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Short communication

How aggressive calls of a Neotropical treefrog vary among different levels of social tension?

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The treefrog *Pithecopus nordestinus* emits aggressive calls during agonistic interactions between males, but how these vocalizations vary between contexts with different levels of aggression remains unknown. Herein, we compared the acoustic parameters of aggressive calls emitted during physical disputes and out of fight contexts. The aggressive calls emitted during physical clashes (Fighting Calls) presented a repetition rate, duration and number of pulses significantly higher than those emitted out of fight contexts. Our results expand the knowledge about acoustic repertoire of Phyllomedusidae treefrogs and contribute to the understanding of the effects of social tension on the vocal behavior of anurans.

KEY WORDS: aggressive interaction, agonistic interaction, bioacustic, fighting call, *Pithecopus nordestinus*, physical disputes, vocalization.

INTRODUCTION

The main form of communication used by anurans in intersexual and intrasexual interactions is vocalization. Anurans can emit different types of calls associated with

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specific functions, such as advertisement calls to attract females and aggressive calls to intimidate or challenge rival males (Wells 2007; Toledo et al. 2014). Changes in the level of aggression can occur during acoustic interactions among males (e.g. Wogel et al. 2004; Reichert & Gerhardt 2011), varying, for example, in relation to the relative proximity between rivals, which might promote modifications in call parameters (Wells 1988). Given that spectral parameters (e.g. call frequency) are more static (Reichert & Gerhardt 2013), whilst temporal parameters (e.g. call duration) are more dynamic, these latter are more likely to change according to the social context (Wagner 1989).

Pithecopus nordestinus (Caramaschi 2006) is a Phyllomedusidae treefrog with geographical distribution along northeastern Brazil (Caramaschi 2006). In this species, agonistic interactions among males may vary from acoustic dispute to physical clashes (Vilaça et al. 2011; Brasileiro et al. 2020). Until recently, it was assumed that this species presented three distinct calls, the Advertisement Call, the Territorial Call I and the Territorial Call II. However, in the last years, it has been suggested alternative names for the hitherto considered Territorial Calls (Toledo et al. 2014; Mângia et al. 2019; Brasileiro et al. 2020). Specifically, the Territorial Call I was first suggested (Toledo et al. 2014) and posteriorly demonstrated (Brasileiro et al. 2020) to be a Fighting Call, due to its occurrence and prevalence during fights. On the other hand, it is also known that the considered Territorial Call I can be also emitted out of fight contexts, considered an Encounter Call when performed by males in a close-range (Toledo et al. 2014), or simply be treated as a nonspecific type of Aggressive Call, when executed by rival males relatively more distant from each other (Brasileiro et al. 2020).

Some aggressive calls (e.g. encounter and fighting calls) are very difficult to investigate in nature (Köhler et al. 2017), due to their relatively less occurrence when compared to other call types (e.g. advertisement and territorial calls). Thus, it is still unknown whether the acoustic parameters of some aggressive calls vary along different levels of social tension. Herein we compared the acoustic parameters of the aggressive calls of *Pithecopus nordestinus* emitted during physical disputes and out of fights, testing the hypothesis that the investment in these calls would be positively related to the level of social tension between males. In this sense, we predicted that the repetition rate and the duration of these calls would be higher when emitted during fights.

MATERIALS AND METHODS

The fieldwork was carried out at four bodies of water: two dams (reservoirs) and two temporary ponds, in the municipality of Groaíras, Ceará State, northeastern Brazil (see details in Brasileiro et al. 2020). All studied sites were exposed to similar climatic conditions and surrounded by the same type of environment. The locality is inserted in semiarid Caatinga domain, being predominantly composed by herbaceous and shrubby vegetation. The climate of the region is tropical hot semi-arid, with mean temperatures around 27 °C and mean annual rainfall of 900 mm (IPECE 2016).

