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(ÁREA DE ZOOLOGIA)

BIOLOGIA REPRODUTIVA DE *BOKERMANNOHYLA IBITIGUARA* (ANURA, HYLIDAE) EM RIACHOS NO SUDOESTE DE MINAS GERAIS

RENATO CHRISTENSEN NALI

Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia).

Fevereiro - 2012

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“Sábio é aquele que conhece os limites da própria ignorância.”

Sócrates, filósofo grego

SUMÁRIO

RESUMO

O Brasil possui a maior diversidade de espécies de anfíbios anuros do mundo, com quase 850 espécies descritas. Apesar dos crescentes esforços e aumento dos estudos, muito pouco ainda se conhece sobre a biologia básica das espécies de anuros brasileiros. A biologia reprodutiva de *Bokermannohyla ibitiguara*, um hilídeo endêmico do Cerrado brasileiro, provavelmente a savana mais ameaçada do mundo, foi estudada em 17 expedições a campo, entre agosto/2010 e julho/2011. A espécie apresenta reprodução prolongada de outubro a junho. Os machos vocalizaram sobre diversos tipos de substratos em matas de galeria ao longo de riachos. O investimento reprodutivo (massa das gônadas/massa do corpo) de machos foi 0,29% e o de fêmeas 20,26%. Fêmeas grávidas apresentaram uma média de 202 ovócitos com diâmetro médio de 1,96 mm e o modo reprodutivo da espécie é semelhante ao modo 4: ovos e estágios larvais iniciais em bacias naturais ou construídas em riachos. Foi descrito o comportamento de corte complexo da espécie, com estímulos acústicos e táteis, além de briga entre dois machos e canto de briga. Machos satélites foram observados e a média de tamanho corporal não diferiu da de machos vocalizadores. A vocalização da espécie é mista, composta de notas curtas e longas, sugerindo diferentes funções. Houve correlação negativa entre frequência dominante do canto e tamanho do corpo do macho e positiva entre duração da nota longa e tamanho do macho, características que podem estar sob seleção pela fêmea. Houve correlação positiva entre a temperatura do ar e duração da nota longa e negativa entre a temperatura do ar e a taxa de repetição de pulsos. Durante os experimentos de *playback*, os machos não alteraram a proporção de notas curtas e longas quando simulamos um intruso, mas sim, alteraram parâmetros das notas curtas originais; esse canto modulado foi classificado como canto agressivo. Nossos resultados sugerem que as notas curtas são o componente agressivo do canto misto, enquanto as notas longas devem ser o componente de anúncio. Sendo esta espécie endêmica e fortemente associada a matas de galeria, acreditamos que nossos dados serão extremamente úteis para ações de manejo e conservação.

Palavras-chave: Anuros; reprodução; territorialidade; atividade de vocalização; Cerrado.

ABSTRACT

Brazil is the country with the greatest amphibian anuran diversity, with almost 850 species described. Despite the increasing efforts and increase in the studies, the basic biology of the Brazilian anuran species is poorly known. The reproductive biology of *Bokermannohyla ibitiguara*, a hylid frog endemic to the Brazilian Cerrado, probably the most threatened savanna in the world, was studied in 17 field expeditions between August/2010 and July/2011.This species is a prolonged breeder, with males calling from October to June. Males used a variety of calling sites along the gallery forests of streams. The reproductive investment of males (gonad mass/body mass) was 0.29% and that of females was 20.26%. Females contained a mean of 202 mature oocytes in the ovaries, each with a mean diameter of 1.96 mm, and the reproductive mode of the species is similar to mode 4: eggs and early larval stages in natural or constructed basins in streams. Herein, we described the complex courtship behavior of the species, involving tactile and acoustic stimuli, a male-male fight, and the fight calls. Satellite males were observed and mean body size was similar to that of calling males. Males emit a mixed call, composed by short and long notes, suggesting different functions. There was a negative correlation between dominant frequency of the call and male body size, and a positive correlation between long note duration and male body size, which might be under sexual selection pressure. Air temperature correlated positively with long note duration and negatively with pulse rate. During the playback experiments with males, they did not alter the proportion of long to short notes when simulating an intruder, but rather they vary some acoustic parameters of the original short notes; this modulated call was classified as aggressive call. Our results suggest that short notes are the aggressive component of the mixed call, while long notes might be the advertisement component. Since this species is endemic and strongly associated with gallery forests, we believe that our data might be very useful for conservation practices.

