

# Nightly calling patterns in a Neotropical gladiator frog

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**Abstract** In anurans, vocalization is the primary communication form and acoustic parameters are influenced by climatic conditions, but also by social contexts. We investigated calling site use and within-individual variation of acoustic parameters throughout the night in the gladiator-frog *Hypsiboas goianus*. We expected that large males would call closer to the water and at higher perches to avoid dehydration and maximize sound propagation. Furthermore, we tested the prediction that males would emit more aggressive calls early in the night and more advertisement calls late at night. Male size was not correlated with either distance from the water or perch height. However, as expected, males of *H. goianus* adopted a calling strategy that consisted of emitting more aggressive calls early in the night and more advertisement calls later in the night. Furthermore, repetition rate and interval between notes of the aggressive calls and repetition rate of the advertisement calls showed within-individual variation throughout the night that agreed with our expectations. The calling strategy of *H. goianus* is probably related to the establishment of calling sites early in the night and an investment in female attraction in the middle of the night when males' aggressive interactions

have faded away, or due to habituation increasing the males' aggressive thresholds. This is the first study investigating within-individual patterns of acoustic parameters of calls throughout the night for anurans.

**Keywords** Anura · Calling site use · Calling pattern · Intra-individual variation · Male territoriality

## Introduction

Communication is a key aspect that mediates all important animal social behaviours (McGregor and Peake 2000), involving the exchange of information in contexts as diverse as aggression (Herberholz et al. 2007), competition (Heavener et al. 2014), mating (Berry and Breithaupt 2010), and parental care (Aquiloni and Gherardi 2008). Among many communication pathways, acoustic communication is widespread among animals (Cinková and Policht 2015; Morais et al. 2015) and particularly important in social interactions of anurans (Ryan 1988; Wells 2007). During the reproductive period, males of most anuran species establish themselves in calling sites at the beginning of the night prior to the females' arrival (Given 1987). Males can defend their calling sites by emitting different call types (e.g. advertisement and aggressive calls) toward intruders, or even engaging in physical combats (Wells 1977; Bastos et al. 2011; Lemes et al. 2012). Advertisement calls are also used to attract conspecific females and, once the chorus is established, males face competition with other males (Wells 2007). As calling behaviour is energetically costly, males may adjust energy expenditure in calling activity according to the females' arrival throughout the night (Bevier 1997). Some studies have shown a pattern of reduction of aggressive calls throughout the night, probably because males settle their disputes early in the evening

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before the females' arrival, when they start to emit more advertisement calls (Reichert 2010; Bastos et al. 2011).

In anurans, females choose males based on acoustic parameters of the calls, e.g. frequency call, call duration, repetition rate, which may signal size and quality of males (Licht 1976; Gehardt 1991; Pfennig 2000; Byrne 2008). However, given that acoustic parameters of the calls may vary within the same individual (e.g. Rogers and Cato 2002; Pettitt et al. 2013) and that the social context of anuran breeding sites may change throughout the night, male acoustic parameters may also change according to the social context (Given 1987; Bevier 1997; Wells 2007). Such variation in the call parameters has the potential to affect anuran reproductive activity. Males can be influenced by the calling activity of their neighbours, altering the acoustic parameters of their calls or their behaviour (Wells 1977; Bastos et al. 2011; Lemes et al. 2012). For example, males can increase calling repetition rate in response to an increase in competitor density (e.g. Given 1987; Bastos et al. 2011), and some species can also change their calling activity due to habituation (Reichert 2010). Although some studies have compared within-individual variation in call parameters between different days or without considering time (e.g. Bee et al. 2001; Pettitt et al. 2013), very few studies have investigated within-individual variation throughout the night in anurans (e.g. Runkle et al. 1994; Schwartz et al. 2002). Moreover, none of these studies had made distinctions between call types, nor did they consider the time of the night where the calls were recorded.