Between February and May 2017, we recorded vocalizations of *P. nordestinus* in agonistic contexts among rival males (see details in Brasileiro et al. 2020). Each individual was registered in a single type of context: during physical disputes or out of fights. Vocalizations were obtained using a Marantz Professional PMD620 sound recorder and Shotgun Sennheiser ME62 unidirectional microphone, at a sampling rate of 44 kHz, 16-bit sample size and .WAV format. The audio files were deposited in the audiovisual collection Coleção Audiovisual do Semiárido, CASA (voucher numbers CASA 111 – CASA 134) at the Universidade Federal Rural do Semi-Árido, UFERSA. Posteriorly, we described the following acoustic parameters of each aggressive call: number of notes by call, repetition rate (call.min^{-1}), duration (msec), number of pulses, and

dominant frequency (kHz). The terminology and definition of acoustic parameters follow Köhler et al. (2017). All acoustic variables were obtained in software Audacity® (http://audacity.fr/) and, specifically to determine the dominant frequencies, we use the automatic peak frequency function, with Fast Fourrier Transformation (FFT = 1024).

For analytical purposes, we considered the mean values of all acoustic parameters for each male, and evaluated the data regarding the premises for the use of parametric tests. When the distribution did not meet the premises even after transformation, we applied non-parametric tests. We evaluated the differences in the acoustic parameters of aggressive calls emitted out of and during physical disputes by Student's t-test or Mann-Whitney tests, according to the compliance with the respective premises (significance level of $\alpha = 0.05$). We conducted all statistical analyses and graphic illustrations in R software v.3.3.0 (R Development Core Team 2016), using the package "seewave" (Sueur et al. 2008) for construction of oscillograms and spectrograms with the following parameters: window length = 256, frame = 100, overlap = 75.

RESULTS

The bioacoustic dataset totalized 41 min, distributed in contexts out of fights (25.6 min, mean of 2.3 min per individual) and during physical clashes (15.5 min, mean of 3.2 min per individual). We analyzed 72 aggressive calls emitted out of fights (n = 11 individuals, mean of 6.3 calls per individual) and 72 aggressive calls emitted during fights (n = 6 individuals, mean of 25.2 calls per individual). All aggressive calls were always composed by a sole note with amplitude modulation, irrespective of emission context (Fig. 1).

The temporal parameters of aggressive calls differed significantly between contexts out of fights and during physical clashes (Fig. 2). The repetition rate ($W = 62$; $n = 17$; $P < 0.01$; Fig. 2), duration ($W = 66$; $n = 17$; $P < 0.01$; Fig. 2) and number of pulses (with outliers: $W = 66$; $n = 17$; $P < 0.01$; without outliers: $t = 8.29$; $df = 13$; $P < 0.01$; Fig. 2) of calls emitted during physical clashes were higher than those emitted out of fights. On the other hand, the dominant frequency did not differ between calls emitted during and out of fights ($t = 1.73$; $df = 14$; $P = 0.11$; Fig. 2).

DISCUSSION

We corroborated the hypothesis that investment in aggressive calls would be positively related to the aggression level of the social context. In fact, we observed a clear distinction in the temporal parameters of aggressive calls emitted during and out of fights between males of *P. nordestinus*. During the physical disputes, there was a substantial increase in the investment of temporal parameters when compared to the context out of fights. Despite acoustic displays might be influenced by sort environmental factors, such as air temperature and vegetation structure (Ziegler et al. 2011), the independent effects of the presence and proximity of neighbours on call attributes is also well documented (Wells 1977, 1988). This ability to shape dynamic parameters of acoustic emissions in relation to different social contexts can represent the adequacy of the calls function to specific contexts (Wells & Schwartz 2007). Thus, our results demonstrated that the so-called Territorial Call I, in fact, combines two calls with acoustic configurations and possibly different functions. In the context of ongoing fights, these findings reinforce the suggestion of Toledo et al. (2014) and Brasileiro et al. (2020), that the aggressive calls of *P. nordestinus* emitted during

