Universidade Federal de Goiás

Instituto de Ciências Biológicas

Programa de Pós-graduação em Ecologia & Evolução

Ecologia reprodutiva, comportamento acústico e territorial de uma

perereca neotropical (*Hypsiboas goianus*) no Brasil Central

Tailise Marques Dias

Goiânia, Goiás

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Orientador: Dr. Rogério Pereira Bastos

Coorientadora: Dr.ª Cynthia P.A. Prado

Dissertação apresentada ao Instituto de Ciências Biológicas da Universidade Federal de Goiás, como requisito para obtenção do título de mestre em Ecologia e Evolução.

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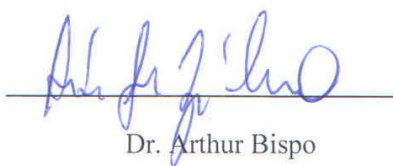
Dr. Mirco Solé

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(Membro Suplente)

Dr. Rogério Pereira Bastos

(Presidente)

Aos meus pais, que

sempre me incentivaram a estudar.

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"Descobrir consiste em olhar para o que todo mundo está vendo

e pensar uma coisa diferente."

Roger Von Oech

SUMÁRIO

Resumo

Em anuros, a comunicação acústica é a comunicação primária e está envolvida em diferentes comportamentos, sejam eles reprodutivos ou territoriais. Nesse sentido, este trabalho investigou a reprodução e o comportamento acústico e territorial de *Hypsiboas goianus*. O estudo foi realizado na Floresta Nacional de Silvânia, município de Silvânia, estado de Goiás, Brasil. Primeiramente, nós investigamos o uso do sítio de vocalização, o padrão de vocalização dos machos ao longo da noite e a variação intraindividual dos parâmetros acústicos ao longo da noite. Nós encontramos uma relação positiva entre o comprimento rostro-cloacal dos machos e a distância deles até a água. Os machos emitiram mais cantos agressivos no início da noite e o número de cantos de anúncio emitidos aumentaram no meio da noite. Além disso, os parâmetros acústicos taxa de repetição e intervalo entre as notas variaram ao longo da noite. Adicionalmente, nós investigamos a ecologia reprodutiva e a dinâmica do comportamento territorial de *H. goianus*. Nós não encontramos relação entre o tamanho da fêmea e o tamanho da desova, e entre o tamanho dos ovos e o número de ovos. Ainda, os acasalamentos não foram assortativos. Além disso, nós realizamos dois experimentos de territorialidade, os quais incluíram manipulação de distância entre os machos e introdução de machos na área do macho residente. Em ambos experimentos o número de cantos emitidos pelos machos foi influenciado pelos seus tamanhos e os combates físicos foram raros. Os resultados encontrados neste trabalho mostram que os machos de *H. goianus* mantêm uma estratégia reprodutiva ao longo da noite e que a comunicação acústica é muito importante em diferentes contextos sociais para essa espécie.

Abstract

In anurans, acoustic communication is related to many different behaviors, either reproductive or territorial. In this sense, this study investigated the reproduction and the acoustic and territorial behavior of *Hypsiboas goianus*. The study was performed in the Floresta Nacional de Silvânia, municipality of Silvânia, state of Goiás, Brazil. Firstly, we investigated the use of calling sites, the calling pattern of the males along the night and the within-individual variation of the acoustic parameters along the night. We found a positive relationship between male snout-vent length and their distance from the water. The males emitted more aggressive calls at the early hours of the night and the number of advertisement calls emitted increased in the middle of the night. Besides, the repetition rate and interval between notes varied along the night. Additionally, we investigated the reproductive ecology and the dynamics of the territorial behavior of *H. goianus*. We found no relationship between the size of the female and clutch size, and also between egg size and number of eggs. Matings were not assortative. We also performed two territoriality experiments, which included manipulation of the distance between males and the placement of intruder males next to an established resident. In both experiments the number of calls emitted was influenced by size, and physical combat was rare. The results from this study show that male *H. goianus* maintain a reproductive strategy along the night and that, for this species, acoustic communication is greatly important in different social contexts.

Key-words: Clutch; Calling, Calling site; Reproduction; Territoriality.

Introdução geral

Os sinais específicos emitidos por indivíduos podem influenciar o seu sucesso reprodutivo (Gerhardt, 1974). Em anuros, os mecanismos de reconhecimento específico de origem pré-zigótica dependem principalmente de características morfológicas e de cantos de corte (Lodé & Pagano, 2000). Além disso, a comunicação acústica nesse grupo tem importante papel no reconhecimento de parceiros, entre outras funções, com importantes implicações no processo de seleção sexual e especiação (Ryan, 1988). Machos de anuros podem emitir diferentes tipos de cantos em diferentes contextos, sendo que um dos cantos mais comuns é o canto de anúncio, que é emitido pelos machos para anunciar sua posição aos machos vizinhos e para atrair fêmeas para o acasalamento (Wells, 1977). Além disso, os machos podem variar alguns parâmetros acústicos do canto para se tornarem mais atrativos às fêmeas, uma vez que elas podem avaliar a aptidão dos seus potenciais parceiros através de seus cantos (Morris, 1989; Passmore et al., 1992; Pfennig, 2000; Pettitt, 2013). Outro tipo de canto comum entre os anuros é o canto agressivo. Esse canto parece ter a função de manter uma distância entre os machos e servir como alerta para um intruso que está se aproximando do macho residente (Wells, 1977; Dyson & Passmore, 1992).

Em contextos sociais de anuros é comum que machos exibam comportamento territorial, uma vez que a manutenção e defesa de um território também pode influenciar no sucesso reprodutivo (Wells, 1977). A territorialidade dos anuros pode estar relacionada com a defesa de recursos necessários à sobrevivência, tais como alimento ou abrigos, bem como defesa de sítios reprodutivos (Wells, 1977). Assim, a defesa de

um determinado sítio pode trazer vantagens se ele oferecer ao macho um acesso a recursos limitados (Wells, 1977; Howard, 1978a). Os machos podem ser influenciados pela atividade de canto de seus vizinhos, emitindo cantos agressivos e também podendo entrar em combates físicos (Wells, 1977; Bastos et al., 2011; Lemes et al., 2012). No entanto, o custo dos combates físicos pode ser alto e, nesse contexto, as vocalizações são importantes para diminuir o gasto energético e evitar as injúrias que podem ser causados por esses combates (Martins et al., 1998). Além disso, um macho pode reconhecer o tamanho e as habilidades de seu oponente a partir de características do canto e, assim, evitar confrontos nos quais ele não teria chances de vencer (Wells, 1978; Bee & Perrill, 1996; Bee, 2002). Da mesma forma, machos intrusos também podem avaliar o tamanho do macho residente e decidir se engajar em uma disputa ou não, uma vez que o custo de perder um território é maior para o macho residente, que poderá defender vigorosamente seu território contra um intruso (Wells, 1978; Bee et al., 1999; Owen & Gorder, 2005).

Os sítios reprodutivos de anuros compreendem os sítios de vocalização e os sítios de oviposição, e em espécies territoriais os machos maiores podem defender os melhores sítios de vocalização (Howard, 1978a; Bastos & Haddad, 1996; Wogel et al., 2002). Para algumas espécies de anuros o sítio de vocalização é o mesmo do sítio de oviposição, sendo que os machos defendem um sítio que seja adequado para deposição dos ovos (Howard, 1978a; Howard, 1978b). Assim, o modo reprodutivo e as características morfológicas e fisiológicas dos indivíduos podem influenciar o uso dos sítios de vocalização (e.g. Crump, 1971; Cardoso et al., 1989). Os machos de algumas espécies também podem escolher os sítios mais altos, uma vez que esses permitem uma melhor propagação do som (Wells & Schwartz, 1982). Por outro lado, machos de outras espécies podem preferir sítios que mantenham maiores distâncias entre seus vizinhos, o

que pode reduzir a interferência acústica, evitar combates físicos e aumentar as chances de encontrar fêmeas (Whitney & Krebs,1975; Shepard, 2004; Nali & Prado, 2012).

As fêmeas preferem os machos mais aptos, assim a escolha das fêmeas pode ser determinante no sucesso reprodutivo dos machos (Wells, 1977). A aptidão dos machos pode estar relacionada positivamente com as taxas de repetição de cantos ou com o tamanho dos machos, uma vez que as fêmeas de várias espécies conseguem acessar essas características por meio da avaliação dos parâmetros acústicos dos cantos (Poole & Murphy, 2006; Pettitt, 2013; Tárano & Fuenmayor, 2013). Nesse sentido, por exemplo, os machos maiores ou que emitem maiores taxas de repetição dos cantos podem ser os escolhidos pelas fêmeas, uma vez que essas características indicam a qualidade dos machos, com consequências para a aptidão da prole (Pfennig, 2000; Pettitt, 2013).

O sucesso da prole também pode estar relacionado com as diferentes estratégias adotadas pelas fêmeas em relação às características das desovas. *Trade-offs* entre o tamanho dos ovos e o número de ovos (menos ovos maiores ou mais ovos menores) são estratégias reprodutivas que podem ser observadas nos anuros (Crump, 1984; Dziminski & Roberts, 2006; Dziminski et al., 2009; Pupin et al., 2010). No entanto, dependendo das condições ambientais do local onde os anuros se reproduzem, a estratégia de variar o número de ovos e o tamanho dos ovos em cada desova pode maximizar o sucesso no desenvolvimento da prole (Crump, 1981; 1984; Dziminski & Roberts, 2006; Dziminski et al., 2009).

A espécie do presente estudo, *Hypsiboas goianus* (B. Lutz, 1968), é um hilídeo que pertence ao clado de *Hypsiboas polytaenius* (Faivovich et al., 2005). Esse clado compreende 12 espécies sul-americanas, incluindo: *H. beckeri* (Caramaschi & Cruz,

2004); *H. botumirim* (Caramaschi et al., 2009); *H. buriti* (Caramaschi & Cruz, 1999); *H. cipoensis* (B. Lutz, 1968); *H. goianus* (B. Lutz, 1968); *H. jaguariaivensis* (Caramaschi et al., 2010); *H. latistriatus* (Caramaschi & Cruz, 2004); *H. leptolineatus* (Braun & Braun, 1977); *H. phaeopleura* (Caramaschi & Cruz, 2000); *H. polytaenius* (Cope, 1870); *H. stenocephalus* (Caramaschi & Cruz, 1999); e *H. bandeirantes* (Caramaschi & Cruz, 2013). *Hypsiboas goianus* ocorre no Cerrado da região central do Brasil, nos estados de Goiás, Minas Gerais e no Distrito Federal (Frost, 2014). Os machos vocalizam sobre folhas e galhos da vegetação ao redor dos corpos d'água (Menin et al., 2004). Os machos dessa espécie são considerados fiéis aos sítios de vocalização, sendo encontrados durante várias noites consecutivas no mesmo micro-habitat (Menin et al., 2004). Como é característico deste grupo de espécies, denominadas de rãs-gladiadoras (Faivovich et al., 2005), os machos apresentam comportamento territorial, emitindo cantos agressivos e entrando em combates físicos (Menin et al., 2004). No entanto, pouco se sabe sobre a ecologia, comportamento reprodutivo e dinâmica do comportamento territorial desta espécie (Bastos et al., 2003; Menin et al., 2004).