Keywords: Anurans; reproduction; territoriality; calling activity; Cerrado.

INTRODUÇÃO GERAL

Atualmente são conhecidas aproximadamente 6000 espécies de anfíbios anuros (Frost, 2011), sendo que a maior riqueza encontra-se na América tropical (Duellman, 1988). A maior diversidade de anuros do mundo, 849 espécies, é encontrada no Brasil (SBH, 2011), com uma taxa de endemismo de mais de 60% (Vasconcelos & Rossa-Feres, 2005). Diante das ameaças que os anfíbios vêm sofrendo nas últimas décadas, com a extinção de espécies e o declínio de populações em várias partes do mundo, provocadas, principalmente, por alterações nos hábitats e doenças (e.g. Becker et al., 2007; Loyola et al., 2008; Becker & Zamudio, 2011), estudos sobre a biologia das espécies são de extrema urgência. Vários aspectos podem ser abordados dentro da biologia dos anuros, como comportamento social e reprodutivo (cuidado parental, vocalização, territorialidade), distribuição espacial e temporal, predação, competição intra e interespecífica, defesa, entre outros (Duellman & Trueb, 1994; Wells, 2007).

Estudos sobre a biologia reprodutiva das espécies de anuros têm aumentado na região Neotropical (e.g. Martins, 1988; 1993; Haddad & Cardoso, 1992; Brasileiro, 1998; Freitas et al., 2001; Wogel et al., 2002; Giasson, 2003; Menin & Giaretta, 2004; Toledo, 2004), descrevendo, por exemplo, a temporada de atividade reprodutiva das populações, geralmente baseada na atividade de vocalização de machos (Pombal Jr., 1997; Bernarde & Machado, 2001; Toledo et al., 2003), e discutindo a interferência de fatores abióticos – como a pluviosidade – sobre a atividade reprodutiva (Aichinger, 1987; Praderio & Robinson, 1990). A própria atividade de vocalização tem sido objeto de estudo dentro desta área (Martins & Haddad, 1988; Zina & Haddad, 2005; Juncá e Lugli, 2009). Entretanto, apesar destes crescentes esforços, existem menos herpetólogos na região Neotropical comparado às regiões temperadas, e a biologia da maioria das espécies é praticamente desconhecida (Kriger, 2010). Outro ponto crítico é que nas últimas décadas a descrição de novas espécies tem sido mais acelerada (e.g. Araújo et al., 2009), cujo ritmo não é acompanhado pelos estudos em biologia das espécies.

Estudos sobre seleção sexual e territorialidade em anuros da região Neotropical são ainda mais escassos (e.g. Lips, 2005; Giasson & Haddad, 2007), se comparado aos estudos em regiões temperadas (e.g. Licht, 1976; Davies & Halliday, 1977; Howard & Kluge, 1985; Woolbright, 1989; Howard et al., 1994; Katsikaros & Shine, 1997). Dada a complexidade dos ecossistemas neotropicais, é de se esperar que o estudo e entendimento dos processos de seleção sexual e interações territoriais entre machos que aqui ocorrem contribuam de forma expressiva para o conhecimento que já é bastante avançado nas regiões temperadas (Wells, 2007). Muitos grupos de pesquisa nas regiões temperadas desenvolveram inúmeros e consistentes estudos sobre poucas espécies (e.g. Ryan, 1983; Gerhardt & Doherty, 1988; Ryan et al., 1990; Gerhardt & Brooks, 2009), enquanto nossa grande quantidade de espécies permanece inexplorada.