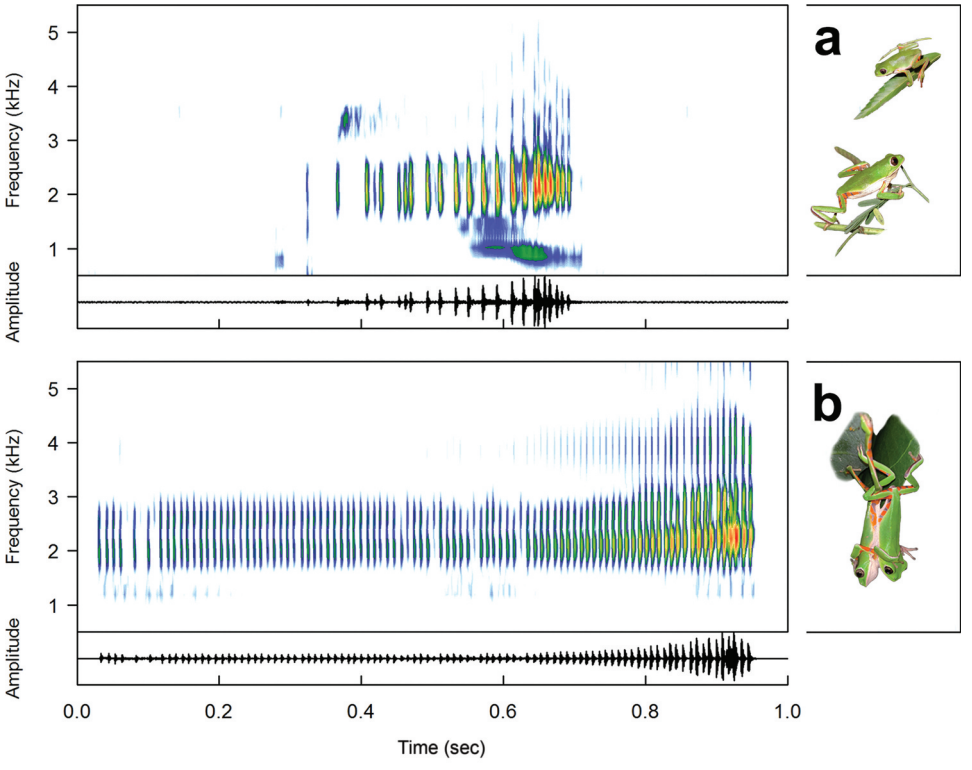

Fig. 1. — Spectograms and oscillograms of the aggressive calls of *Pithecopus nordestinus* from Groaíras municipality, Ceará State, northeastern Brazil. (a) Aggressive call emitted out of fight context; (b) Aggressive call emitted during physical clash (Fighting Call). Photographs by Carol Brasileiro (a) e Rogério Rumão (b).

physical disputes must be properly called Fighting Calls, not only due to differences in the social context, but mainly due to peculiarities in their acoustic parameters.

Regarding the social context out of fights, the literature indicates that the so-called Territorial Call I, could be treated with an Encounter Call when emitted by rival males a short distance from each other, preceding physical disputes (Toledo et al. 2014). The evidence provided here reveals that these calls emitted out of fights, even if they do not involve males substantially close to each other, present acoustic peculiarities consistent with specific functions. Thus, one possibility is to treat these calls emitted out of fights as Encounter Calls and necessarily to make the conceptual delimitation of this subcategory of aggressive calls more flexible, relativizing the distance between rival males according to the target species (in the case of *P. nordestinus* it varied from a few centimeters to 3 m). However, another genuine possibility is to propose a new additional subcategory of aggressive calls, specific to this context involving males relatively more distant from each other. Instead of arbitrarily suggesting one of these possibilities, we claim to more specific behavioral studies which might test the functions of different calls in species with diversified acoustic repertoires (e.g. *Scinax centralis*, Bastos et al. 2011; *Hylodes japi*; Sá et al. 2016).

