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Physiological basis for diurnal activity in dispersing juvenile *Bufo granulosus* in the Caatinga, a Brazilian semi-arid environment ☆

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Abstract

Diurnal activity is characteristic of many toad species, including *Bufo granulosus* from the Brazilian semi-arid biome called the Caatinga. Because of their patterns of activity, juvenile toads are exposed to hot and dehydrating conditions. Our investigation focuses on temperature and water relationships, and is based on the prediction that anuran diurnal activity in a semi-arid environment must be associated with morphological, physiological and behavioral traits enhancing thermal tolerances, capacity for performance at high temperatures and water balance. To test specific hypothesis related with this prediction, we investigated postmetamorphic *B. granulosus* and collected data on thermal tolerances and preferences, thermal safety margins, thermal dependence of locomotor behavior, thermal and kinetic properties of citrate synthase (CS), and skin morphophysiology. This information was compared with additional data from adult conspecifics and adult toads from sympatric species or from species from more moderate environments. We found that juvenile *B. granulosus* exhibit the highest critical maximum temperature reported for toads (44.2 °C) and are well suited to move at high temperatures. However, and in contrast with juveniles of other *Bufo* species, they do not show thermal preferences in a gradient and appear to hydroregulate more than thermoregulate. The CS of adult and juvenile toads shows typical patterns of thermal sensibility, but the thermal stability of this enzyme is much higher in juveniles than in adult Bufo of any other species studied. The inguinal skin exhibits a complex folding pattern and seems highly specialized for capillary water uptake. Diurnal activity in juvenile *B. granulosus* is possible given high thermal tolerances, keen ability to detect and uptake water, and avoidance behaviors.

Keywords: Dehydration; Toad; Water uptake; Thermal preference; Thermoregulation; Citrate synthase; Diurnal activity

1. Introduction

A number of anuran amphibians are able to fulfill life cycles in environments characterized by severe temperatures (Navas, 1996a,b; Wu and Kam, 2005). Such extreme temperatures might affect whole life cycles or specific developmental stages, and are offset by a number of traits among which physiological adjustments apparently play a fundamental role. In species from tropical high altitudes, for example, evolutionary shifts in physiology fully compensate for the effects of low activity temperatures on locomotor performance, whereas observed thermoregulatory behaviors apparently play only a secondary role in modulating the activity temperatures of individuals (Navas, 2003, 2005). It is possible, therefore, that adjustments of thermal physiology are generally more important than behavioral adjustments in the evolutionary expansion of thermal niches in anurans (Navas, 2003).

One example of anuran activity at high temperatures that apparently follows a pattern of conserved behavior and specialized thermal physiology was recently reported for juvenile toads in the Brazilian semi-arid biome called the Caatinga (Navas et al., 2004). Although this environment it is characterized by hot and dry conditions and by a rather

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unpredictable annual rain cycle (Souzareis, 1976), it is home to a number of amphibian species including two toad species in the genus *Bufo* (Trefaut-Rodrigues, 2000). Juvenile toads of these two species are predominantly diurnal, a trait that seems common in the genus and has been considered advantageous in the context of the thermal biology (Lambrinos and Kleier, 2003; Lillywhite et al., 1973; O'Connor and Tracy, 1992; Seymour, 1972; Tracy et al., 1993). In the Caatinga, however, diurnal activity exposes toadlets to hot and dry conditions, even more given their small size. Despite such apparent challenge, juveniles move over long distances along the dispersion phase and can be observed in large numbers at daytime, even after a few no-rain days (Navas et al., 2004).

This paper is based on preliminary data suggesting that diurnal activity in the Caatinga juvenile *Bufo* would not be possible without significant physiological adjustments (Navas et al., 2004). Our investigation focuses on the temperature and water relationships of postmetamorphic *Bufo granulosus*, a topic we treat at three levels of organization. At the organismal level, we study basic elements of the thermal ecology of juvenile toads, asking whether or not they become fully exposed to the harsh thermal conditions of the Caatinga, what are their thermal tolerances, and what are their thermal safety margins during field activity. Given that the critical maximum temperatures (CMT) of anurans vary among species but rarely reach values above 40 °C (Brattstrom, 1968), we hypothesized that this species either exhibits an exceptionally high CMT or it is often active near its upper thermal limit. Additionally, and given that our preliminary data indicated that the curve describing hopping performance *versus* temperature in this species is shifted to the right (Navas et al., 2004), we performed additional investigations on the influence of temperature on the behavioral performance of juvenile toads. We also evaluated the jumping stamina of juveniles as a function of body mass to better understand thermal effects on locomotion and the possible role of growth rate on this ecologically relevant behavioral trait.