Diante do exposto acima, nosso objetivo geral foi investigar o comportamento e a ecologia reprodutiva de *Hypsiboas goianus* na Floresta Nacional de Silvânia, município de Silvânia, Estado de Goiás. A dissertação está estruturada em dois capítulos, como segue:

Capítulo 1:

Título: From where, when, and how to call? The calling strategy of a Neotropical tree-frog (*Hypsiboas goianus*) along the night

Objetivos: Investigar o uso dos sítios de vocalização; o padrão de vocalização ao longo da noite; e a variação intraindividual dos parâmetros acústicos de *H. goianus*.

O manuscrito está submetido à revista *Behaviour.*

Capítulo 2:

Título: Reproductive ecology and territorial behavior of *Hypsiboas goianus*, a Gladiator Frog from the Brazilian Cerrado

Objetivos: Investigar a ecologia reprodutiva e a dinâmica do comportamento territorial de *H. goianus.*

Nós pretendemos submeter o manuscrito à revista *Herpetological Journal.*

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Capítulo

1

From where, when, and how to call? The calling strategy of a Neotropical tree-frog (*Hypsiboas goianus*) along the night

From where, when, and how to call? The calling strategy of a Neotropical tree-frog (*Hypsiboas goianus*) along the night

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Short title: **Calling strategy of a Neotropical tree-frog**

Summary

In anurans the acoustic pathway is the primary communication form. We investigated the use of calling sites vocalization pattern along the night and within-individual variation of acoustic parameters in the Neotropical tree-frog *Hypsiboas goianus*. Male SVL was significantly correlated with their distance from the water, and smaller males occurred closer to the stream, but SVL was not correlated with perch height. Perch height did not vary during the reproductive season. Males emitted more aggressive calls at the early hours of the night and the number of advertisement calls emitted increased in the middle of the night. Our study revealed that males of *H. goianus* adopt a calling strategy along the night, emitting more aggressive calls early in

the night and emitting more advertisement calls in the middle of the night. This strategy probably is related to the establishment of territories and the attraction of females to the calling site. This is the first study analysing within-individual variation of acoustic parameters of calls along the night for frogs in general.

Key-words: calling site; within-individual variation; calling pattern; calls; repetition rate.

Introduction

Acoustic communication is important in different behavioural contexts, such as aggressive interactions, courtship behaviour, territory maintenance, conspecific recognition and individual recognition (Ryan, 1991; Lemes et al., 2012; Sandoval et al., 2014). Recognition of specific signals of a species is crucial for reproductive success of many animals (Gerhardt, 1974; Berry & Breithaupt, 2010; Nemeth et al., 2012). The parameters of acoustic signals, however, may vary within the same individual (e.g. Rogers & Cato, 2002; Pettit, 2013). This intra-individual variation has been observed in many animals and can be related to male dominance, resource holding-potential, signal detectability or sexual selection (Ryan, 1991; Cowlishaw, 1996; Rogers & Cato, 2002; Delgado, 2006).

In anurans, the acoustic signals are the main communication form and are involved in mate recognition and sexual selection (Ryan, 1988). The use of calling sites by anuran males can be related to the morphological traits of the individuals (e.g. Howard, 1978; Wogel et al., 2002). The choice for specific sites can increase the reproductive success of males, and male size can influence site use, with the largest males defending better territories (e.g. Howard, 1978; Wogel et al., 2002). High calling sites close to the water may be considered the best sites. This happens because the

vegetation is less dense then near the ground, presenting less obstacles to attenuate the acoustic signals, and because the proximity to the water prevents dehydration (Bellis 1962; Wells & Schwartz, 1982).

At the beginning of the night, males establish themselves in the calling sites and defend their territories before females arrive in the reproductive area (Given, 1987). The maintenance of a territory by anuran males may be advantageous when resources are limited (Wells, 1977). Males can defend their calling sites employing different strategies, including the emission of advertisement and aggressive calls toward intruders, or even engaging in physical combats (Wells, 1977; Bastos et al., 2011; Lemes et al,. 2012). Advertisement calls emitted by anuran males are also used to attract conspecific females and may be highly variable within-individuals regarding acoustic parameters, resulting in differential attractiveness (Wells, 1977; Passmore et al., 1992; Pettitt, 2013). As females choose males based on calling traits, which may signal physiological conditions and size of males (Licht, 1976; Morris, 1989; Pfennig, 2000), the attractiveness of males´ call is important in determining their reproductive success (Wells, 1977; Smith & Roberts, 2003; Byrne, 2008). Besides individual variations, acoustic parameters can also be influenced by many environmental variables and 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 influenced by male size, while temporal parameters are more influenced by air temperature (e.g. Lemes et al., 2012; Morais et al., 2012; Bee et al., 2013). Some studies analyzed the within-individual variation of acoustic parameters of calls of anurans males (Bee et al., 2001; Pettit, 2013), however, none studies analyzed this variation within-individual along the night.

The tree-frog *Hypsiboas goianus* (B. Lutz, 1968) belongs to the *Hypsiboas polytaenius* clade (Faivovich, 2005) and occurs in the Brazilian Cerrado, in the states of

Goiás, Minas Gerais and in the Distrito Federal, central Brazil (Frost, 2014). Males of *H. goianus* call from branches and leaves in the banks of water bodies and calls of this species have harmonic structure and variable number of pulses (Guimarães et al., 2001; Menin et al., 2004; Dias et al., 2014). Little is known about the acoustic and reproductive behaviour of this and other closely related species (Bastos et al., 2003a; Menin et al., 2004). Thus, here we investigated the use of calling sites, the pattern of calling strategy along the night and the within-individual variation of the acoustic parameters of *H. goianus*. We tested the following predictions: i) that larger males will occupy higher calling sites, which favour call propagation and will also prefer those closer to the water, which decreases dehydration; ii) that perch height will vary along the breeding season due to variance in climatic conditions; iii) that aggressive calls will be more commonly emitted early in the night, when males are delimiting calling sites; iv) that same male will vary the acoustic parameters of their calls along the night accordingly with presence of nearby females or males.

Material and methods

Study area

The study was conducted in the Floresta Nacional de Silvânia (Flona), a national forest reserve (16º39'32'' S, 48º36'29'' W, elevation ca. 900 m), Silvânia municipality, Goiás state, central Brazil. Local vegetation comprises different phytophysiognomies of the Cerrado (Morais et al., 2012). The investigated site included the pool areas of a slow-flowing second-order stream. The stream remains for the whole year, thus being considered a permanent water-body. It is surrounded on both sides by gallery forest. The climate is tropical (AW of Koppen´s classification), with two well defined seasons:

a rainy season from October to March and a dry season from April to September. The mean temperature is 26° C and annual mean rainfall is 1600 mm (Bastos et al., 2003b).

Data collection

We visited the reproductive site during the rainy season, in March and April 2013 and from October 2013 to March 2014, totalling 50 sampling nights. We searched for males in calling activity after sunset (around 19:00 h) and characterized the calling sites where they were found recording the following traits: perch height, horizontal distance from the water, vegetation type, and distance from the nearest male. We categorized the vegetation types based on their height and complexity according to Vidal & 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 grass of the Family Poaceae (Gramineae). We measured the height of vegetation with a measuring tape.

To analyse the calling pattern, we searched for males in calling activity, from right after sunset, around 18:30 h, until the decrease of their activity around 24 h. For each male found, we recorded the calls emitted during two minutes. The number of advertisement and aggressive calls was counted for each recording. Afterwards, the recordings were organized according to the following periods of the night: 1st period - from 18:00 to 20:00 h; 2nd period - from 20:01 to 21:00 h; 3rd period- from 21:01h to 22:00 h; 4th period- from 22:01 to 23:00 h; and 5th period- after 23:01 h.

To investigate the existence of within-individual variation of the acoustic parameters along the night, we searched for males around 20:00 h, when they were already well established at the calling site, and recorded them during 2 minutes separated by 30 minute intervals, totalling six sessions (sessions "a", "b", "c", "d", "e"

and "f"). After each recorded 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, intensity of the calls of the focal male, and noise intensity. Air temperature and air humidity were recorded with a digital thermo-hygrometer (Instrutemp, ITHT 2210 model).

We measured the snout-vent length (SVL) with a digital caliper (0.01 mm) and mass with a digital scale (0.01 g) of all males sampled in the present study. Furthermore, we marked them with subcutaneous implants (alphanumeric fluorescent tags visible under ultraviolet light, Northwest Marine Technology, Inc.) to avoid pseudoreplication. The recordings were made using a digital recorder Tascam DR-40 coupled to a Rode NTG-2 microphone and the intensities and noise were registered with decibel-meter Minipa MSL 1325. The callings were analysed in the Raven Pro 64 1.4 software (Cornell Lab of Ornithology, Ithaca, NY, USA). The acoustic parameters analysed were: dominant frequency (Hz), note duration (s), number of pulses, interval between notes (s) and repetition rate (calls per minute). Bioacoustics terminology followed Toledo et al. (2014).

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.

To examine whether the largest males occupied highest calling sites and those closest to the water, we performed two simple linear regressions: between male SVL (predictor variable) and perch height (response variable); and between male SVL (predictor variable) and distance from the water (response variable). To test if perch

height (response variable) varied among months (predictor variable), we performed a one-way ANOVA.