Esta dissertação, dividida em dois capítulos, teve como objetivo estudar a biologia reprodutiva de *Bokermannohyla ibitiguara* Cardoso (1983), endêmica do Cerrado brasileiro, cuja biologia praticamente desconhecida conferiu à espécie o status de Dados Deficientes pela IUCN (Caramaschi and Eterovick, 2004). O primeiro capítulo aborda sua biologia básica, descrevendo sítios de vocalização dos machos, organização do coro, padrão temporal de reprodução, distribuição temporal, modo reprodutivo, investimento reprodutivo de machos e fêmeas e interações sociais entre machos e fêmeas (e.g. comportamento de corte, briga entre machos, macho satélite). O primeiro capítulo está apresentado em inglês, nas normas da revista Amphibia-Reptilia, para a qual foi submetido recentemente. O segundo capítulo, também redigido em inglês na forma de manuscrito, aborda a questão da atividade de vocalização dos machos em si, através de observação direta e experimentos de playback com machos em campo. A função de diferentes partes do canto foi investigada, bem como a descrição de um canto agressivo especializado e de um canto de briga, além de avaliar correlações entre parâmetros do canto e tamanho do corpo dos machos e temperatura.

O estudo foi realizado na área rural do município de Sacramento (19°51’S; 47°26’O), estado de Minas Gerais, em fazendas próximas à represa de Peixoto (fig. 1A). As observações e coletas foram realizadas em três riachos: i) riacho permanente 1 (20º16’21,9”S; 47º04’24,5”O; altitude 677 m; fig. 1B), ii) riacho temporário (20º13’09,9”S; 47º06’21”O; altitude 880 m; fig. 1C) e iii) riacho permanente 2 (20º12’20,1”S; 47º07’53,6O”, altitude 839 m; fig. 1D).

O clima da região é classificado como mesotérmico de inverno seco (Araujo et al., 2009). O relevo é bastante montanhoso, apresentando topos achatados e arredondados, com encostas escarpadas em forma de cuestas e cânions, predominando os latossolos. A paisagem é formada por cerradões, cerrados e campos nos interflúvios, florestas de galeria contínuas no fundo e nos flancos dos baixos vales, também estando presente a floresta estacional decidual (ver Araujo et al., 2009). Romero & Nakajima (1999), em referência a Serra da Canastra, localizada a aproximadamente 60 km da área de estudo, descrevem os tipos de vegetação como sendo florestas mesófilas de encosta, capões, cerradão, cerrado, campo cerrado, campo limpo e campo rupestre. Haddad et al. (1988) caracterizam a vegetação do Parque Nacional da Serra da Canastra como constituída de campos rupestres, com pequenas manchas de cerrado e matas de galeria. O clima é marcadamente sazonal, com um verão quente e chuvoso (outubro a março) e um inverno seco (abril a setembro), com precipitação total anual entre 1300 – 1700 mm de chuva (Queirolo & Motta-Junior, 2007).

