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Breeding under unpredictable conditions: Annual variation in gonadal maturation, energetic reserves and plasma levels of androgens and corticosterone in anurans from the Brazilian semi-arid

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ABSTRACT

Anurans living in arid and semi-arid habitats are subjected to unpredictable rain patterns. Consequently, they should be prepared to reproduce at the onset of rain events. We investigated the covariation between calling behavior, testicular maturation, abdominal fat body index (FBI), plasma levels of androgens (T-DHT) and corticosterone (CORT) of males from three species of anurans in the Brazilian semi-arid during the reproductive period and drought. One of these species aestivates during the drought, while the other two species remain foraging. Although the three species display different behavioral strategies during the dry period, they present the same general reproductive patterns. T-DHT levels on the plasma and germinative cyst diameters were higher during the reproductive and breeding period compared to the drought. Additionally, the germinative cysts had all cell stages including sperm bundles during the dry season, however, it was only during the breeding event that free spermatozoa were found in the cyst lumen. These results suggest that these species present the reproductive pattern typical of desert anurans, consisting of opportunistic breeders that reproduce when triggered by a rain stimulus. *Rhinella jimi* and *Pleurodema diplolister* had higher CORT when males were calling. Moreover, *Rhinella granulosa* and *P. diplolister* showed lower FBI during breeding event, when males were calling. The high levels of CORT and lower FBI during reproductive period are associated, indicating that CORT modulates the recruitment of energy stores to prepare and maintain reproduction, particularly the expensive calling effort.

1. Introduction

Vertebrates from mesic habitats are usually characterized by predictable breeding patterns synchronized by environmental clues, including seasonal changes in temperature, precipitation, prey availability and photoperiod (for a review, see Wingfield, 2008). While environmental clues indicate the proximity of the reproductive period, increased plasma levels of androgens in males promote the maturation of testicular cells and development and maintenance of secondary sexual characters (Carr, 2011). Consequently, the peak of circulating androgens usually occurs during the reproductive period, when males are ready to display the courtship behavior and mate (Baum, 2002; Emerson and Hess, 1996). In addition, species that live in arid and semi-arid habitats are subjected to unpredictable rain patterns (Shine and Brown, 2008). These conditions have been associated with the occurrence of dissociated reproductive patterns, characterized by a disruption of temporal association between high androgen plasma levels and reproductive activity (Shalan et al., 2004). Given their preclusion to physiological anticipation of the breeding event, these animals should present year-round mature gonads, enabling immediate reproduction with the onset of unpredictable rain events (Lofts, 1984; Harvey et al., 1997). However, given that testicular maturation is dependent on elevated androgen levels (Emerson and Hess, 1996; Moore et al., 2005), the pattern of annual covariation between levels of circulating testosterone (T) and testicular maturation in desert and semi-arid species merits further investigation.

In addition to androgens, several groups of vertebrates show higher plasma levels of glucocorticoids (GC) during the reproductive season. Such moderately elevated GC levels may enhance the mobilization of energy substrates and facilitate reproduction through indirect effects on physiology and behavior (see Moore and Jessop, 2003; Landys et al., 2006; Carr, 2011). Testosterone (T) has a well-established effect on the stimulation and maintenance of calling behavior, but the relationship between corticosterone (CORT) and calling behavior seems to be more complex

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(Narayan et al., 2012a,b; Narayan et al., 2013a). While some studies provide evidence that CORT may inhibit calling activity (Leary et al., 2006a,b) and correlate inversely with circulating androgen levels (Orchinik et al., 1988; Marler and Ryan, 1996; Burmeister and Wilczynski, 2001), other studies have reported positive correlations of calling rates with both androgens and CORT plasma levels in natural choruses (Emerson and Hess, 1996, 2001; Harvey et al., 1997; Moore et al., 2005; Assis et al., 2012; Narayan et al., 2013b). Elevated CORT plasma levels during reproductive activity may facilitate calling activity by recruiting energy stores, but they are also certainly a consequence of calling exercise *per se* (Moore et al., 2005; Woodley, 2011; Bevier, 1995; Carvalho et al., 2008; Pough et al., 1992). In this context, the investigation of patterns of covariation between CORT plasma levels and energetic depots along different phases of the life cycle of anuran species from semi-arid and arid regions is also relevant.