To evaluate whether aggressive calls were more commonly emitted early in the night, we performed three one-way ANOVAs (one test for advertisement calls, one for aggressive calls and one for total number of calls) with the number of calls emitted (response variable) for each period of the night (predictor variable). Because we were interested in how each call type changed among the periods, each response variable was tested separately (Huberty & Morris, 1989). To investigate whether the same male varied the acoustic parameters of their calls along the night, we performed five different ANOVAs, one test for each acoustic parameter (response variable) considering the individual and the six recording sessions (predictor variable). Because we were interested in how each acoustic parameter changed among the periods, each response variable was tested separately (Huberty & Morris, 1989). Afterwards, we performed simple linear regressions to test the influence of air temperature (predictor variable) on acoustic parameters that varied significantly along the nigh (response variable). The other factors (humidity, noise, number of males, distance to the nearest male and humidity) were not included in the regressions because they were correlated to each other or did not vary significantly along the night.

Results

Males´ calling activity started around 6:45 p.m. Substrates commonly used as calling sites were herbaceous plants (53%, $n = 37$), small trees (23%, $n = 16$), Poaceae (23%, $n = 16$) and ground (1%, $n = 1$) (Figure 1). The minimum, maximum and average values of perch height, distance of males from the water and distance to the nearest

calling male are shown in table 1. All calling males (except one) were perched externally to the pond margin (i.e. above the ground, never directly above the water).

Male SVL ranged from 28.53 to 37.48 mm (average $= 32.55 \pm 1.46$, $n = 217$). Male SVL was significantly correlated with distance from the water; i.e., smallest males were found closer to stream, while largest males were further away from it ($F_{(1,22)} = 4.59$, $R^2_{adj} = 0.13$, $beta = 0.41$, $p = 0.04$) (Figure 2). On the other hand, male SVL was not correlated with perch height ($F_{(1,102)} = 1.65$, $R^2_{adj} = 0.0062$, $beta = 0.13$, $p = 0.20$). Furthermore, perch height did not vary among months ($F_{(4,125)} = 2.12$, $p = 0.08$).

Number of advertisement calls, aggressive calls and total number of calls emitted differed along the night ($F_{(4,193)} = 2.58$, $p = 0.04$; $F_{(4,193)} = 15.77$, $p < 0.001$; $F_{(4,192)} = 14.46$, $p < 0.001$, respectively) (Figure 3). Males emitted more aggressive calls early in the night (1st and 2nd period) and number of advertisement calls was higher in the 3rd period (Figure 3).

Regarding acoustic parameters of the advertisement calls, only repetition rate showed within-individual variation along the night, with the highest repetition rate observed in the session "d" compared to other sessions ($F_{(5,87)} = 2.65$, $p = 0.03$, Table 2, Figure 4A). All other parameters of the advertisement calls did not exhibit significant variations (Table 2). For aggressive calls, the repetition rate and interval between notes within-individuals varied along the night ($F_{(5,69)} = 4.59$, $p = 0.001$; $F_{(5,69)} = 2.58$, $p = 0.03$, Table 3, Figure 4B and 4C). The repetition rate was higher and the interval between notes was smaller in the first session ("a") (Figure 4B and 4C). All other parameters of the aggressive calls did not exhibit significant variations (Table 3). The parameters of the advertisement and aggressive calls which varied significantly were not correlated with air temperature (Table 4).

Discussion

In the present study, males called from the ground, perched on grasses, small trees and mainly on herbaceous plants. Previous studies observed that this species used mainly grasses and shrubs as calling sites, despite the presence of trees in the area (Bastos et al., 2003b). However, neither this nor other published studies regarding this species actually quantify on which vegetation type the males were calling, and only offer circumstantial observations (Bastos et al., 2003b; Menin et al., 2004). This suggests that males of *H. goianus* can use a great variety of microhabitats as calling sites, which might be related with the availability of vegetation types in the banks of the water bodies (Godwin & Roble, 1983; Nali & Prado, 2012; Dias et al., 2013; Dias et al., 2014).

Environmental conditions, such as air temperature and humidity, can influence anuran calling activity (Brooke et al., 2000; Prado & Pombal, 2005; Nali & Prado, 2012). We expected that largest males would occupied sites closer to the water, which decreases dehydration, however smaller males vocalized closer to the water compared to larger males. Hylids typically lose more water than ground species (e.g. Leptodactylids) because they remain exposed on the vegetation and larger frogs are more tolerant to lower humidity conditions (Bellis, 1962; Prado & Pombal, 2005). In this sense, smaller males of *H. goianus* might be occupying sites closer to the water in order to avoid excessive water loss.

For territorial anurans, larger males may defend the best calling sites (Howard, 1978; Bastos & Haddad, 1996; Wogel et al., 2002). Higher calling sites may be considered the best to emit calls, because the vegetation is denser near the ground and may function as a barrier that attenuates the acoustic signals (Wells & Schwartz, 1982).

Males of *Hypsiboas goianus* present territorial behaviour, however male SVL was not correlated with perch height. This species is typical of gallery forests, a phytophysiognomies of the Cerrado characterized by dense vegetation where plant density remains the same along the vertical strata (Ribeiro & Walter, 2008). Given this, perch height can be more important for anurans of open areas, such as *Phyllomedusa iheringii*, an endemic hylid of the Pampa domain (unpublished data). However, because *H. goianus* males defend territories and show fidelity to the calling sites (Menin et al., 2004), some other traits of the site may be preferred by males. Males of *H. goianus* may be selecting sites which maintain large distances among calling individuals, reducing acoustic interference and consequently increasing female attraction chances (Whitney & Krebs, 1975; Shepard, 2004; Nali & Prado, 2012).

For *H. goianus* males perch height did not differ among months. This difference could be explained by habitat use among species. *Hypsiboas goianus* is a forest species, thus within the height interval used by males (0 – 2 m) vegetation density is homogeneous and may provide humidity and equal protection from higher temperatures for calling males, as well as good visibility and/or sound propagation (Abrunhosa & Wogel, 2004; Bartelt & Peterson, 2005). Thus, perch height is expected to remain stable along the reproductive season.

Males of *H. goianus* emitted more aggressive calls early in the night. This result is similar to the pattern observed for other anurans (Wells, 1978; Given, 1987; Bastos et al., 2011). At the beginning of the night, males establish themselves in the calling sites and defend their territories before females arrive in the reproductive area (Given, 1987). Following that, the number of advertisement calls of *H. goianus* males increased in the 3rd period of the night. The advertisement call plays an important role in female

attraction, and competition among males may be higher during the peak of the calling activity (Wells, 1977; Ryan, 1991). Thus, the greater investment in calling activity by males in the 3rd period of the night is possibly associated with females´ arrival in the chorus, and this adjustment of the calling activity by males was already observed for other anuran species (e.g. Bevier, 1997; Murphy, 1999).

The interval between notes and repetition rate of the aggressive calls varied within-individuals along the night. Because of the higher repetition rate and smaller interval between notes in the first session, our results also support the prediction of establishment and defense of territories by males early in the night, before females arrive at the chorus, as mentioned above (Given, 1987). Similarly, the repetition rate of the advertisement calls varied within-individuals along the night being higher in the session "d", possibly associated with females´ arrival at the chorus as was observed for other species, although we have not observed the arrival of *H. goianus* females in our study area (e.g. Bevier, 1997; Murphy, 1999). High repetition rate has been correlated with increased metabolic costs in many anurans and may indicate male's competitive and resource-acquiring abilities (Wells, 2001; Pettitt, 2013). Thus, highest repetition rates indicate the fitness and good physiological condition of males, which may be the ones chosen by females, as observed for several species of anurans (e.g. Morris & Yoon, 1989; Pauly et al., 2006; Tárano & Fuenmayor, 2013). This preference may benefit females through increasing fertilization success and/or offspring fitness (Bourne, 1993; Pfennig, 2000; Pettitt, 2013). Thus, future studies on *H. goianus* should investigate female preferences and benefits correlated with their choices.

Although the influence of air temperature on temporal acoustic parameters has been verified for other species (Lemes et al., 2012; Morais et al., 2012; Gambale &

Bastos, 2014), here neither temporal parameters of the aggressive nor the advertisement calls correlated with air temperature. Moreover, we did not detect within-individual variation for the spectral parameter (dominant frequency) analysed for both the advertisement and aggressive calls along the night. Contrary to temporal acoustic parameters, which commonly vary within-individuals (dynamic properties; Gerhardt, 1991; Pettitt, 2013), the dominant frequency is determined by morphological traits of the individual and, thus, is considered a static property of the call (Gerhardt, 1991; Morais et al., 2012; Bee et al., 2013).

Hypsiboas goianus males seem to be generalist regarding vegetation types used as calling sites. Furthermore, perch height does not appear be an import trait for this species, given the homogeneous vegetation density in the habitat. Future studies should investigate factors determining habitat preferences and its relationship with male mating success. Our study revealed that males of *H. goianus* adopt a calling strategy along the night, emitting more aggressive calls early in the night and emitting more advertisement calls in the middle of the night. These behaviours are probably related to the establishment of territories and attraction of females to the site, respectively. Future studies should focus on the dynamics of female arrival in the chorus and test the existence of female preferences for males that present the highest repetition rates. This is the first study analyzing within-individual variation of acoustic parameters of calls along the night and these studies will be fundamental to better understand the reproductive strategy of this and other ecologically similar Neotropical anurans.

Acknowledgments

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Table 1. Characterization of the use of calling site by males of *Hypsiboas goianus*, in central Brazil.

	Minimum (cm)	Maximum (cm)	Average ± SD (cm)	*n*
Perch height	0	200	64 ± 42	105
Distance of male from the water	-30	2250	783 ± 570	24
Distance to the nearest male	20	1000	243 ± 150	101

Table 2. Results of the repeated ANOVAs for the within-individual variation of the acoustic parameters of advertisement calls along the night. Statistically significant values of "p" are shown in bold.

	$F_{(5,87)}$	p
Dominant Frequency	0.34	0.88
Note duration	0.46	0.80
Number of pulses	0.28	0.92
Interval between notes	0.93	0.46
Repetition rate	2.65	**0.03**

Table 3. Results of the repeated ANOVAs for the within-individual variation of the acoustic parameters of aggressive calls along the night. Statistically significant values of "*p*" are shown in bold.

	$F_{(5,69)}$	*p*
Dominant Frequency	0.64	0.67
Note duration	0.82	0.53
Number of pulses	0.42	0.83
Interval between notes	4.59	**< 0.01**
Repetition rate	2.58	**0.03**

Table 4. Results of the linear regressions of the acoustic parameters with air temperature (the only environmental factor that varied significantly along the night).