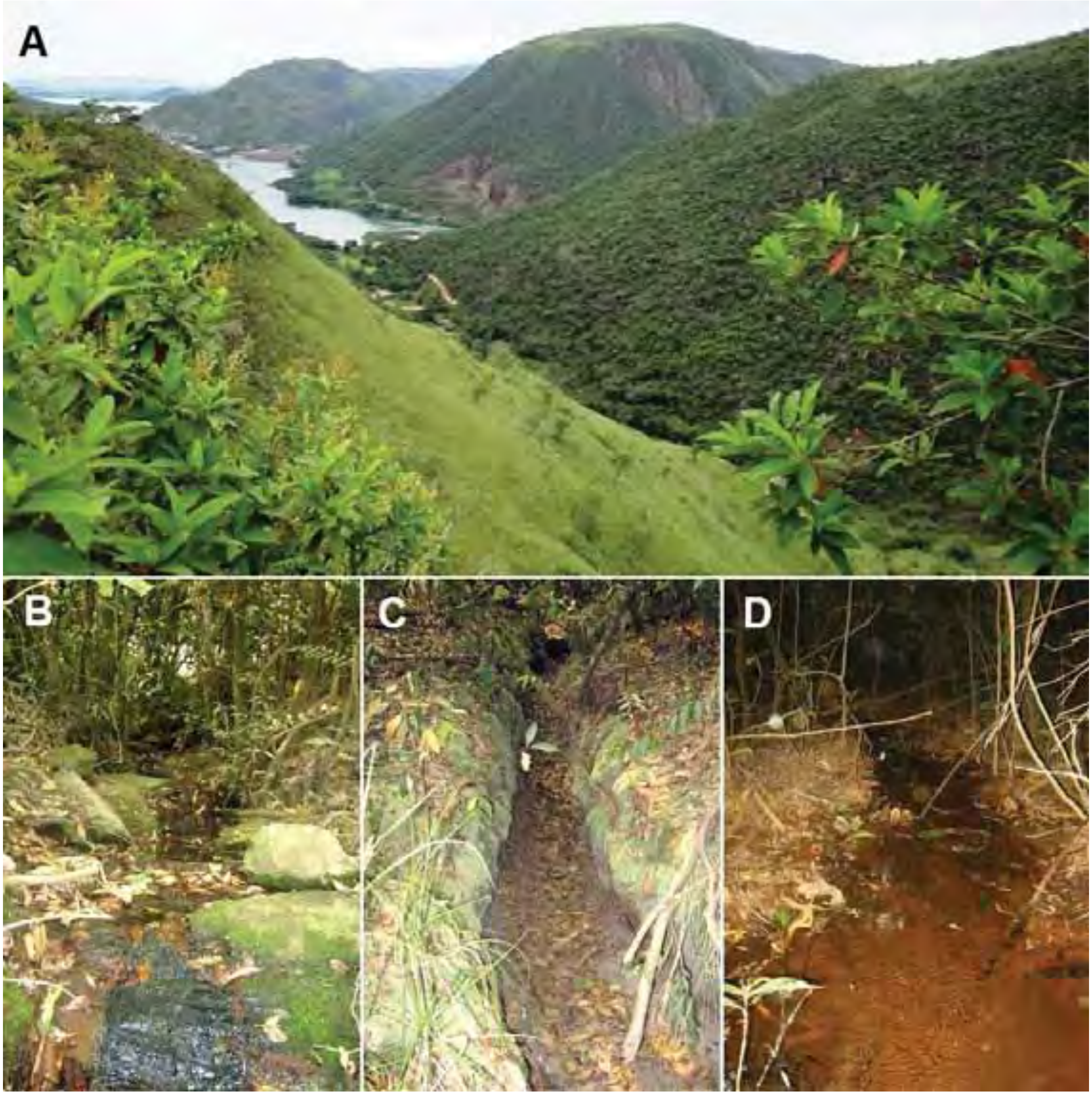

Figura 1. Local de estudo. A) Vista geral, mostrando a represa de Peixoto ao fundo, município de Sacramento, Minas Gerais; B) riacho permanente 1; C) riacho temporário (fotografia tirada em mês de seca - agosto); D) riacho permanente 2, onde formam-se vários poços interligados por corredeiras.

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Chapter 1

HABITAT USE, REPRODUCTIVE TRAITS AND SOCIAL INTERACTIONS IN A STREAM-DWELLER TREEFROG OF THE BRAZILIAN CERRADO

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Habitat use, reproductive traits and social interactions in a stream-dweller treefrog of the Brazilian Cerrado

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ABSTRACT

The richest anuran fauna is found in the Neotropics, but natural history of most species is unknown. This is the case of *Bokermannohyla ibitiguara*, a stream-dweller treefrog endemic to the Cerrado of southeastern Brazil. The species is classified as "Data Deficient" by the IUCN, thus, herein we describe the species’ reproductive biology. Fieldwork was conducted from August 2010 to July 2011. The reproductive activity occurred from October to June and was positively correlated with air temperature, air humidity, and monthly rainfall. Individuals were observed only in gallery forests at the margins of streams and males used different substrates as calling sites. Males did not aggregate in specific parts of the streams and distance among males was higher in the dry season. Males and females did not differ in lenght, but females without mature oocytes were lighter than males. The reproductive investment of males (gonad mass/body mass) was 0.29% and that of females was 20.26%. Females contained a mean of 202 ± 75 mature oocytes in the ovaries, each with a mean diameter of 1.96 ± 0.16 mm. We describe, for the first time for the genus, a complex courtship behaviour and also male-male fight and satellite behaviour. The Cerrado is probably the most threatened savanna in the world, being intensively modified in the last decades. Endemic species associated to gallery forests, such as *B. ibitiguara*, are highly vulnerable to habitat alterations. Thus, our study provides important data on the species’ biology, which might be very useful for conservation practices.

