mm ± 0.56 SD (n = 15). They were actively swimming 24 hours after hatching and started to feed 48 hours later. The maximum length of the larvae was 18 mm, corresponding to Gosner stages 40 and 41. Metamorphosis was completed in 20 to 30 days and the newly metamorphosed juveniles were 5 to 7 mm in length. Coloration of juveniles was black, without the spots that are characteristic of the adult stage.

Discussion

According to its clutch characteristics and larval development, *Melanophryniscus* sp. can be assigned to "reproductive mode one" as defined by Duellman and Trueb (1986). This mode (eggs and feeding tadpoles in lentic waters) is considered the most primitive and generalized. However, according to Lavilla and Rouges (1992), who outlined 16 reproductive modes for Argentinean anurans, *Melanophryniscus* sp. matches the description of their mode 5 (eggs in masses laid in the bottom of the water body; embryonic development and hatching in the same environment; typical larvae).

Clutch characteristics and timing of embryonic and larval development could be explained as adaptations to ephemeral aquatic environments, which is in agreement with features reported for all other species in the genus studied in terms of their reproductive biology, i.e., explosive breeding highly synchronized with rainfall and rapid larval development (Fernández, 1927; Prigioni and Garrido, 1989; Bustos Singer and Gutiérrez 1997; Vaira, 2005; Goldberg *et al.* 2006). Nevertheless this reasoning is based solely on observational data, and alternative explanations for the observed features could be proposed in relation to competition, predation and/or phylogenetic constraints. Bustos Singer and Gutiérrez (1997) reported an acceleration of larval development in *Melanophryniscus stelzneri* as ponds evaporated and water temperature rose, so that metamorphosis was completed before the pond dried out. At our study site, pond evaporation usually takes place so quickly that larval development is frequently interrupted, resulting in massive death of larvae. Population persistence in the area probably depends on episodic recruitment related to certain climate events, such as heavy or successive rains that prevent pond drying.

The maximum duration of the amplexus for *M. rubriventris* in captivity is 96 hours (Lavilla and Rouges 1992); that is 24 hours less than those recorded in our work. However, amplexus duration in captivity is remarkably higher than in the field in both species. Regarding clutch size, Goldberg *et al.* (2006) recorded a maximum of 11 ovipositions for a pair of *M. rubriventris* breeding in a natural pond, with 8.6 eggs per oviposition event in average and a maximum of 18 eggs in a clutch. Fernández (1927) reported a maximum of 16 oviposition events during the amplexus of three females of *M. stelzneri.* Based on that we calculated an average of 17.2 and a maximum of 46 eggs per event. Bustos Singer and Gutiérrez (1997) reported clusters ranging from 5-35 eggs for *M. stelzneri* breeding in natural ponds. The maximum number of oviposition events recorded in our work for *Melanophryniscus* sp. in captivity was ten (with an average of six) with up to 80 eggs in one egg mass. These variations occurred despite the fact that all these taxa belong to the same phenetic group of small to mid-sized toads. The increased number of eggs per oviposition event could be due to the absence of intraspecific interference. Goldberg *et al.* (2006) proposed that laying multiple clutches is a strategy that avoids aggressive interactions between ampletic pairs and intruder males. We did not observed these aggressive interactions in wild populations at Sierra de la Ventana, although we found unmated males calling in the proximity of amplectic pairs. Alternatively, the dispersal of eggs by oviposition as multiple clutches in a pond could be an adaptation to increase the chances

of survival of the eggs when parts of the pond retain water for longer periods of time. This is supported by observations at the study site where we found dried egg clutches on the edges of ponds whereas clutches were still viable in the deeper part of the same pond. At our study site, there could be a trade off between oviposition sites in deeper central sections of the pond -against high speed waterflow.

Some clutches in our study completely failed, i.e., no larvae hatched, despite containing 50-200 eggs. We were not able to detect any pattern associated with those clutches which came from different natural ponds, corresponded to amplexus of standard duration, and were produced by males and females that did not significantly differ from the average size.

A lack of correlation between female size and fecundity found in our study is in agreement with previous studies for other amphibians (e.g., Crump 1974, Kluge 1981,Basso 1990). In our study, this may be due to the small size range of the females or to differences caused by variations in the amount of energy available for reproduction, as suggested by Duellman and Trueb (1986). As far as we could find in the literature, the significant positive correlation between male size and the number of hatched larvae has not been previously reported in amphibians. Additional research is needed to explore possible explanations related to morphology, age or experience of the male, or to sperm quality.

A dark pigmented animal pole of *Melanophryniscus* sp eggs agrees with descriptions for other species in this genus (Bustos Singer and Gutiérrez 1997; Echeverría, 1998, but see also Fernández, 1927). Dark pigmented eggs and oviposition in sunny and shallow sites cause acceleration of egg development by absorbing heat and by the gelatinous egg capsules acting as thermal insulator, features that have been described for amphibians living in other temperate and cold areas (Duellman and Trueb, 1986).

Further studies are needed to increase the knowledge about the breeding biology of this species and in particular to elucidate factors that allow its survival in such unpredictable environments.

Resumo

Pouco se sabe da biologia reprodutiva de espécies do gênero *Melanophryniscus*. Neste trabalho provê-se a primeira descrição do comportamento de amplexo, de sítios de oviposição, de características de posturas e do desenvolvimento larval para *Melanophryniscus* sp., uma espécie de status taxônomico indefinido que habita o extremo sul da área de distribuição do gênero (Sierra de la Ventana, Argentina), e discute-se sua relação com características de hábitat e pressões de seleção. Os resultados dos estudos foram comparados à biologia reprodutiva de outras espécies do gênero.

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