Call type	Model	df	F	R^2_{adj}	beta	p
Advertisement	Repetition rate ~ temperature	1,91	0.82	-0.002	0.09	0.37
Aggressive	Interval among 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

Figure legends

Figure 1. Male of *Hypsiboas goianus* calling perched on an herbaceous plant, in the Cerrado of Goiás state, central Brazil.

Figure 2. Simple linear regression between male SVL and distance from the water in *Hypsiboas goianus*, in central Brazil.

Figure 3. Mean number of advertisement calls (ADV), aggressive calls (AGG), and total number of calls (TOT) emitted by *Hypsiboas goianus* males in each period of the night, in central Brazil.

Figure 4. Within-individual variation of the acoustic parameters of *Hypsiboas goianus* along the night, in Central-Western Brazil. (A) Repetition rate of the advertisement calls; (B) Repetition rate of aggressive calls; (C) Interval between notes of aggressive calls.
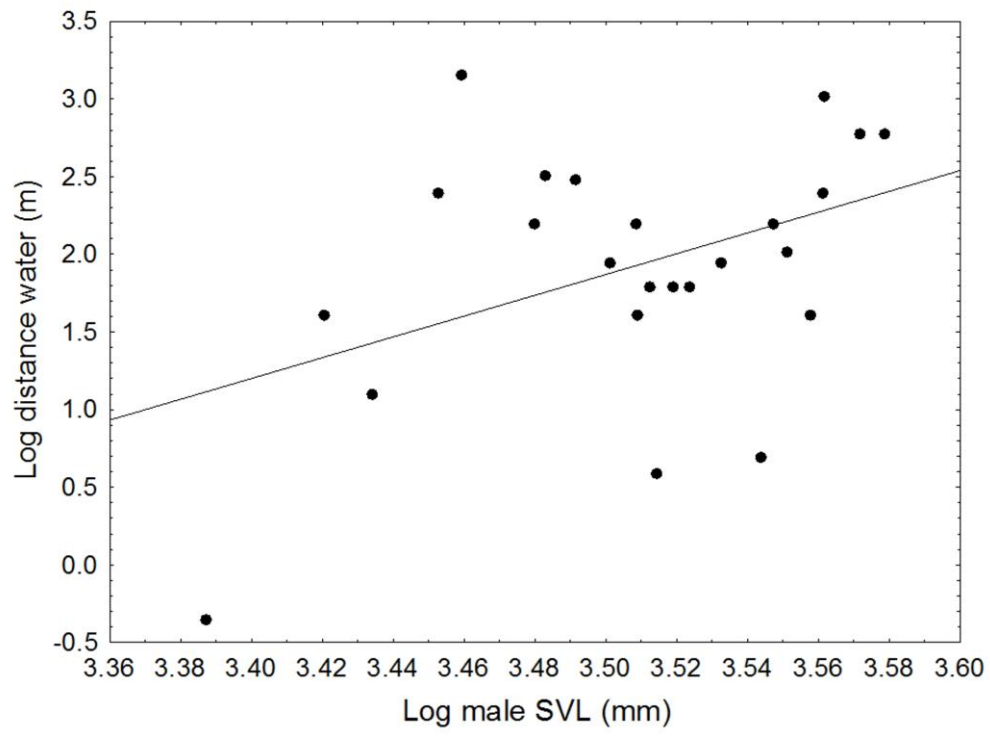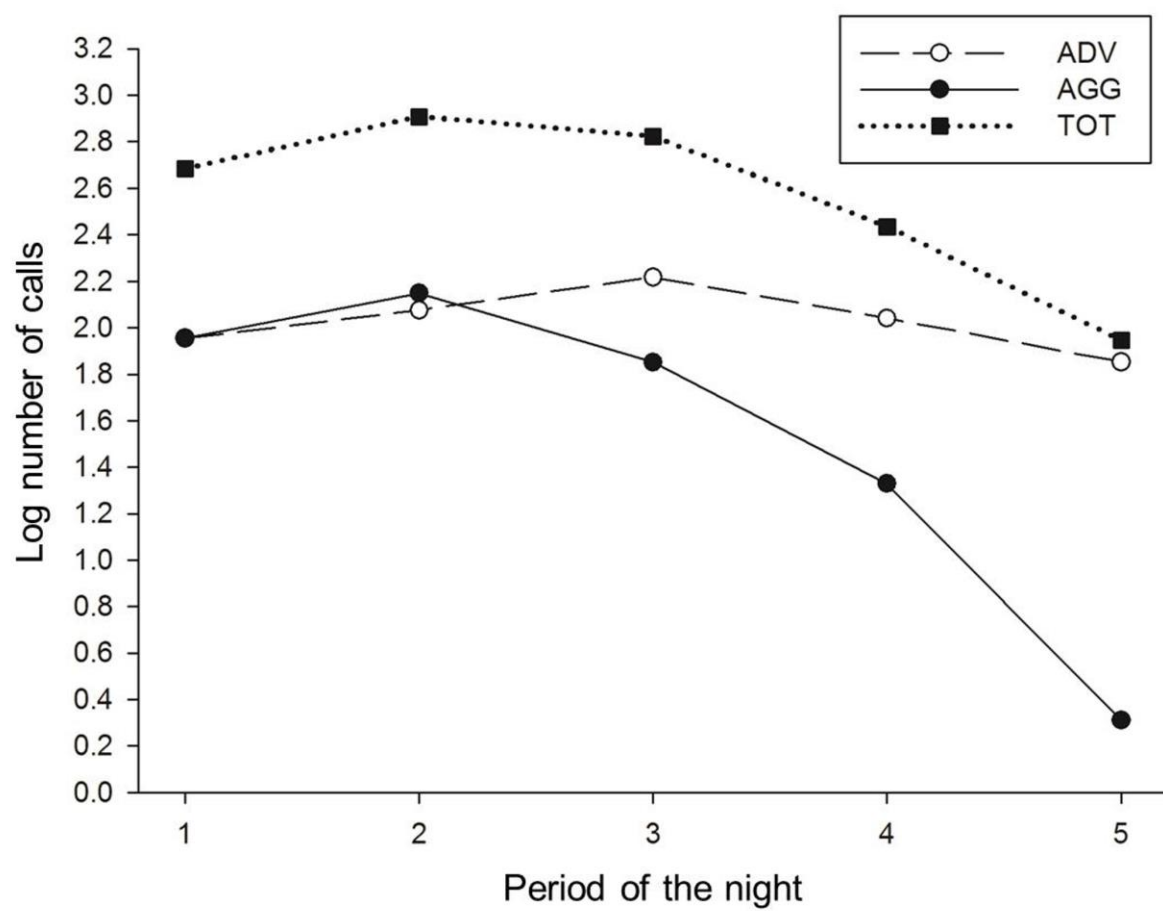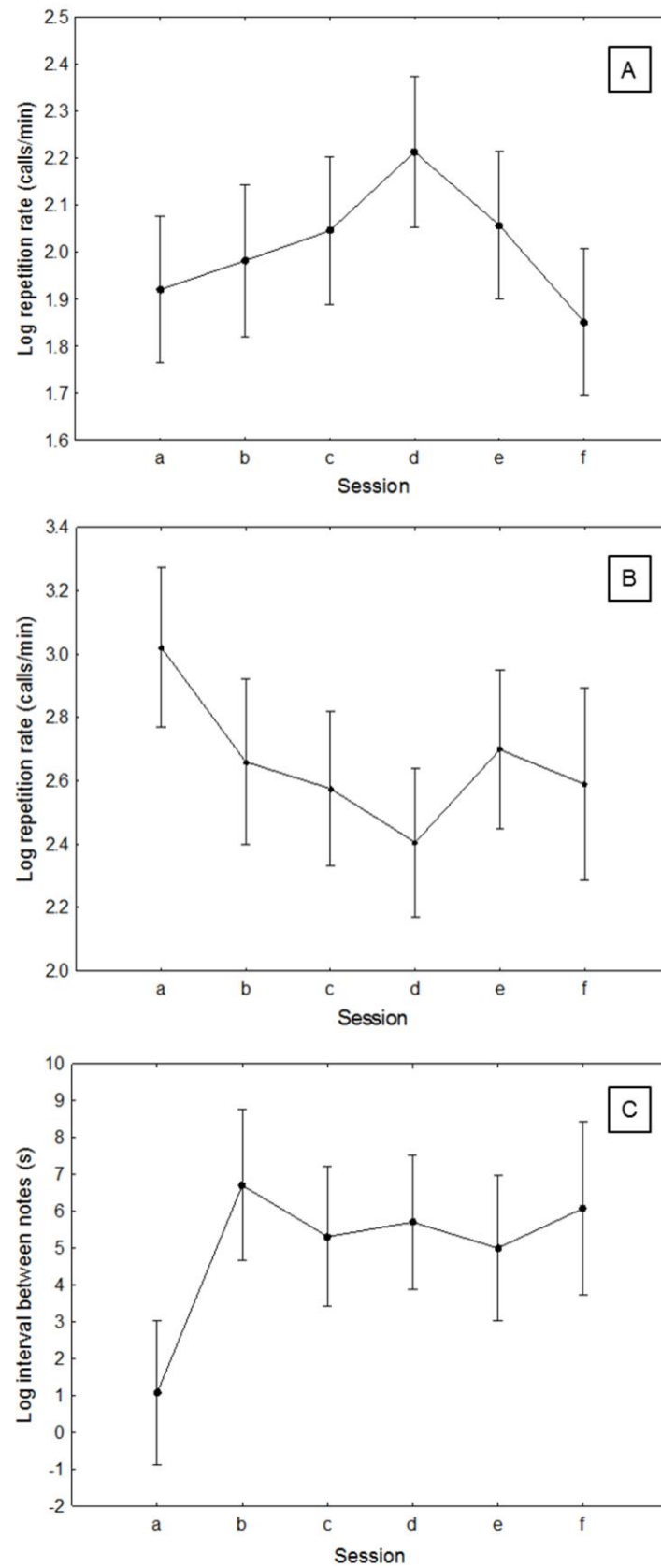
Figure 1.

Figure 2.

Figure 3.

Figure 4.