Besides the within-individual variation and social context, anuran acoustic parameters can also be influenced by environmental variables and/or be limited by male morphological traits (e.g. Lemes et al. 2012; Morais et al. 2012; Bee et al. 2013). The dominant frequency of the calls is usually correlated with male size, while temporal parameters are more influenced by air temperature (e.g. Wagner 1989; Morais et al. 2012; Bee et al. 2013). Male size can also influence calling site use, with larger males defending better calling sites, which may increase reproductive success (e.g. Howard 1978; Wogel et al. 2002). The calling site occupied by a given male can affect the propagation of calls, influencing its ability to attract females (Wells and Schwartz 1982; Kime et al. 1999; Llusia et al. 2013). Elevated calling sites, for example, may allow for reduced call degradation by favouring sound propagation (Wells and Schwartz 1982; Kime et al. 1999). Another factor that may influence the choice of calling site is the desiccation risk (Rittenhouse et al. 2007), and thus, males may choose calling sites closer to the water to prevent desiccation (Bellis 1962; Lemckert and Slatyer 2002).

The gladiator frog *Hypsiboas goianus* (B. Lutz 1968) is included in the *Hypsiboas polytaeniatus* clade (Faivovich et al. 2005) and occurs in the Brazilian Cerrado in central Brazil (Frost 2016). Males of *H. goianus* call from the vegetation at the banks of water bodies and the repertoire of males include advertisement,

aggressive, and release calls, which have a harmonic structure and a variable number of pulses (Guimarães et al. 2001; Menin et al. 2004; Dias et al. 2014a) (Fig. 1). Males exhibit fidelity to calling sites for at least a few days and the species reproduces in the summer, mainly during the rainy months (Oct–Mar; Bastos et al. 2003a; Menin et al. 2004), and the frequency of the advertisement call is negatively correlated with male size (Morais et al. 2015). Because little is known about the calling behaviour of the species, here we investigated the use of calling sites, the pattern of calling strategy adopted by males throughout the night, and the within-individual variation of the acoustic parameters of *H. goianus*. We tried to answer the following questions: (i) do *H. goianus* males emit more aggressive calls early in the night when establishing calling sites? (ii) do males exhibit within-individual variation in acoustic parameters of the calls throughout the night according to social context? Furthermore, in order to test if large males (presumably the best mates) occupy higher quality perches, we also tried to answer the following: (iii) do larger males call from higher perches and sites located closer to the water?

