

One of the conditions for the parasite-mediated sexual selection model to be fulfilled is that male ornament condition or sexual display rate decreases with increased parasite intensity, an assumption that has gained mixed support from studies in different phylogenetic groups (Anderson, 1994).

Intersexual selection in anurans is based largely on properties of male calls that were classified by Gerhardt (1991) as static or dynamic according to their coefficient of variation.

Given that dynamic properties of calls could be subjected to intersexual directional selection and entail high physiological costs, they may signal the quality of males, including their genetic resistance to parasites, to females. Among dynamic properties, calling rate is particularly suited to intersexual selection. We tested the hypotheses that:

Objetivo/Hipótese

Study Animal and Sites.—*Hypsiboas prasinus* is a treefrog occurring in the Atlantic Forest in southeastern Brazil at moderate altitudes. These frogs reproduce year round (Haddad

Behavioral Observations.—Calling males were located visually and observed for 30 min to quantify calling behavior. This period of observation was sufficient to evaluate the calling rate because males of this species call at relatively constant rates throughout the night.

Frog Capture and Parasite Collection, Quantification, and Identification.—The frogs were captured by hand and placed in individual plastic containers provided with water-soaked foam and artificial plants. Animals were then transported to the laboratory where they were euthanatized with sodium tiopental solution (Thiopentax®). Mass was recorded to the nearest 0.001 g and the digestive tract, body cavity, lungs, liver, and urinary bladder of each individual were examined for helminth parasites under a stereomicroscope. Helminths were fixed, quantified, and identified to family, genus, or species (sensu Madelaire et al., 2012).

Jessop, 2003). In anuran amphibians, males produce advertisement calls during the breeding season, and several aspects of these calls are subjected to intersexual selection (Wells, 2007a). In particular, the dynamic properties of advertisement calls, such as calling rates, are subjected to intersexual directional selection (Gerhardt, 1991; Wells, 2007a; Castellano et al., 2009), entail high energetic costs (Pough et al., 1992; Wells, 2001), and are positively correlated to glucocorticoid and androgen plasma levels (corticosterona é um glicocorticoide)

Assis et al., 2012). However, calling rates have been found to be more closely associated with corticosterone plasma levels (hereinafter referred to as CORT) in anurans

Although glucocorticoids are necessary for the maintenance of reproductive behavior (Moore and Jessop, 2003), high glucocorticoid plasma levels associated with the response to stressors have been associated with physiological changes that could potentially decrease fitness, including immunosuppression

Objetivo/Hipótese

Hypsiboas albopunctatus is an arboreal midsize tree frog

We collected behavioral data and blood samples from 35 adult males on February 4–8 (N = 11) and March 1–7 (N = 24), 2012, in São Luiz do Paraitinga (23°13'23 "S, 45°18'38 "W). São Paulo, Brazil.

2008; Kiss et al., 2009). Focal observations lasted 30 min, during which observers recorded calling behavior (number of vocalizations).

At the end of the behavioral observations, focal males were collected for blood sampling. We obtained blood samples (~80 µL) by cardiac puncture using heparinized 1 mL syringes and 26g × 1/2" needles.

The remaining blood was centrifuged for 4 min at 3000 rpm, and separated plasma was pipetted off and stored at –80 °C for later CORT measurements.

After blood collection, the animals were kept in individual plastic containers and transported to the laboratory for a test of the inflammatory response to subcutaneous injection of phytohemagglutinin (PHA).

The swelling in response to PHA was calculated from the proportional change in foot thickness before and 12–24 h after injection.

Among tetrapods, amphibians are generally characterized by highly permeable skin and comparatively higher rates of evaporative water loss (Toledo & Jared, 1993), and dehydration can affect both locomotor performance and thermal tolerance in this taxon (Anderson & Andrade,

In response to several stressors, including dehydration (Moeller, Demare, Davies, & DeNardo, 2017), the hypothalamic-pituitary-adrenal/interrenal (HPA/I) axis is activated resulting in glucocorticoids (GCs) secretion in vertebrates with corticosterone (CORT) being the main GC present in amphibians (Rollins-Smith, 2017). The

Along with GC changes, the neutrophil:lymphocyte (N:L) ratio increases in response to stressors in vertebrates (Davis, Maney, & toads from genus *Rhinella* show increased plasma CORT levels and N:L ratio in response to a standardized restraint stress protocol (Assis et al., 2015, in press; Gomes et al., 2012). Moreover, the magnitude of the stress response, represented by higher CORT and N:L ratio, is dependent on the intensity of the stressor (Assis et al.,

Objetivo/Hipótese _____

Adult males of *R. ornata* (N = 42) were collected in São Paulo, SP, toads were randomly divided into four groups: control, dehydration 10%, dehydration 20%, and restraint. Thereafter, a blood sample was collected.

Blood samples were transferred to microcentrifuge tubes and were centrifuged (4 min at 604g), the plasma was isolated and kept in a -80°C freezer for further CORT determination.

The leukocyte profile was performed according to Campbell & Trall (2012) and N:L ratio was calculated as the number of neutrophils divided by the number of lymphocytes on each slide.

Calling Behavior and Parasite Intensity in Treefrogs, *Hypsiboas prasinus*

selection. We tested the hypotheses that: 1) calling characteristics are correlated negatively to parasite intensity; and 2) the To test these hypotheses we studied the relationships between call parameters and helminth parasite intensity in males of a Brazilian subtropical tree frog, *Hypsiboas prasinus*. Because *H.*

Calling rate, corticosterone plasma levels and immunocompetence of *Hypsiboas albopunctatus*

In this study, we investigated the association between calling rates, variables associated to reproductive behavior and stress response, including CORT, body condition index, NLR, testes mass and an aspect of the immunocompetence (phytohaemagglutinin skin-swelling response) in males of the tree frog *Hypsiboas albopunctatus*. We predicted that calling rates are positively associated with CORT and other variables commonly interpreted as proxies of stress response, and negatively associated with immunocompetence.

Dehydration as a stressor in toads (*Rhinella ornata*)

In the present study, we tested the hypothesis that the dehydration is a stressor for *R. ornata* toads, a species occurring in habitats associated with Atlantic forest (Baldissera, Caramaschi, &

we compared physiological indicators of the stress response (CORT, N:L ratio, and Hct) in three groups of toads submitted to different levels of dehydration (0%, 10%, and 20% of standard body mass) and one group with 100% hydrated toads submitted to restraint with movement restriction. We predicted that: (a) in response experimental dehydration, toads would increase CORT, Hct, and N:L ratio; (b) increased CORT, Hct, and N:L ratio would be directly proportional to the level of dehydration; (c) toads subjected to the more intense dehydration (20%) would show values of CORT and N:L ratio comparable to those restrained.