Capítulo 2

Reproductive ecology and territorial behavior of

***Hypsiboas goianus*, a Gladiator Frog from the**

Brazilian Cerrado

Reproductive ecology and territorial behavior of *Hypsiboas goianus*, a Gladiator Frog from the Brazilian Cerrado

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Running title: **Reproductive ecology and territorial behavior of *Hypsiboas goianus***

ABSTRACT

Females and males of anurans can adopt different reproductive and behavioral strategies depending of the biotic and abiotic factors of their habitat. Here we investigate the reproductive ecology and the dynamics of the territorial behavior of a Neotropical tree-frog (*Hypsiboas*

goianus) from the Cerrado of central Brazil. We found no relationship between female size and clutch size, and between egg size and number of eggs per clutch. Matings were not assortative. The largest males arrived at the reproductive area early in the reproductive season, which occurs during the rainy season (usually from October to May). In the first territoriality experiment, one of the two males emitted more calls and was larger than the other male. In the second experiment, the largest males emitted more calls, and the more aggressive calls were emitted by the resident male, and distance to the nearest male was larger. In both experiments the number of calls emitted by the males was influenced by their size. The low incidence of physical combats along with the spacing pattern among calling males indicate that this species relies almost solely on calls to solve contests. Thus, the acoustic communication seems to be extremely important for *H. goianus* in multiple social contexts.

Key-words: clutch size, non-assortative mating, male competition, aggressive interactions, arrival dynamics.

INTRODUCTION

Reproductive effort of females can be divided among those with a few large eggs, those with many small eggs or size diverse of eggs within of the same clutch (Crump, 1984). Additionally, large females can produce more eggs than small ones, and this positive relationship between clutch size and female size was already described for several species of anurans (e.g. Kusano & Hayashi, 2002; Wogel et al., 2002; Dziminski & Alford, 2005). In this context, females may also adopt different reproductive strategies depending on the environmental conditions (Crump, 1984).

In anurans, females often choose their mates (Morris, 1989). In general, the female chooses the male that it deems the most suitable in order to grant her offspring the greatest success, but strategies may vary among different species. In species with

assortative matings, females prefer males of sizes similar to their own, because this allows a closer juxtaposition of the cloacae during mating. This behavior can increase egg fertilization rate (Licht, 1976). Alternatively, when there is no assortative mating, females may be choosing males according to some traits other than a similar size (Poole & Murphy, 2006; Tárano & Fuenmayor, 2013).

The reproductive success of males can be related to their arrival at the reproductive area, and males that arrive early may increase their chances of mating (Wells, 2007). Furthermore, the formation and maintenance of a territory can also be essential for the reproductive success of some anurans (Wells, 1977a). In this sense, males can be influenced by the calling activity of their neighbors, changing their acoustic behavior, and sometimes engaging in physical combats (Wells, 1977a, Bastos et al., 2011; Lemes et al., 2012). Males can also assess the size of rival males through the calls, thus avoiding a conflict with a larger opponent (Bee & Perrill, 1996; Bee, 2002). Furthermore, residence of one of the males can affect the outcome of the contest by increasing motivation to defend its territory (Owen & Gorder, 2005).

Hypsiboas goianus (B. Lutz, 1968) is a hylid of the *Hypsiboas polytaenius* clade (Faivovich et al., 2005). It is distributed in the central Brazilian states of Goiás, Minas Gerais and in the Distrito Federal (Frost, 2014). Males of this species call perched on the vegetation that surrounds water bodies (Menin et al., 2004). Furthermore, males of *H. goianus* are known to show territorial behavior and to be faithful to calling sites (Menin et al., 2004). However, little is known about the reproductive ecology and of the dynamics of their territoriality (Bastos et al., 2003; Menin et al., 2004).

In the present study, we investigated the reproductive ecology and the dynamics of the territorial behavior of *H. goianus*. We tried to answer the following questions: i)

Is there a positive relationship between female size and clutch size? ii) Is there a trade-off between egg size and number of eggs per clutch? iii) Are the matings assortative? iv) Do larger males arrive at the reproductive area earlier in the reproductive season? v) Does male size define dominance in contests without a prior resident? vi) Do the presence of intruders and the distance between males affect the behavior of the resident male?

MATERIALS AND METHODS

Study area

The study was conducted at the Floresta Nacional de Silvânia (FNS; 16º39'32'' S, 48º36'29'' W, elevation ca. 900 m), Silvânia municipality, Goiás state, central-western Brazil. The FNS is located in the Cerrado domain and presents many vegetation types, one of which is the gallery forest, where we conducted this study (*sensu* Oliveira-Filho & Ratter, 2002). According to Koppen's classification, the climate is defined as tropical, and can be separated in two well defined seasons, a rainy season from October to March and a dry season from April to September.

Data collection

Reproductive ecology We visited the reproductive area during the rainy season, from January to May 2013, and from October 2013 to May 2014. We searched for pairs in amplexus after sunset (around 07:00 p.m.). When they were found, we captured the pair and waited for them to spawn, and after returned the pair and the clutch to nature. For each clutch we counted the number of eggs and collected ten eggs to be measured. The eggs were fixed in 10% formalin and deposited at the Coleção Zoológica da

Universidade Federal de Goiás (ZUFG). We measured the eggs in the laboratory using a Zeiss stereomicroscope with a scaled background, to nearest 0.01 mm.

We measured the snout-vent length (SVL) with a digital caliper (0.01 mm) and mass with a digital scale (0.01 g) of all males and females collected in the present study. We also marked them with subcutaneous implants (alphanumeric fluorescent tags visible under ultraviolet light, Northwest Marine Technology, Inc.) to avoid pseudoreplication.

Territoriality We placed the males 100 cm above the ground because this is the most commonly used height in nature (see chapter 1 and Menin et al., 2004). The usual distance between males in natural environments is around 200 cm, and distances smaller than 100 cm are rare (see chapter 1 and Menin et al., 2004). Thus, we placed the males 50 cm apart to maximize their behavioral response in the establishment and defense of the territory.

We performed two experiments of territoriality. In the first experiment, we chose two males in calling activity that were approximately 2 m from each other. Next, the two males were taken to another place within the reproductive area. There, the males were placed on a small tree 100 cm above the ground and 50 cm apart (Fig. 1). We waited until one of them started calling and counted the number of the advertisement calls and aggressive calls emitted by both males during two minutes. The counting of the calls was repeated 15 and 30 minutes later, totaling three counting periods. We also measured the distance between the males. After the third counting period the experiment was terminated and then the males were measured as described above.

For the second experiment, we arrived at the reproductive area and searched for two males that were up to 400 cm apart, so that we could see both simultaneously. These males were considered the resident males. During two minutes we counted the number

of advertisement calls and aggressive calls emitted by them. After, we searched for a third male (first intruder), which was placed between the resident males (Fig. 2). We waited until the intruder male started to call and counted again the number of advertisement and aggressive calls emitted by the resident males. We also measured the distance between all males. This procedure was repeated until a third intruder was placed in the area (Fig. 2). After counting the calls, the experiment was terminated. A total of four counting periods were performed. Males were also measured as described above.

Data analyses

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.

Reproductive ecology To analyze if mating was assortative by body size, we performed a Spearman's Rank Correlation test between the SVLs of males and females in amplexus. To examine if clutch size was correlated to female size, and whether egg size was correlated with the number of eggs per clutch, we performed simple linear regressions. To examine if SVL of males differed among the months of the reproductive season we performed a one-way ANOVA.

Territoriality For each replicate of the first experiment, we chose one of the males to be the alpha (always the one who called more) and the other was deemed the beta. To determine the establishment of dominance between the males and the validity of our alpha-beta classification, we tested if the difference between the supposed alphas and

betas was significant or not using a paired t-test. Since these differences were statistically significant ($t_{[14]}$=6.35, p<0.001), we deemed our classification as valid.

The variable "size" was determined by multiplying male SVL and male mass. Furthermore, to analyze whether number of calls emitted and sizes of alpha and beta males differed, we performed two paired t-tests.

To verify whether number of calls emitted by males varied among the counting periods, distance between males and male size, we performed three repeated ANCOVAs (one test for the advertisement calls, one for the aggressive calls and one for total calls), using the distance between males and male size as covariates. For these tests, only one randomly chosen male was included in the analyses. For each two-minute counting periods performed, the behavioral responses of males were also recorded following the focal animal method (Altman, 1974).

In the second experiment, to evaluate whether number of calls emitted by males varied for the periods counting periods performed, distance between males, and male size, we performed three repeated ANCOVAs (one test for the advertisement calls, one for the aggressive calls and one for total calls), using the distance between males and male size as covariates. The variable "size" was also determined as described for the experiment one. For these tests, only one randomly chosen resident male was included in the analyses. For each two-minute counting periods performed, the behavioral responses of males were also recorded, also following the focal animal method (Altman, 1974).

RESULTS

Reproductive ecology

From the nine clutches we sampled, two were fertilized by the same male (clutches 1 and 2) and two were placed by the same female (clutches 5 and 7) (Table 1). The number of eggs per clutch ranged from 105 (clutch 6) to 207 (clutch 7) and egg size ranged from 1.39 (clutch 6) to 1.96 mm (clutch 2) (Table 1).

Male SVL ranged from 28.53 to 37.48 mm (average=32.55 $\pm$ 1.46, n=217) and female SVL ranged from 34.40 to 37.48 mm (average=35.84$\pm$0.10, n=8). We did not find any relationship between female size and clutch size, nor between egg size and number of eggs per clutch ($F_{[1,7]}$ =1.32, r^2_{adj}=0.04, p=0.28; $F_{[1,7]}$=0.43, r^2_{adj}=-0.076, p=0.53, respectively). Body size of males and females in amplexus were not correlated (r_s=0.53, p=0.14). Male SVL varied along the months: in the last months of the reproductive season (March and April), males were smaller compared to males of all other months ($F_{[5,211]}$=4.59, $p<0.001$) (Fig. 3).

In the reproductive season of 2012/2013 we marked 108 individuals (four females and 104 males) and in the reproductive season of 2013/2014 we marked 128 individuals (five females and 123 males). However, no individual marked in the first season was recaptured in the second reproductive season. The total number of individuals captured and recaptured are shown in Table 2.

Territoriality

In the first experiment, alpha males were significantly larger than beta males ($t_{[14]}$=2.23, p=0.04, respectively) (Fig. 4). The number of advertisement calls and total calls emitted

by males were influenced only by male size, and the number of aggressive calls was not influenced by any of the tested variables (Table 3).

Regarding the results of experiment one, the frequencies of the observed behavioral responses were as follows: (a) males only walked away from each other ($n=3$); (b) males only approached each other ($n=2$); (c) males walked away and then approached each other ($n=2$); (d) males approached and then walked away from each other ($n=6$); (e) one male amplected a female ($n=1$); (f) one male approached the other, initiated physical contact and caused both males to fall to the ground ($n=1$). Some of these behaviors occurred simultaneously within a same replicate.