In the Brazilian semi-arid Caatingas, rainfall is an unpredictable event that determines the anuran breeding activity (Arzabe, 1999; Vieira et al., 2009). In this type of environment, reproductive patterns characterized by some level of temporal dissociation between events of courtship behavior, spermatogenesis and peak circulating androgens might be selected (Crews, 1984; Crews and Moore, 1986). Additionally, anuran species from the Caatingas may respond to the dry period in different ways. While some species aestivate buried near underground water resources, other species remain in foraging activity around artificial permanent lakes or moist areas where these water bodies are available during drought (personal observation, Carvalho et al., 2010). Such different interspecific patterns of activity during the annual cycle may also affect the patterns of association between reproduction, energy deposition and mobilization, and plasma levels of the main steroids that modulate these activities. The objective of this study is to investigate the patterns of covariation between calling behavior, testicular maturation, energy reserves, and plasma levels of testosterone and dihydrotestosterone (T-DHT), and CORT of males from three species of anurans from the Brazilian semi-arid, during reproductive period and drought. The three species of anurans studied includes one species that aestivates during drought, and two species which remain foraging during this period. We expect that: (1) given the unpredictability of rain events, males should present testicles ready to reproduce during the dry period, containing germ cells in advanced stage of development; (2) higher plasma levels of T-DHT will occur during the reproductive period, when compared to the dry period; (3) higher CORT plasma levels will occur during a reproductive event, when males are calling; (4) there will be a negative correlation between fat body index (FBI) and CORT plasma levels; (5) lower FBI during the reproductive period when compared to the dry period, as lipid reserves accumulated at the end of the rain season would sustain most of the energetic requirements during drought and the next breeding event discharged with the onset of unpredictable rains in Caatingas; and (6) the differences in steroid plasma levels and energy reserves between the reproductive period and drought will be more accentuated in the aestivating species when compared to the species that remain foraging during drought.

2. Materials and methods

2.1. Field characterization and procedure

Field work was conducted at Fazenda São Miguel, a private area located near the city of Angicos, in the State of Rio Grande do Norte, Brazil (5°30′43″S. 36°36′18″W). The area is within the domain of Brazilian Caatingas, and is characterized by high temperatures. January is the hottest month, presenting an average temperature 27.4 °C (minimum: 22.8 °C, maximum: 32.0 °C), and July is the coldest month, with an average temperature of 24.3 °C (minimum: 20.3 °C, maximum: 28.3 °C) (http://pt.climate-data.org/location/312354/). The annual average temperature from 1950 to 2000 is 26.6 °C (worldclim.org) and there are two distinct seasons: a rainy season (January to May, 96.4 mm of precipitation/month) and a dry season (August to November, 2.5 mm of precipitation/month). During the rainy season, anurans reproduce when heavy rain occurs (150 mm or higher precipitation – personal observation; Arzabe, 1999). However, there are years that it does not rain in the Caatingas, and since anuran reproductive activity depends on precipitation (Arzabe, 1999), they do not reproduce in these years (personal observation). In response to the challenges of the dry season, anurans from this locality have adopted different behavioral strategies. While *Rhinella granulosa* and *Rhinella jimi* remain active, foraging close to water sources (personal observation), *Pleurodema diplolister* aestivate borrowed in the sandy soil under the beds of temporary rivers (Carvalho et al., 2010). For this study, animals were collected during three different periods in 2011: (A) during the reproductive season, but when there was no precipitation and breeding activity (January, 11–21th); (B) during a breeding event, when animals were calling (January, 22–24th); and (C) during the dry season (October, 6–17th). Males of *R. granulosa* ($N = 59$) and *R. jimi* ($N = 42$) were collected during the three periods cited above, and males of *P. diplolister* ($N = 38$) were collected only during periods B and C.

Blood samples were collected by cardiac puncture using heparinized 1 ml syringes with 26G × ½ inch needle for *R. granulosa* and *R. jimi*. Blood samples were obtained with heparinized microtubes following decapitation for *P. diplolister*, given the small body size of individuals from this species. Blood samples were collected within 3 min of capture to avoid interference of manipulation stress on steroid plasma levels (Romero and Reed, 2005). Blood samples were maintained on ice for up to 2 h, and submitted to centrifugation for 5 min at 5000 rpm. Plasma samples were frozen in liquid nitrogen, transferred to the laboratory in the University of São Paulo and stored at −20 °C for later analyses. Sampled males from *R. jimi* and *R. granulosa* were individually maintained in plastic containers with access to water until they were weighted in the following morning, before being euthanazited with sodium tiopental solution (25 mg/ml) (Thiopenthax®). After decaptation of the individuals of *P. diplolister*, the head and the body were individually maintained in aluminum foil on the 4 °C fridge until they were weighed and submitted to dissection procedures.

The individuals were dissected, testicles were immersed in Bouin's fixative solution for 24 h, and data on body mass (precision of 0.001 g) and abdominal fat mass (precision of 0.001 g) were collected. To calculate the FBI for each individual, the mass of abdominal fat bodies was divided by body mass. All the experiments and fieldwork were conducted under approved permission of Comissão de Ética no Uso de Animais do IB (CEUA) (Protocol number: 140/2011), and Ministério do Meio Ambiente, ICMBio, SISBio (License number: 3747–1).

2.2. Histological analyses

For *R. jimi*, we used the middle region of one testicle, for *R. granulosa* and *P. diplolister* we used one of the testicles. The tissue was dehydrated in a series of alcohols, clarified in xylene, and embedded in paraffin. After, they were then sectioned at 10 μm and stained with haematoxylin–eosin. The cross sectional diameter of 50 randomly selected germinative cysts were measured, and the stage of spermatogenic cells were classified by microscopy DMLB (Leica) and QWin Lite 3.1 image analyzer software (100× magnification) to determine the spermatogenetic activity (van Oordt, 1956; Loft, 1974).