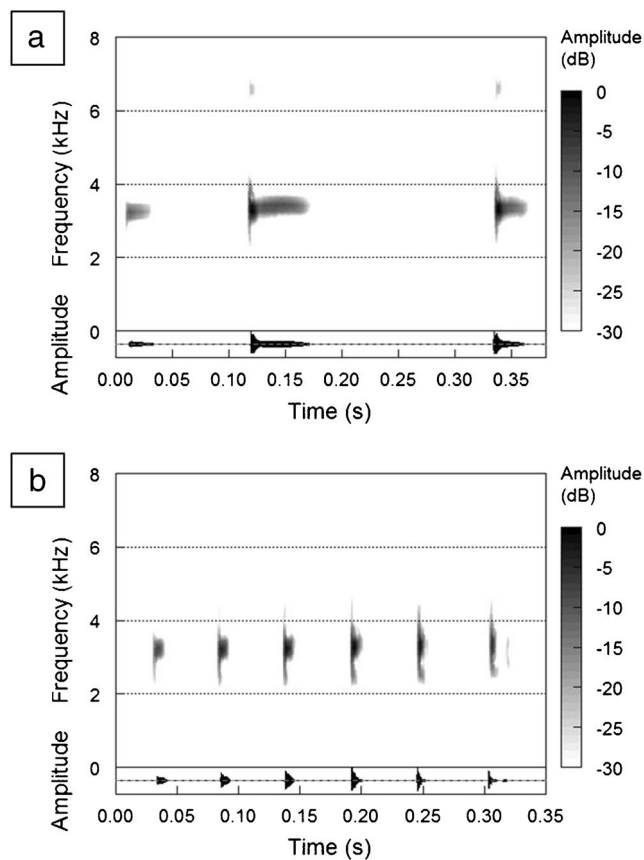
## Methods

### Study area

The study was conducted at the Floresta Nacional de Silvânia (Flona), a national forest reserve (16° 39' 32" S, 48° 36' 29" W, elevation ca. 900 m), Silvânia municipality, Goiás state, central Brazil. Local vegetation comprises different phytophysiognomies of the Cerrado (Morais et al. 2012). The study site included a slow-flowing second-order permanent stream with a surrounding gallery forest. The climate is tropical (AW of Köppen's classification), with two well-defined seasons: a rainy season usually from October to March and a dry season usually from April to September. The mean annual temperature is 26 °C and mean annual rainfall is 1600 mm (Bastos et al. 2003b).

### Data collection

We visited the study site during the reproductive season, in March and April 2013 and from October 2013 to March 2014, totalling 50 sampling nights. We searched for calling males after sunset (around 19:00) and characterized their calling sites based on the following variables: perch height, horizontal distance from the water, substrate type (ground or vegetation), and distance from the nearest male. We further categorized the vegetation types based on their height and complexity, according to Vidal and Vidal (2003): herbs (little or no lignification); shrubs (branches from the base, less than 5 m); small trees (same tree architecture, with a maximum of 5 m height); trees (clear trunk, more than 5 m); or grasses of the Poaceae family (Gramineae). We measured the height of vegetation with a measuring tape.



**Fig. 1** Spectrogram and oscillogram of the calls emitted by *Hysiboas goianus* recorded at Silvânia municipality, Goiás state, central Brazil. **(a)** One note of the advertisement call with three pulses and **(b)** one note of the aggressive call with six pulses; the spectrogram is shown above, and the oscillogram is shown below. Frequency information was obtained through fast Fourier transformation (FFT) of 1024 points width; recordings were made at a sample rate of 44.1 kHz and a 16-bit resolution (.wav file)

To analyse the calling pattern (i.e. the number of aggressive and advertisement calls throughout the night), we searched for calling males from 19:00, until the calling activity decreased significantly, around 24:00. For each male sampled, we recorded the calls emitted during 2 min ( $n = 100$  males). These recordings were used only for the counting of the number of advertisement and aggressive calls emitted by the males. We marked each male with subcutaneous implants (alphanumeric fluorescent tags visible under ultraviolet light, Northwest Marine Technology, Inc.) to avoid pseudoreplication, so each male was tested only once for each purpose. The vocal activity recorded during five periods of recording was compared: first period—from 19:00 to 20:00, second period—from 20:01 to 21:00, third period—from 21:01 to 22:00, fourth period—from 22:01 to 23:00, and fifth period—from 23:01 to 24:00.

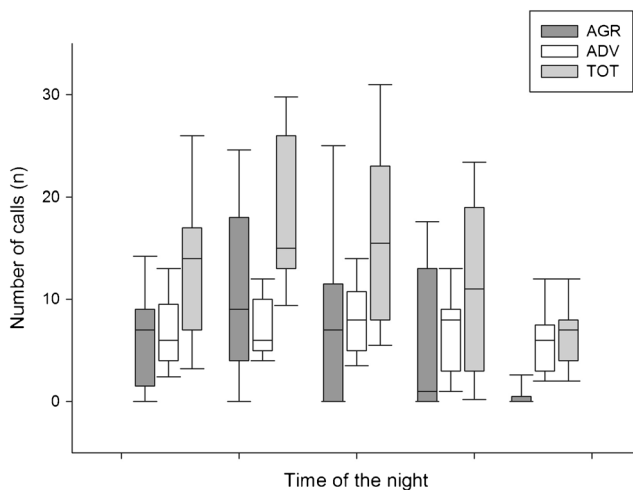
To investigate the existence of within-individual variation in acoustic parameters during the most active period of the night, we searched for males ( $n = 16$  males) around 20:00, when they were already well established at the calling sites (i.e. when the male