In the second experiment, the number of aggressive calls was positively influenced by male size and the distance between males, while the total number of calls was positively influenced only by male size (Table 4) (Fig. 5). The number of advertisement calls was not influenced by any of the tested variables (Table 4).

Regarding the results of experiment two, the frequencies of the observed behavioral responses were as follows: (a) the introduced male behaved as satellite male ($n=2$); (b) one resident male and one introduced male behaved as satellite males ($n=1$); (c) one resident male walked away ($n=1$); (d) the introduced male walked away ($n=2$); (e) one resident male engaged in physical combat with one introduced male, both fell to the ground and resumed calling activity after a few minutes ($n=1$). Some of these behaviors occurred simultaneously within a same replicate.

DISCUSSION

Reproductive ecology

Our results differ in some aspects from what is known for *H. goianus*. For the population of *H. goianus* studied in Minas Gerais state, the mean number of eggs was larger and the mean egg size was smaller compared to our results (Menin et al., 2004). Our number of samples was larger than that of the other studied population of *H. goianus*, and a larger sample can increase the chances of finding extreme values within the population. Additionally, depending on the environmental conditions of the reproductive areas (i.e. competition, predation, food resources), females may differ in reproductive investment (i.e. fewer and larger eggs, many smaller eggs, eggs variable in size) (Crump, 1984; Dziminski et al., 2009; Pupin et al., 2010).

Most studies on anurans report a positive relationship between female size and clutch size (e.g. Berven, 1988; Martins, 1988; Bastos et al., 1996; Kusano & Hayashi, 2002; Wogel et al., 2002; Dziminski & Alford, 2005). However, we did not find this relationship for *H. goianus*, and this result was already observed for other congeneric species (Lips, 2001; Hartmann, 2004). We observed that at least one female mated and spawned two times and, in another occasion, after a female had spawned, we looked throughout the transparent skin of its abdomen and observed that the female still carried several eggs. This shows that it is extremely likely that *H. goianus* females spawn more than once during each reproductive season, as observed for other anuran species (Rafinska, 1991; Bastos & Haddad 1996; Vaira, 2001). In these species it has been suggested that the correlation between female size and size of the eggs might be weak (Parker & Begon, 1986; Kusano & Hayashi, 2002). Considering that egg size may represent a similar energetic cost for the female as the number of eggs per clutch, the same explanation could explain the absence of relationship between clutch size *versus* female size and between egg size *versus* eggs per clutch in this multiple-clutch species.

A female that produces only large eggs in an unpredictable environment may have her fecundity decreased (Crump, 1984). In this sense, an option in some habitats may be the production of eggs of varying sizes, because this may increase the fitness of the female and the chances of survival of at least some of her offspring (Crump, 1981; 1984). As mentioned, egg size and number of the eggs per clutch were not correlated in *H. goianus*. Thus, females of this species may be varying the size of the eggs in order to increase the success of the offspring according to the predictability of the environment, presenting a bet-hedging strategy, as proposed for other species (e.g. Lips, 2001; Diziminski & Alford, 2005). Furthermore, to determine trade-offs between egg size and number, it is necessary to know exactly how many clutches and eggs were produced by a female throughout the entire reproductive season (Lips, 2001), which is particularly difficult to determine in multiple-clutch species, such as *H. goianus*.

Assortative mating allows a close juxtaposition of cloacae (Licht, 1976), which has been observed, for instance, in the hylid *Dendropsophus elegans* (Bastos & Haddad, 1996). However, we did not find a relationship between the body sizes of males and females of *H. goianus* in amplexus. Female anurans are able to use the calls to assess males´ size and thus judge their fitness (Licht, 1976; Morris, 1989). In our field samplings, we observed four matings, and in three of them, the female mated with a male that was larger than the other nearest male. In this context, the females may prefer largest males for mating or, alternatively, choose males that present high calling efforts (i.e. highest repetition rate), because these traits may indicate male quality (e.g. Morris & Yoon, 1989; Poole & Murphy, 2006; Tárano & Fuenmayor, 2013). Alternatively, because this species spawns on the water (Menin et al., 2004), juxtaposition of cloacae may be less important.

Males' SVL varied along the months of the reproductive season. Males were larger at the beginning of the reproductive season. Differences in the timing of male arrival may affect mating success, mainly when the majority of the females arrive at the beginning of the reproductive season (Wells, 2007). Following this, large males arrive earlier than small males, although the reason for this is unclear (Wells, 2007). One explanation could be that large males are older and more experienced, and thus know the reproductive area better and are able to arrive first (Reading, 2001). However, this may not apply for *H. goianus*, because this species probably does not live for more than one year: no individual captured in the first season was recaptured in the second. This suggests that males of this species may go to the reproductive area only once during their lifespan. One possible explanation could be that larger juvenile males of *H. goianus* may spend the non-reproductive season near the reproductive areas, thus reducing distances to arrive at the reproductive area (Wells, 2007). Additionally, large males of *H. goianus* may simply have better physical conditions, arriving faster at the area, as observed for *Rana dalmatina* (Ranidae) (Lodé et al., 2004).

A third explanation would be that the smaller size of the males in the end of the reproductive season actually reflects the recruitment phenomenon. The reproductive season of this species starts in October, and it is possible that males born in the first months of the season may already be sexually mature around May. If this is true, the entrance of these young, small males into the reproductively active population may lower the average size of the males and thus explain our results. An interesting technique that could help to discern between these explanations is the marking of individuals since their tadpole phase, although these techniques are not always very effective (e.g. Grant, 2007; Martin, 2011; Courtois et al., 2013). This would allow researchers to fully understand from where the males came and how old they are.

Territoriality

Male anurans emit advertisement calls to attract females and announce their position to nearby males competing for the same females (Wells, 1977a). They can recognize the size of their opponents through the advertisement calls, thus allowing males to avoid fights which they have little chances of winning, because large males tend to win more contests than smaller males (Wells, 1978; Ramer et al., 1983; Robertson, 1986; Bee & Perril,1996; Bee, 2002). When the asymmetries between males are large and more easily assessed, less intense encounters are expected, because weaker individuals will assess their low probability of winning and give up earlier (Bee, 2002). Males of *H. goianus* present territorial behavior and interact with conspecific males through calls and/or physical combats (Menin et al., 2004). In our first experiment, the alpha males were larger than the betas, and the number of advertisement calls and total calls was explained by male size. This suggests that males of *H. goianus* may be assessing the size and abilities of their opponents through their calls, making smaller males to usually give up a contest with larger rivals by reducing their calling activity, remaining silent, or moving away from the alpha male.

The aggressive calls probably have the role of maintaining a spatial separation between calling males, and thus may serve as a warning to an intruder that it is calling too close to a resident male (Wells, 1977a; Dyson & Passmore, 1992). Thus, the maintenance of a distance between males may be more important than the possession of a particular calling site (Whitney & Krebs, 1975; Wells, 1977a). Furthermore, male intruders can assess the resident's size through their aggressive calls, and the resident males are more likely to win territorial disputes, because the cost of losing a territory is

higher for a resident (Wells, 1978; Bee et al.,1999; Owen & Gorder, 2005). Additionally, as stated above, larger males usually win over smaller males (Howard, 1978; Wells, 1978). In the second experiment, larger males emitted more calls and the more the resident males emitted aggressive calls, the larger was the distance adopted by the nearest male. This suggests that males of *H. goianus* can detect the spatial position of other males and that intruder males can assess resident's size through their calls (Wells, 1977b). Thus, maintaining a suitable distance from the resident male or avoiding areas where they are calling may be strategies adopted by intruding males of *H. goianus* to avoid undesired physical combats. Similar behavior has been described for another gladiator frog, *Bokermannohyla ibitiguara* (Nali & Prado, 2012), a species in the same clade of *H. goianus* (Faivovich et al., 2005).

Some males behaved as satellite males during the territorial experiments. This strategy was already observed for many anuran species (e.g. Wells, 1977b; Toledo & Haddad, 2005; Leary et al., 2005; Nali & Prado, 2012), and may represent an attempt to intercept females approaching calling males (Wells, 1977b). Another strategy would be engaging in a fight in order to obtain the resident's territory. However, physical combats were rare during both experiments, and never exceeded 5 s. Aggressive behaviors can include both aggressive calls and physical combats (Wogel et al., 2002; Costa et al., 2010; Lemes et al., 2012). However, as costs of physical combats can be high, males of *H. goianus* may be investing more in aggressive calls, what may be important to decrease energy expenditure and avoid injuries that could be caused by fights (Kluge, 1981; Nali & Prado, 2012).

Males and females of *H. goianus* appear to be capable of adopting different reproductive strategies according to environmental conditions. The species much likely

presents multiple-clutches, which can vary in number of eggs and egg size. It would be particularly interesting to follow females throughout an entire reproductive season, so that we can have accurate information about the relationship between egg size, egg number and female size in this species. Females could be obtaining information about calling males, and thus selecting those with higher fitness (e.g. the largest, or those with the highest repetition rates). Calling males are apparently able to assess the size and abilities of their opponents through calls, and to choose a different strategy (e.g. move away, behave as satellite males, engage in physical combat) based on these information. Males of *H. goianus* seem to avoid fights in which individuals have low probabilities to win, because physical combats were rare. This is reinforced by the distance between calling males, which increases with the number of aggressive calls of the resident male. Acoustic communication seems to be extremely important for *H. goianus* in many social contexts, and future studies should investigate which information, exactly, are being assessed by individuals.

ACKNOWLEDGEMENTS

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Table 1. Number of eggs and egg size (*n*=9) for each clutch of *Hypsiboas goianus* sampled in Central-Western Brazil. Values are shown as mean $\pm$ SD.

Clutch	Number of eggs	Egg size (mm)
1	176	1.76 $\pm$ 0.18
2	150	1.96 $\pm$ 0.11
3	159	1.73 $\pm$ 0.11
4	163	1.54 $\pm$ 0.04
5	151	1.67 $\pm$ 0.06
6	105	1.39 $\pm$ 0.06
7	207	1.64 $\pm$ 0.07
8	172	1.58 $\pm$ 0.05
9	201	1.65 $\pm$ 0.04
Mean	164.88 $\pm$ 30.18	1.68 $\pm$ 0.16

Table 2. Captures and recaptures of individuals of *Hypsiboas goianus* in Central-Western Brazil. First season: January 2013 to May 2013; Second season: October 2013 to May 2014.