At the organ level, we focused on the skin, the permeable interface between the toads and their environment. Skin morphology, thermal biology and water balance are highly integrated functions in amphibians (Snyder and Hammerson, 1993), and have played a primary role in the extension of their ecological ranges. Anurans might exhibit specialized secretions that help in body cooling (Lillywhite, 1971) or reduce water loss (Lillywhite et al., 1997a,b; Wygoda, 1984), or morphological attributes that increase water uptake by enhancing capillarity (Kobelt and Linsenmair, 1986; Lillywhite et al., 1997a,b; Schmuck and Linsenmair, 1997; Toledo and Jared, 1993). In general, species in the genus *Bufo* are regarded as fully permeable and susceptible to dehydration (Carey, 1978; Tracy et al., 1993), whereas dehydration is known to produce a strong dipsogenic effects in this genus (Goldstein et al., 2003). Accordingly, we propose that the skin morphology of Caatinga toads enhances opportunistic water uptake. To verify this, we compared the dorsal and the ventral skin of juvenile *B. granulosus* (including ventral inguinal skin as a separate category), and compared the skin of diurnal juveniles with that of nocturnal adults.

A more mechanistic approach of our study relates to the question of whether or not ontogenetic shifts in physiology are compatible with the contrast in the thermal ecology of adult and juvenile toads. We focused on muscle biochemistry because the ability of animals to survive acute thermal changes (relevant for juvenile toads in the Caatinga) depends on preserving enzyme function (Somero, 1995). Because juvenile toads are characterized by extensive hopping, a type of aerobic locomotion (Wells and Taigen, 1984), we studied the enzyme citrate synthase (CS), a catalytic agent of aerobic metabolism. To test the hypothesis of enzyme specialization in juvenile toads, we investigate the effects of temperature on the activity and thermal stability of CS obtained from the leg muscles, and compared results on juvenile *B. granulosus* with those on adult conspecifics and adult toads representing non-xeric species.

2. Material and methods

2.1. Study site

The Brazilian Caatinga is a semi-arid environment that covers some 800,000 km^2 in the Northeast of Brazil. It is characterized by high temperatures and unpredictable rainfall, usually between 300 and 800 mm over the year (Souzareis, 1976). The rainy season usually arrives in January and continues over a few months, but non-rain years and even longer draughts might occur. The families Leptodactylidae, Hylidae, Bufonidae, Microhylidae, Phyllomedusidae and Pipidae are represented in this environment, but only the first two mentioned are highly diversified (>15 species). Other families are represented by one or two species (Trefaut-Rodrigues, 2000). The family Bufonidae is represented by two species in the genus *Bufo*, *B. granulosus* and *B. jimi*.

This study was performed at Fazenda São Miguel, a private area located near the city of Angicos in the state of Rio Grande do Norte, Brazil. Field work was carried out from February 3 to 29, 2004, during the rainy season. On February 13 and 29, groups of juveniles and adults were transported to laboratories at Instituto Butantan and University of São Paulo for additional analyses. *Bufo ictericus*, another species cited in this paper, is common in the city of Sao Paulo and was captured on campus in March, 2004.

2.2. Natural history, ecology, and characteristics of the study animals

South American toads in the genus *Bufo* constitute a monophyletic group in which the ecologically diverse *B. granulosus* is placed within a small clade. The other Caatinga toads, *B. jimi*, is a close relative of *B. schneideri*, and possibly belongs to a sister clade containing also *B. ictericus* (Pramuk, 2006). The species mainly focused in this paper, *B. granulosus,* is a medium sized Bufonid with an adult body length of about 5 cm, whose reproductive season in the Caatinga starts with the first rains and continues while water is available (couples in amplexus were still observed by mid-February). Metamorphosis usually occurs within a few weeks, and juveniles disperse