was less likely to leave the site), and recorded them during 2 min separated by 30-min intervals. These recordings were performed in the time range when males were already established in their calling sites (around 20:00) until the time when most males would have left or ceased to call (around 22:30), totalling six sessions (termed sessions “a”, “b”, “c”, “d”, “e”, and “f”). After each recording session, we registered the distance from the nearest male, number of calling males (i.e. the number of additional males that were calling within a ~10-m radius of the focal male), air temperature, air humidity, amplitude of the calls of the focal male, and noise amplitude (i.e. calls from other *H. goianus* and other frogs) ( $n = 16$  replicates for all variables). To measure call amplitude, the sound level meter was positioned at about 50 cm from the calling male. Air temperature and air humidity were recorded with a digital thermo-hygrometer (Instrutemp, ITHT 2210 model). A reduced number of males was used here because this analysis required that we record the same male through the whole night. Some males left the study site in the time between two recording sessions, some suddenly stopped calling, and some succeeded in mating. Whenever this happened, we excluded the male from the dataset. Each male was used only once and only for one objective. Males recorded for within-individual call variation ( $n = 16$ ) were different from the males used to analyse the call pattern ( $n = 100$ ).

We measured male snout-vent length (SVL) with a digital caliper (0.01 mm) and mass with a digital scale (0.01 g). The recordings were made using a digital recorder Tascam DR-40 coupled to a Rode NTG-2 microphone and the amplitudes of male calls and background noise were registered with a Minipa MSL 1325 sound level meter (Type II; Time weighting = Fast; A-Weighted). For all recordings, we placed the microphones at 50 cm from a calling male. We analysed five calls of each type for each recorded male using the Raven Pro 64 1.4 software (Cornell Lab of Ornithology, Ithaca, NY, USA). The statistical analyses were performed with average values for each recorded male. To investigate the within-individual variation of the acoustic parameters of the calls through the night, we analysed the following variables: dominant frequency (Hz), note duration (s), interval between notes (s), number of pulses per note, and repetition rate (calls per minute). Bioacoustics terminology followed Toledo et al. (2015). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

## Data analysis

Before performing the analyses, all variables were tested for normal distribution and log-transformed (Zar 1999). A  $\ln(x + 1)$  transformation was adopted if the data matrix included zeroes, whilst a  $\ln(x)$  transformation was adopted for the rest. All tests were performed in the R software (R Development Core Team 2013). To examine whether larger males occupied higher perches or called closer to the water, we calculated the Pearson correlation coefficient between male SVL



**Fig. 2** Number of advertisement calls (ADV), aggressive calls (AGR), and total number of calls (TOT) emitted by *Hypsiboas goianus* males in each period of the night, in central Brazil ( $n = 100$ ). Period 1: from 19:00 to 20:00 h; 2: from 20:01 to 21:00 h; 3: from 21:01 to 22:00 h; 4: from 22:01 to 23:00 h; and 5: from 23:01 to 24:00 h. Central horizontal bars represent medians, box heights represent first and third quartiles, and whiskers represent maximum and minimum values

and perch height and between male SVL and distance from the water.

To evaluate whether aggressive calls were more commonly emitted early in the night, we performed three generalized linear models (GLMs—one test for advertisement calls, one for aggressive calls and one for the total number of calls) with the number of calls emitted as response variable and the period of the night as predictor variable. Since the response consisted of count data, our GLMs used a Poisson error structure and a log link, with non-transformed (i.e. raw) data. Because we were interested in how each call type changed among the periods, each response variable was tested separately (Huberty and Morris 1989). To investigate whether the same male varied the acoustic parameters of their calls throughout the night, we performed five different generalized least squares analyses (GLS) (following Pékar and Brabec 2016). For each model, we included the acoustic parameter as the response variable, the individual as a repeated-measure factor, the recording session as a predictor variable with increasing levels, and an autoregressive process of order 1 (corAR1) as correlation structure, to account for the fact that measurements that are closer in