	First season	Second season
Total of individuals captured	108	128
Total of recaptures	68	63
Recaptured one time	28	30
Recaptured two times	11	10
Recaptured three times	3	3
Recaptured four times	1	1
Recaptured five times	1	0

Table 3. Territorial experiment one: results of the repeated measures ANCOVAs for the effect of the period, distance between the males and male size on the variation of the number of calls. Distance and size were used as covariates. Statistically significant values of "p" are shown in bold.

Number of	df	F	p
Advertisement calls			
Period	2,34	0.89	0.42
Distance between the males	1,34	0.19	0.66
Size	1,34	14.66	**< 0.001**
Aggressive calls			
Period	2,34	0.15	0.85
Distance between the males	1,34	0.65	0.42
Size	1,34	3.76	0.06
Total of calls			
Period	2,34	0.77	0.47
Distance between the males	1,34	0.84	0.36
Size	1.34	12.63	**< 0.01**

Table 4. Territorial experiment two: results of the repeated measures ANCOVAs for the effect of the period, distance between the males and male size on the variation of the number of calls. Distance and size were used as covariates. Statistically significant values of "p" are shown in bold.

Number of	df	F	p
Advertisement calls			
Period	3,17	1.03	0.40
Distance between the males	1,17	0.07	0.80
Size	1,17	0.07	0.78
Aggressive calls			
Period	3,17	2.05	0.14
Distance between the males	1,17	10.50	**< 0.01**
Size	1,17	14.42	**< 0.01**
Total of calls			
Period	3,17	1.99	0.15
Distance between the males	1,17	4.04	0.06
Size	1.34	5.58	**0.03**

Figure legends

Figure 1. Scheme representing the first territoriality experiment of *Hypsiboas goianus* in Central-Western Brazil.

Figure 2. Scheme representing the second territoriality experiment of *Hypsiboas goianus* in Central-Western Brazil.

Figure 3. Snout-vent length of the males of *Hypsiboas goianus* along the months of the reproductive season, in Central-Western Brazil.

Figure 4. Size of the alpha and beta males of *Hypsiboas goianus* sampled in the first territoriality experiment, in Central-Western Brazil. (*) indicates statistically significant difference.

Figure 5. Relationship between the number of aggressive calls emitted by the resident male and the distance to the nearest male of *Hypsiboas goianus* in the second territoriality experiment, in Central-Western Brazil.
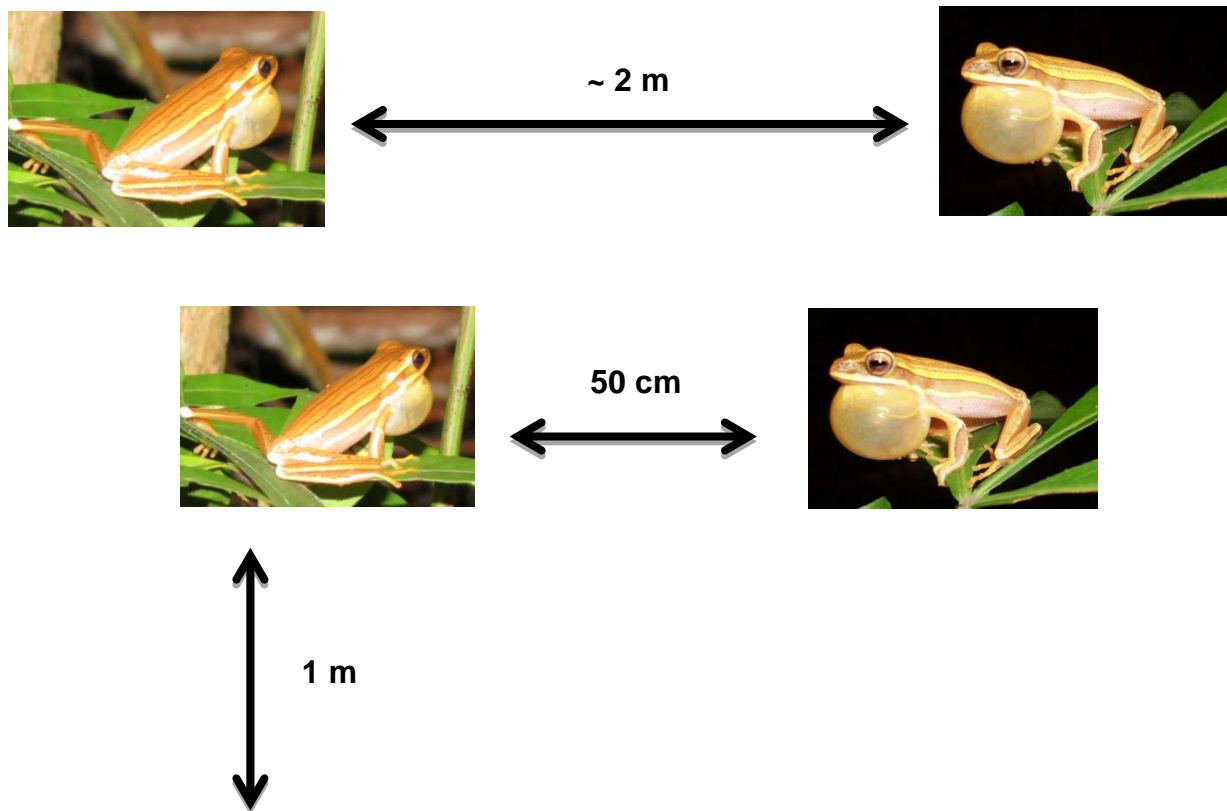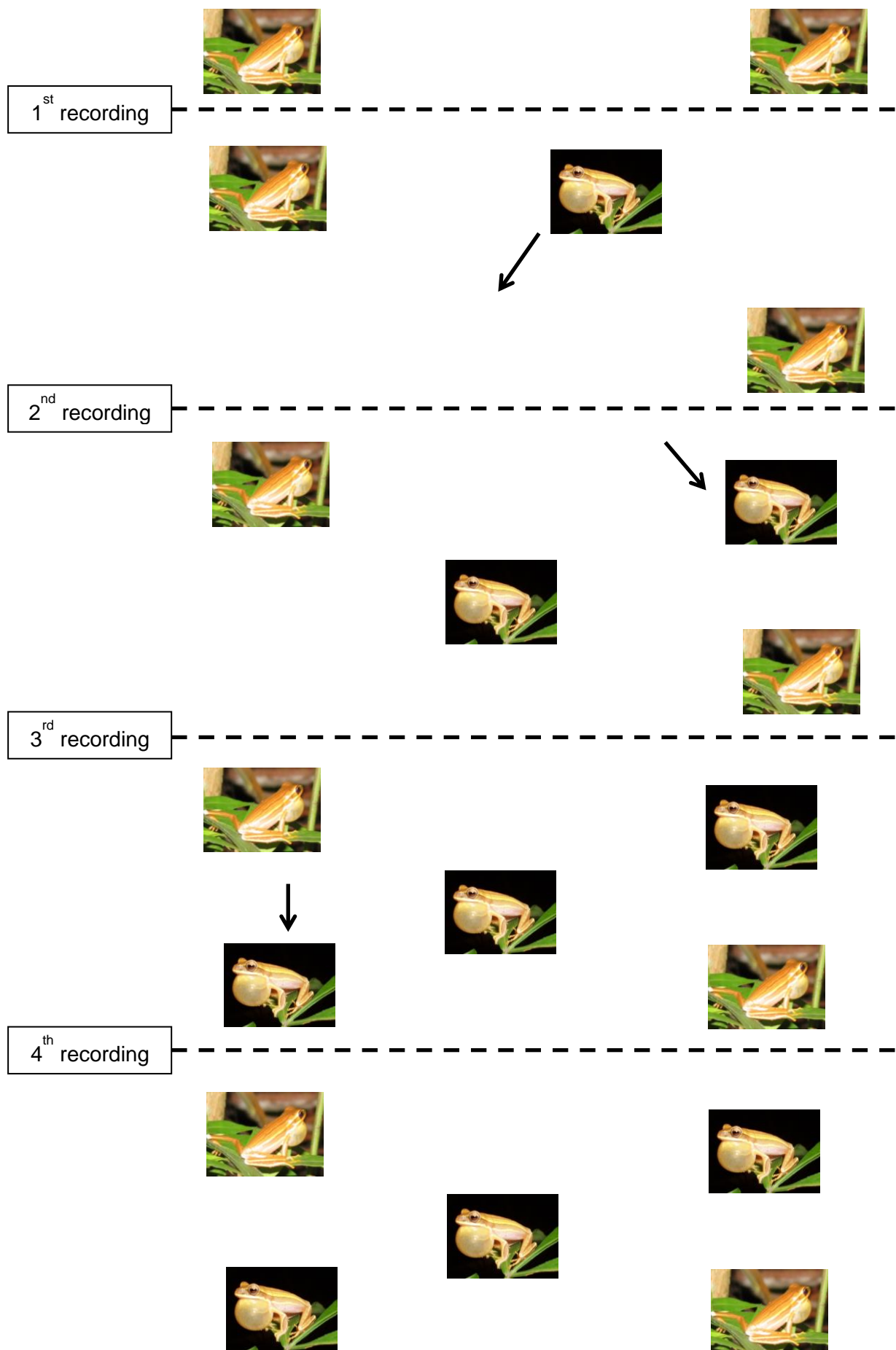
Figure 1.