Keywords: *Bokermannohyla ibitiguara,* Hylidae, courtship behaviour, calling sites, temporal distribution

INTRODUCTION

Currently, 5966 amphibian anurans are known in the world (Frost, 2011), with the highest diversity found in the American tropics (Duellman, 1988). The greatest anuran richness, 847 species, is found in Brazil (SBH, 2011), with approximately 60% of endemism (Vasconcelos and Rossa-Feres, 2005). Despite the great diversity, in the Neotropics, approximately 35% of the amphibians are endangered due to habitat loss or fragmentation, as well as a result of habitat split (Becker et al., 2007; Loyola et al., 2008). Habitat loss is considered the greatest threat to Brazilian amphibians (Silvano and Segalla, 2005), probably because of alteration or complete elimination of specific humid microhabitats used by the species (Beebee, 1996; Young et al., 2000). Furthermore, there are fewer herpetologists in the Neotropics compared to temperate regions and its huge fauna is still poorly known (Kriger, 2010). This is a dangerous combination of facts for anuran diversity conservation, making urgent the study of species’ natural history and ecology.

Although studies on anuran natural history have increased in the Neotropical region, most of them focus on forest species from the Amazon or the Atlantic forest domain (e.g. Canelas and Bertoluci, 2007; Bernarde and Kokubum, 2009; Montanarin, Kaefer and Lima, 2011). The biology of anuran species from open habitats, such as the Cerrado, although widely distributed and commonly observed in the field, are much less studied (e.g. Brasileiro et al., 2005; Giaretta et al., 2008; Guimarães et al., 2011). The Brazilian Cerrado is the second largest South American domain, with an original area of approximately 2.5 million square kilometers (Silva et al., 2006), mostly composed by grasslands, seasonal savannas, and corridors of gallery forests. The Cerrado is the largest, most species-rich, and probably most threatened tropical savanna in the world (Silva and Bates, 2002), thus included among the 25 most threatened hotspots on Earth (Myers et al., 2000). This open formation in central South

America harbors approximately 204 anuran species (Valdujo, 2011) and has been intensively modified in the last 40 years, primarily due to urban and agricultural development; currently only 20% of its original area remains and only about 6% of that area is legally protected (Myers et al., 2000).

The genus *Bokermannohyla* (Faivovich et al., 2005) includes 29 hylid species, distributed in Center, South and Southestern Brazil (Frost, 2011). Together with the genera *Hyloscirtus*, *Miersiohyla*, *Hypsiboas* and *Aplastodiscus* they form the Cophomantini tribe, a well-supported clade (Faivovich et al., 2005; D'Heursel and Haddad, 2007). *Bokermannohyla ibitiguara* (Cardoso, 1983) is a stream-dweller frog, endemic to the Cerrado in Southeastern Brazil. The species is currently placed in the *B. pseudopseudis* group, with *B. alvarengai*, *B. pseudopseudis, B. saxicola, B. oxente*, and *B. itapoty* (Faivovich et al., 2005; Frost, 2011). General information on its natural history can be found in the original description (Cardoso, 1983). It is a medium-size species with robust body, males' call is composed by primary and secondary notes, and adults inhabit rocky environments along small streams (Cardoso, 1983). Population status is unknown and reproductive habits had never been published in detail. Thus, *B. ibitiguara* is currently included in the Data Deficient category by the IUCN (Caramaschi and Eterovick, 2004).