time are more likely to be similar. Afterwards, we performed simple linear regressions to test the influence of air temperature (predictor variable) on acoustic parameters that varied significantly throughout the night (response variable) ( $n = 16$  replicates). Prior to the analysis, we found that only air temperature ( $F_{(5,87)} = 6.09$ ,  $P < 0.001$ ) and humidity ( $F_{(5,87)} = 2.34$ ,  $P = 0.04$ ) varied significantly throughout the night. Moreover, these variables were negatively correlated ( $R = -0.47$ ,  $P < 0.001$ ). Thus, we decided to use temperature in the analysis because we believe this is a more informative variable (e.g. Alcantara et al. 2007; Lignau and Bastos 2007). As number of calling males ( $F_{(5,73)} = 0.52$ ,  $P = 0.76$ ), distance to the nearest male ( $F_{(5,67)} = 0.18$ ,  $P = 0.97$ ), and noise level ( $F_{(5,49)} = 1.53$ ,  $P = 0.19$ ) did not vary throughout the night, they were not included in the analyses.

## Results

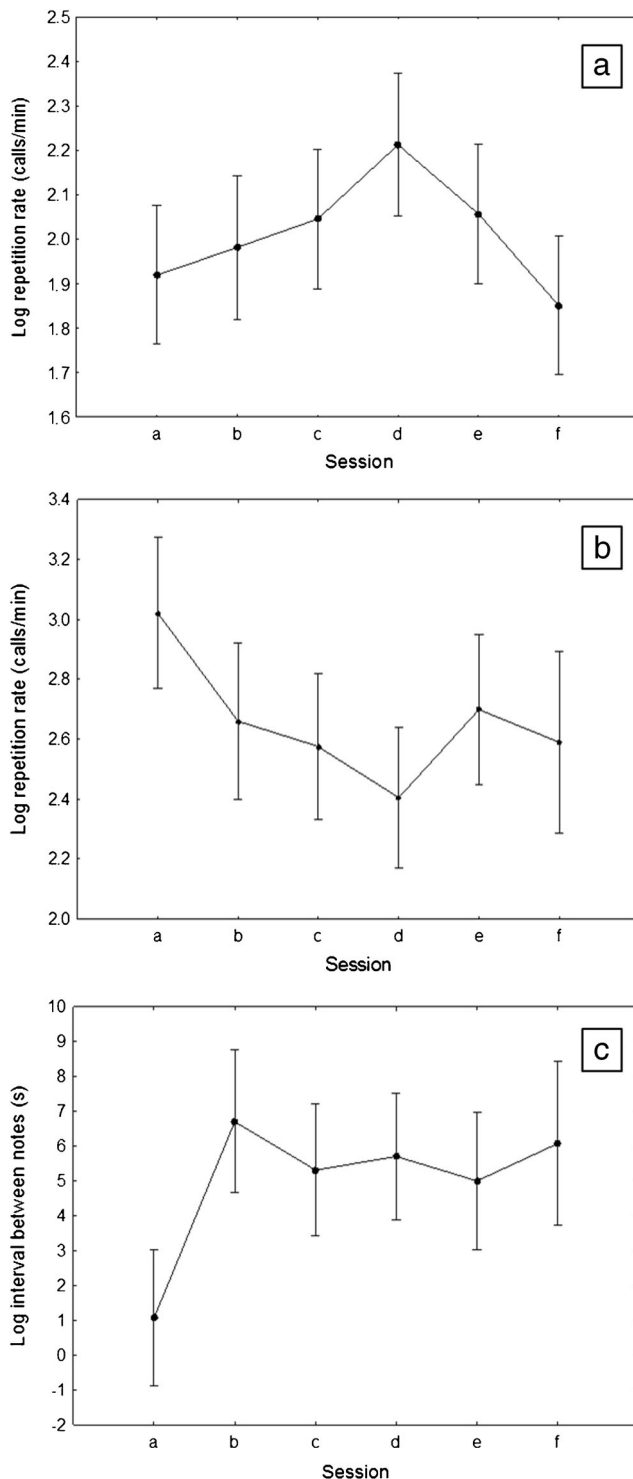
Males' calling activity started around 18:45 h. Substrates used as calling sites were herbaceous plants (53%,  $n = 37$ ), small trees (23%,  $n = 16$ ), grass (Poaceae, 23%,  $n = 16$ ), and ground (1%,  $n = 1$ ). Perch height where males were found ranged from 0 to 200 cm (average =  $64 \pm 42$ ,  $n = 105$ ) and the distance of males from the water ranged from 30 to 2250 cm (average =  $783 \pm 570$ ,  $n = 24$ ). All calling males were perched in the vicinity of the stream margin, i.e. above the ground, never directly above the water. Male SVL ranged from 28.53 to 37.48 mm (average =  $32.56 \pm 1.45$ ,  $n = 105$ ) and was not correlated with distance from the water ( $r = 0.14$ ,  $P = 0.54$ ,  $n = 23$ ), neither with perch height ( $r = 0.11$ ,  $P = 0.21$ ,  $n = 105$ ). The mean distance to the nearest calling male was  $243 \pm 150$  cm (ranging from 20 to 1000 cm,  $n = 101$ ). Air temperature ranged from 19.6 to 23.6 °C (average 20.9 °C) and humidity ranged from 74.3 to 97.6% (average 92.4%).