1st recording

2nd recording

3rd recording
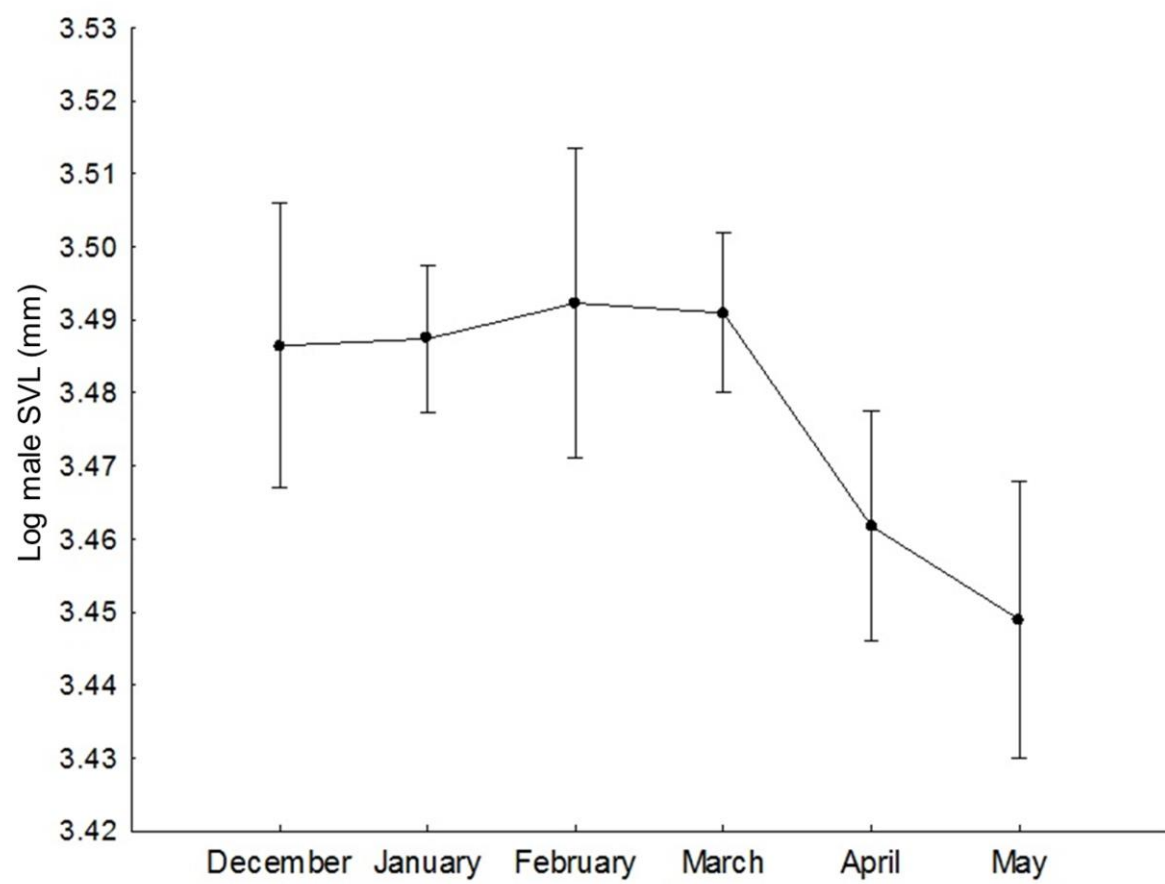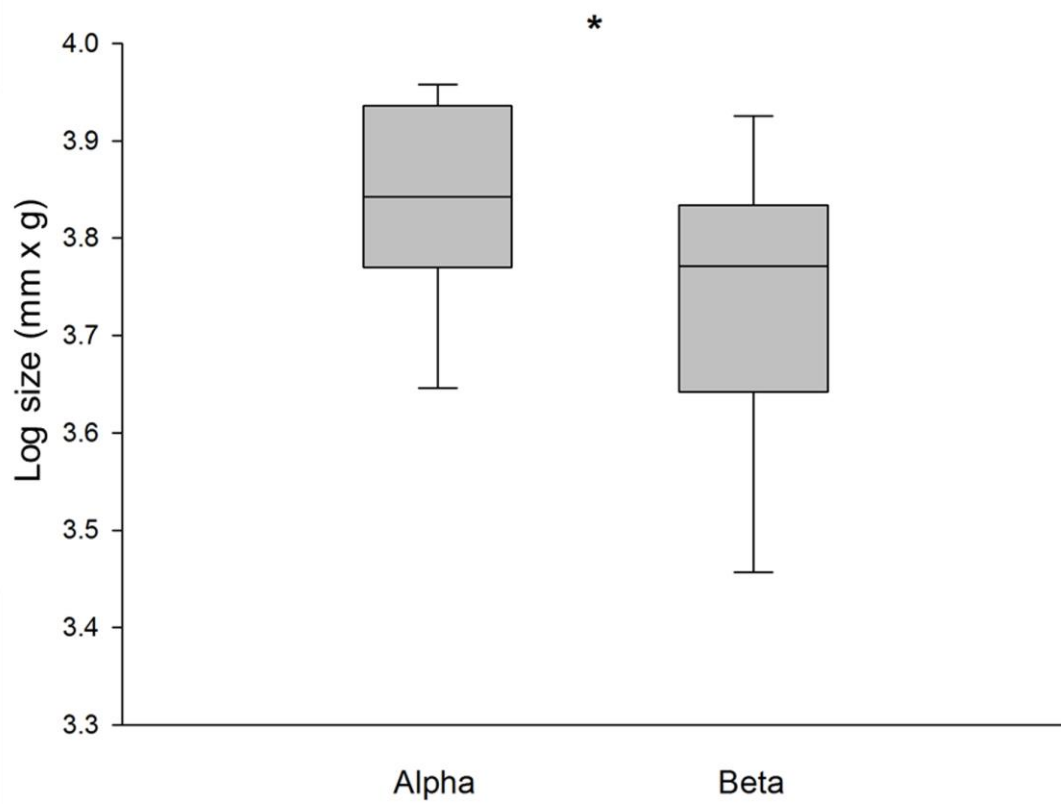
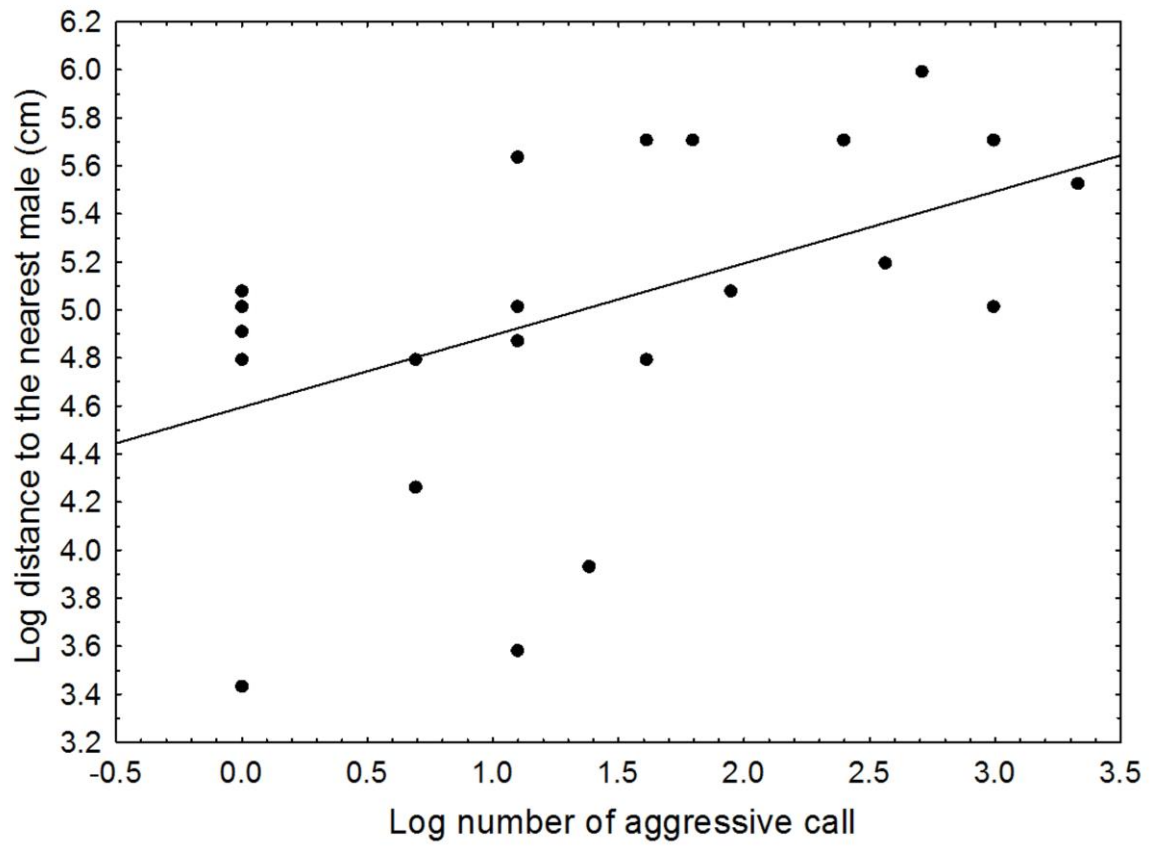4th recording

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Conclusão geral

Os machos de *H. goianus* usaram diferentes tipos de vegetação como sítios de vocalização. O CRC dos machos foi correlacionado positivamente com a distância até a água, sendo que os machos menores estiveram mais próximos da margem do corpo d'água. No entanto, o CRC não esteve correlacionado com a altura do poleiro. Além disso, a altura do poleiro não variou entre os meses da estação reprodutiva. Os machos emitiram mais cantos agressivos no início da noite e mais cantos de anúncio no meio da noite. Dos parâmetros acústicos dos cantos de anúncio, somente a taxa de repetição mostrou variação intraindividual ao longo da noite, e para os cantos agressivos a taxa de repetição e o intervalo entre as notas mostraram variação intraindividual ao longo da noite.

Nós não encontramos relação entre o tamanho da fêmea e o tamanho da desova, nem entre o tamanho dos ovos e o número de ovos por desova, e os acasalamentos não foram assortativos. Além disso, os machos maiores chegaram na área reprodutiva no início da estação reprodutiva. No primeiro experimento de territorialidade, um dos dois machos emitiu mais cantos e foi maior em relação ao outro macho. No segundo experimento de territorialidade, os machos maiores emitiram mais cantos, e quanto mais cantos agressivos o macho residente emitiu, maior foi a distância adotada pelo macho mais próximo. Em ambos os experimentos o número de cantos emitidos pelos machos foi influenciado pelos seus tamanhos. A baixa incidência de combates físicos juntamente com o padrão de espaçamento entre os machos indica que essa espécie usa principalmente os cantos para resolver seus contextos.

Finalmente, os machos de *H. goianus* mantêm uma estratégia reprodutiva ao longo da noite, a qual está relacionada com o estabelecimento de territórios e com a atração de fêmeas para os sítios de vocalização. Além disso, a comunicação acústica

parece ser extremamente importante em múltiplos contextos sociais para essa espécie. Estudos futuros devem investigar quais informações podem ser acessadas pelos indivíduos através dos cantos e os fatores que determinam o sucesso reprodutivo do macho no que se refere ao uso do hábitat e a preferência da fêmea.