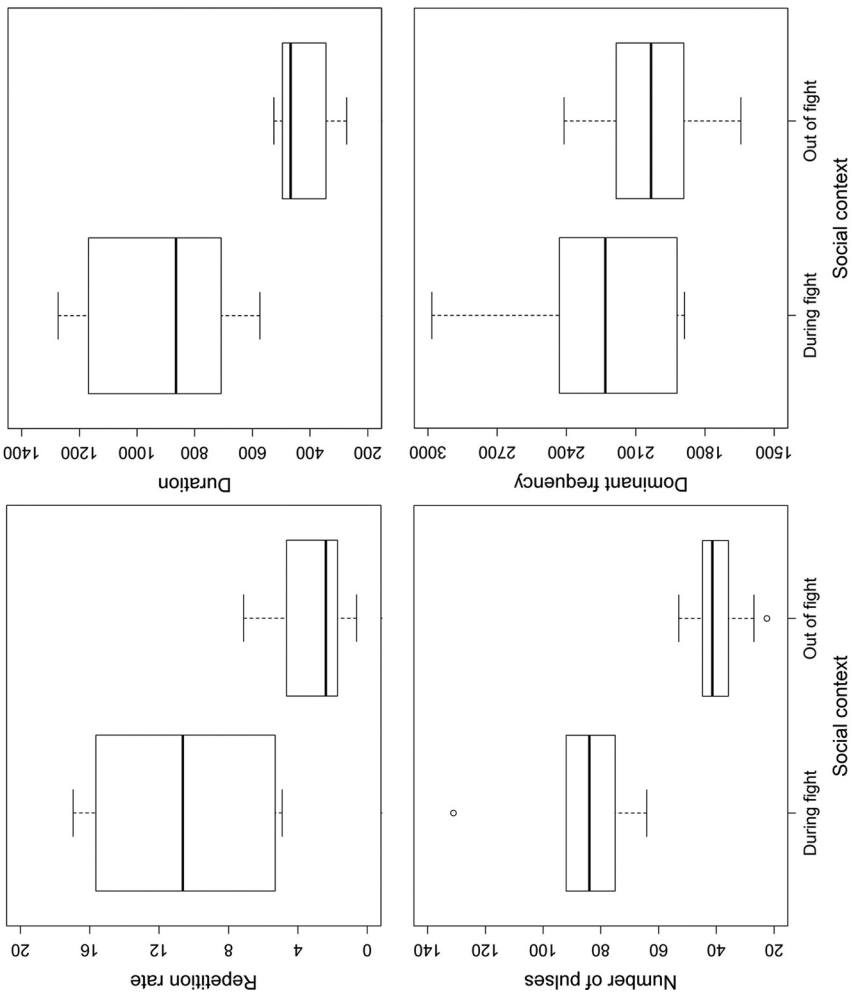

Fig. 2. — Comparisons of acoustic parameters of the aggressive calls of *Pithecopus nordestinus* between contexts out of fights and during physical clashes. Repetition rate is given in calls.min^{-1}, duration in msec, and dominant frequency in Hz.

The increased investment in the emission of certain parameters of the call is usually related to an increase in energy expenditure (Grafe & Thein 2001). However, in intrasexual interactions, the increase in the emission of a temporal parameter (e.g. call duration), may be associated with a reduction in the emission of another temporal parameter (e.g. call repetition rate), in a trade-off that balances the costs and benefits of acoustic activity (Wagner 1989). In *P. nordestinus*, multiple temporal parameters of aggressive calls were intensified during physical disputes. This condition implies a strategy with high energy investment (Wells & Taigen 1986), which may be related to the advantage that the emission of this call causes in physical clashes between rival males (Brasileiro et al. 2020).

On the other hand, the dominant frequency did not vary significantly in relation to the social context. When acoustic parameters have little intraspecific variation, they are considered static. This is the case, in general, for spectral parameters of calls, such as the fundamental and dominant frequencies (Gerhardt 1991). Exceptions, however, are possible, such as a reduction in the dominant frequency with an increase in the level of aggression (Reichert & Gerhardt 2013) and changes in frequency in order to appear larger than it really is (Bee et al. 2001). Nevertheless, in general, the dominant frequency is taken as an honest sign of the males' quality, since it has a strong relationship with the individuals' body attributes (Tárano & Herrera 2003; Dyson et al. 2013). Furthermore, it is natural that the dominant frequency has a considerable degree of phylogenetic conservation, considering that it is primarily involved in the recognition of individuals of the same species (Morais et al. 2012; Gingras et al. 2013).

In conclusion, we found that males of *P. nordestinus* increased the repetition rate, duration and number of pulses of their aggressive calls in relation to the level of social tension. The findings reported here contribute to the understanding of the complexity of acoustic repertoire of *P. nordestinus* as well as to the conceptual delimitation of the types of aggressive calls in anurans. In addition, we expanded the knowledge about the effects of social tensions on the vocal behavior of anurans, through fulfillment of a gap of knowledge concerning Neotropical species.

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DISCLOSURE STATEMENT

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AUTHOR CONTRIBUTION

A.C. Brasileiro and D.C. Passos collected and analyzed the data and wrote the manuscript. P. Cascon supervised all stages of the research and revised the manuscript.

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