Number of aggressive calls and total number of calls emitted differed throughout the night ( $\chi^2_{(95,99)} = 275.75$ ,  $P < 0.001$ ;  $\chi^2_{(95,99)} = 158.03$ ,  $P < 0.001$ , respectively) (Fig. 2;  $n = 100$ ). Males emitted more aggressive calls early in the night (1st and 2nd period) (Fig. 2;  $n = 100$ ). However, the number of advertisement calls did not exhibit significant variations ( $\chi^2_{(95,99)} = 7.17$ ,  $P = 0.13$ ). Sound amplitude levels

**Table 1** Results of the generalized least square regressions for the within-individual variation of the acoustic parameters of advertisement calls and aggressive calls throughout the night; statistically significant values of “ $P$ ” are shown in italics ( $n = 16$  males)

	Advertisement calls			Aggressive calls		
	$F_{(5,87)}$	$P$	Mean $\pm$ SD	$F_{(5,69)}$	$P$	Mean $\pm$ SD
Dominant Frequency	0.80	0.54	$3288.32 \pm 108.62$	1.2	0.32	$3258.95 \pm 113.80$
Note duration	1.17	0.33	$0.26 \pm 0.09$	1.35	0.25	$0.24 \pm 0.06$
Number of pulses	0.99	0.42	$2.58 \pm 0.56$	0.77	0.57	$4.88 \pm 3.02$
Interval between notes	0.95	0.45	$9.72 \pm 11.47$	4.98	<i>&lt; 0.01</i>	$2.93 \pm 8.72$
Repetition rate	2.55	<i>0.03</i>	$7.78 \pm 2.89$	3.58	<i>&lt; 0.01</i>	$14.96 \pm 7.64$





**Fig. 3** Within-individual variation of the acoustic parameters of *Hypsiboas goianus* through the night (sessions from “a” to “f”, see [Material and Methods](#) section) for which the generalized least square analyses showed a significant effect of the recording session, in Central-Western Brazil ( $n = 16$ ); **(a)** Repetition rate of the advertisement calls; **(b)** repetition rate of the aggressive calls; and **(c)** interval between notes of the aggressive calls. The repetition rate of advertisement calls peaks in the middle of the night (session “d”); the repetition rate of aggressive calls peaks in the early night (session “a”) and then steadily decreases; and the interval between notes exhibits the lowest values in the early night (session “a”), which then increases and remain stable