Herein, we describe the reproductive biology of *B. ibitiguara*, including habitat use patterns, breeding period, and social behaviours, such as a complex courtship and male-male fight, both described for the first time for the genus *Bokermannohyla*. Moreover, following the reccomendation of Pimenta et al. (2005), that researchers should concentrate efforts to study Data Defficient species to obtain information on their population status, we believe that the results reported here will not only increase our knowledge on the basic biology of the species, but also might be extremely useful for conservation practices.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in private farms in the municipality of Sacramento (19°51’S; 47°26’W), Minas Gerais state, Southeastern Brazil. Three streams were sampled: (i) permanent stream 1 (20º16’21.9”S; 47º04’24.5”W; 677 m elevation); (ii) temporary stream (20º13’09.9”S; 47º06’21”W; 880 m elevation), and (iii) permanent stream 2 (20º12’20.1”S; 47º07’53.6”W, 839 m elevation).

The study area is located at about 60 km from the Serra da Canastra National Park. The climate is markedly seasonal, with a hot and rainy summer (October to March) and a dry winter (April to September), with total annual precipitation between 1300-1700 mm (Queirolo and Motta-Junior, 2007). The topography is mountainous, with flat or rounded hills, reaching up to 1500 m elevation, with deep valleys and many streams (Dietz, 1984). The landscape consists of savannas, locally named as Cerrado vegetation, grassland fields at higher elevations, gallery forests in the valleys along streams, and patches of semideciduous forest (Dietz, 1984; Araujo, Condez and Sawaya, 2009).

Data collection

Seventeen field expeditions yielded 190 hours of observations on the natural history of *B. ibitiguara* throughout a whole year (August 2010 to July 2011). Two field expeditions per month were made in the rainy season, except for March (*n* = 11 expeditions) and one per month in the dry season (*n* = 6 expeditions).

Individuals were found by active search guided by male vocalizations and were observed with flashlights. To reduce the interference of luminosity on the frogs’ behaviour, red lights were used (e.g. Sazima and Pombal-Jr., 1986; Miranda et al., 2008; Mott and

Sparling, 2009), avoiding, whenever possible, to point the light focus directly toward the individuals (Toledo, 2004). To examine some reproductive traits (see item below), 27 individuals were hand-captured, anesthetized with lidocaine 10%, fixed in formalin 10%, and preserved in alcohol 70% (McDiarmid, 1994).

Habitat use

Habitat use by individuals of *B. ibitiguara* was registered monthly, describing male vocalization site, such as leaf, trunk, branch, inside water, inside hidden places, and on the ground, also evaluating male abundance in each type of substrate. We also measured horizontal distance from the water (DFW), height above water (HAW), height above ground (HAG), and distance to the nearest neighbor (DNN), this last one divided into categories (less than 1 m; 1 to 5 m; 5 to 10 m and over 10 m). We performed a Chi-squared test for the frequencies of less than 5 m and more than 5 m, comparing the DNN between the rainy season (Oct – Mar) and dry season (Apr – Sep).

Temporal distribution and temporal breeding pattern

The numbers of calling males and active females were registered every night. Data on rain and air humidity were obtained from the meteorological station of the municipality of Sacramento, by the National Institute of Meteorology (INMET), and the air and water temperatures were registered at each stream with an annalogical thermometer. To verify abiotic effects on species activity we performed a multiple correlation analysis, using Kendall’s rank coefficient (Zar, 1999). The species’s breeding pattern was classified as prolonged or explosive, according to Wells (1977a).

Body size and reproductive traits

Male and female body mass and snout-vent lenght (SVL) were measured in the field with a dynamometer (0.1 g) and an analogic caliper (0.05 mm), respectively. Males were recognized by the presence of secondary sexual traits: developed prepollex, hypertrophied forearm, and yellowish vocal sac. To verify the occurrence of sexual size dimorphism (SSD), mean values were compared through a Student's t-test (Zar, 1999).

Reproductive mode of the species was determined (sensu Haddad and Prado, 2005). Since we did not find any clutches in the field, mature oocytes in the ovaries of gravid females were analyzed to obtain information on clutch and egg size. Diameter of approximately 10 oocytes per female was measured under a Leica stereomicroscope with a photo capturing software (Leica Application Suite). To examine the relationship between female body size and clutch size, Spearman correlation analysis was performed between the variables: (i) female SVL vs. *n* oocytes in the ovaries, and (ii) female body mass vs. ovary mass. The same analysis was also used to verify if there was a tradeoff between oocyte number and oocyte size.