observed in the session “d”, around 21:30 ( $F_{(5,87)} = 2.55$ ,  $P = 0.03$ , Table 1, Fig. 3a) ( $n = 16$ ). The remaining parameters of the advertisement calls did not exhibit significant variations (Table 1). For aggressive calls, the repetition rate and interval between notes within-individuals varied throughout the night ( $F_{(5,69)} = 3.58$ ,  $P = 0.006$ ;  $F_{(5,69)} = 4.98$ ,  $P < 0.001$ , Table 1, Fig. 3b, c) ( $n = 16$ ). The repetition rate was highest and the interval between notes was smallest in the first session (“a”), around 20:00 (Fig. 3b, c). The remaining parameters of the aggressive calls did not exhibit significant variations (Table 1). The parameters of the advertisement and aggressive calls that varied significantly were not correlated with air temperature (Table 2).

## Discussion

As we expected, males of *H. goianus* emitted more aggressive calls early in the night, similar to what has been reported for other anurans (Reichert 2010; Bastos et al. 2011). At the beginning of the night, males establish themselves in the calling sites and defend them from intruders, mostly through the emission of aggressive calls (Wells 1978; Given 1987; Bastos et al. 2011). Conversely, the advertisement calls did not vary significantly among the periods, despite exhibiting a tendency to increase in the third and fourth periods of the night (see Fig. 2).

The within-individual analyses showed a higher repetition rate and smaller interval between notes of the aggressive calls in the first session (a) compared to the others. These results support our prediction of establishment and defence of calling sites by males early in the night, before the females arrive at the chorus, as mentioned above (Given 1987; Wells 2007). Despite the absence of statistical significance, our results showed that the emission of advertisement calls tended to increase in the third and fourth periods of the night. When we analysed the within-individual variation of the repetition rate of the advertisement calls (which can be used as a proxy of the number of advertisement calls), the tendency observed in the GLM becomes significant: the repetition rate varied within-individuals throughout the night, being higher in the session “d” (around 21:30 h), corresponding to the middle of the night. The advertisement call plays an important role in

ranged from 64.9 to 88.3 d SPL (average 66.36 d SPL) for advertisement calls, from 71 to 87.7 d SPL (average 79.6 d SPL) for aggressive calls, and from 46 to 64.3 d SPL (average 54.6 d SPL) regarding the background noise ( $n = 16$ ).

Regarding the acoustic parameters of the advertisement calls, only the repetition rate showed within-individual variation throughout the night, with the highest repetition rate

**Table 2** Results of the linear regressions of the acoustic parameters with air temperature (the only environmental factor that varied significantly throughout the night,  $n = 16$  males)

Call type	Model	df	<i>F</i>	$R^2_{adj}$	<i>beta</i>	<i>P</i>
Advertisement	Repetition rate~temperature	1.91	0.82	−0.002	0.09	0.37
Aggressive	Interval between notes~temperature	1.71	1.46	0.006	0.14	0.23
	Repetition rate~temperature	1.71	2.89	0.026	0.20	0.09

female attraction, and competition for these females may be more intense during the peak of the calling activity (Wells 1977; Ryan 1991). Thus, the greater investment in calling activity by males could be associated with females' arrival in the chorus (Bevier 1997; Murphy 1999).

We did not actively search for females, as this was not the focus of our study. However, we occasionally encountered mating pairs, both before and during amplexus, approximately between 21:00 and 23:00 h (TM Dias, personal observation). Earlier arrival of males relative to females at reproductive sites is a strategy that has also been observed in other anurans (Bevier 1997; Murphy 1999). High repetition rates have been correlated with increased metabolic costs in many anurans and may indicate the male's competitive and resource-acquiring abilities (Wells 2001; Pettitt et al. 2013). Thus, high repetition rates may indicate good physiological condition of males, which may be evaluated by females, as observed for many anuran species (e.g. Morris and Yoon 1989; Welch et al. 1998; Pauly et al. 2006; Tárano and Fuenmayor 2013). This preference may benefit females through increasing fertilization success and/or offspring fitness (Bourne 1993; Pfenning 2000; Pettitt et al. 2013).

Another possible explanation is that aggressive calls decrease as a result of habituation, defined as a decrement in response to repeated stimulation (sensu Thompson and Spencer 1966). Specifically, continuous stimulation by neighbours' advertisement and aggressive calls may elevate the aggressive threshold of males (i.e. the minimal amplitude of another male's vocalization that elicits an aggressive call response, see Reichert 2010). This habituation effect was already reported for anurans and could explain why aggressive calls and some of its parameters tend to decrease as the night progresses, conversely to advertisement calls, for which the repetition rate varied, but did not steadily decrease (Fig. 2) (Marshall et al. 2003; Reichert 2010; this study). Habituation could also explain why the nearest neighbour distance and number of calling males did not vary through the night despite the variation of the number of aggressive calls, i.e. habituation does not require a variation in social context to occur (Thompson and Spencer 1966). Habituation could occur even if the time of female arrival is an important factor, as these explanations are not mutually exclusive.

Environmental conditions, such as air temperature and humidity, influence anuran calling activity (Brooke et al. 2000; Steelman and Dorcas 2010; Nali and Prado 2012) and the

influence of air temperature on temporal acoustic parameters has been verified for other species (Lemes et al. 2012; Morais et al. 2012; Gambale and Bastos 2014). However, here neither temporal parameters of the aggressive nor those of the advertisement calls that varied throughout the night correlated with air temperature, despite the variation of temperature throughout the night. Moreover, we did not detect within-individual variation in the dominant frequency analysed for both the advertisement and aggressive calls throughout the night. The lack of variation in the dominant frequency can be explained because this parameter is usually correlated with morphological traits, mainly male size, which is stable over time (Morais et al. 2012; Bee et al. 2013).

We expected that larger males would defend and occupy humid sites closer to the water, which decreases dehydration. However, we found no correlation between male size and perch height neither with distance from the water. A possible explanation is that dehydration risk is not such an important factor for *H. goianus*, probably because they call from the gallery forest. This means that the choice of calling sites in *H. goianus* might be related to other factors, such as exposure/visibility of the perches and arrival order (Abrunhosa and Vogel 2004; Shepard 2004; Dias et al. 2014b). Although we did not control for such factors in our study, *H. goianus* males are territorial (Menin et al. 2004), suggesting that social factors are important, and thus likely to explain the choice of calling sites. Hence, the choice of calling sites may be determined simply by the male's arrival order, where males that arrive early choose the best sites available (e.g. Wells 1977; Dias et al. 2014b). It would be particularly interesting to investigate what is the most optimal calling site for *H. goianus* males and how does the social context influence the choice of these sites.

Our study revealed that males of *H. goianus* adopt a calling pattern throughout the night, emitting more aggressive calls, at higher repetition rates and smaller intervals early in the night, while emitting more advertisement calls, at higher repetition rates, in the middle of the night ( $n = 100$  for the number of calls; and  $n = 16$  for the acoustic parameters). This calling strategy could be related to the establishment of males at their calling sites and attraction of females to these sites, respectively; or could also be the result of habituation increasing this species' aggressive threshold. Unfortunately, the data on females' behaviour and the habituation phenomenon in this

species are still lacking, which prevents us from either dismissing or corroborating these hypotheses. Future studies should focus on the dynamics of female arrival at the chorus and test the existence of female preferences for males that emit calls at high repetition rates. They should also investigate the territorial behaviour, aggressive thresholds and the possibility of habituation occurring in this species. Our study is one of the few analysing within-individual variation of acoustic parameters of calls throughout the night in anurans and the first to make a distinction between call types. These studies are fundamental to better understand the reproductive strategy of this and other ecologically similar Neotropical anurans.

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