The reproductive investment (RI) was calculated for both males and females as the percentage of gonad mass/body mass (Prado and Haddad, 2005). Body and gonad mass were measured in a digital balance (0.001 g). Males collected in a preliminary field trip, in January 2010, were also used.

Social interaction observations

To study social interactions, we used the methods of focal-animal sampling, all occurrences sampling, and sequence sampling (Altmann, 1974). Animals were recognized through natural skin marks (spots, scars, etc.; Bradfield, 2004). Females and males were differentiated in the field by the secondary sexual traits described above. Digital photo, audio, and video recordings were made whenever possible.

RESULTS

Habitat use and chorus structure

Males of *Bokermannohyla ibitiguara* were observed calling only inside the gallery forests, along the streams encovered by the canopy. They were found calling from tree or shrub leaves (56.6%; n = 129), trunks (21.9%; n = 50), branches (10.1%; n = 23), inside hidden places – among roots and leaves, under rocks or fallen trunks – (7.5%; n = 17), on the ground (2.2%; n = 5), and partially submersed in the water (1.8%; n = 4; fig. 1). When males called perched, they could be either above the water or above the ground: mean HAW was 1.13 ± 0.89 m (range 0 – 2.93 m; n = 67 observations) and mean HAG was 0.99 ± 0.74 m (range 0 – 2.93 m; n = 136). In the last case, mean DFW was 0.67 ± 0.64 m (range 0.05 – 3.54 m; n = 117). Most commonly, males called parallel to the ground (92%; n = 210), but also in vertical or diagonal position (8%; n = 18) relative to the ground.

Considering chorus structure, calling males did not aggregate at specific sites, but rather they were distributed sparsely along the streams. Only 4.3% of males participating in the chorus were less than 1 m from the nearest calling male, 45.7% were distant between 1 and 5 m, 19.7% between 5 and 10 m, and 30.3% were farther than 10 m from the nearest calling male. Comparing the frequencies between seasons, males tend to be closer (< 5 m) to each other in the rainy season, and more distant (> 5 m) in the dry season (Chi-square χ^2_1 = 10.21, $P < 0.05$).

Temporal distribution and temporal breeding pattern

Calling males of *B. ibitiguara* were found from October to June (fig. 2). Adult females were found from November to February and in April. *Bokermannohyla ibitiguara* exhibited a prolonged breeding pattern (sensu Wells, 1977a; see discussion).

Maximum number of calling males per month correlated significantly with air temperature (Kendall's rank coefficient $\tau = 0.48$, $P < 0.05$), relative air humidity ($\tau = 0.68$, $P < 0.05$), and total monthly rainfall ($\tau = 0.60$, $P < 0.05$).

Body size and reproductive traits

Average SVL and mass of males and females are shown in table 1. The population showed no SSD for SVL (Student's t-test $t_{74} = -0.05$, $P = 0.96$), but females without eggs were significantly lighter than males ($t_{73} = 2.59$, $P < 0.05$).

Although we were not able to observe the oviposition site, as individuals were hidden in rock crevices or under fallen trunks (see description below), the reproductive mode of *B. ibitiguara* is probably similar to mode 4 (sensu Haddad and Prado, 2005): eggs and early larval stages in natural or constructed basins; subsequent to flooding, exotrophic tadpoles in streams. During observation of courtship 1 and 2 (see below), it was possible to recognize two likely oviposition sites: (1) a basin covered by leaves and a fallen trunk, with 10 cm diameter and 5.5 cm deep, and (2) a cavity in the stream's banks, with 20 cm between end of the cavity and beginning of the stream water. The first site is probably a natural basin.

We did not find any clutches in the field, probably because oviposition sites were in hidden locations. Clutch size and egg diameter were then estimated by analyzing mature oocytes from gravid females (table 1). Oocytes were black on the animal pole and beige on the vegetative pole. One female spawned one single egg while kept in the plastic bag, and its diameter was 1.99 mm inside an individual jelly capsule with 3.4 mm of diameter. Males invested approximately 0.3% of their body mass in gonads and females 20.3% (table 1). Female body mass and ovary mass were positively correlated (Spearman correlation r_{s4} = 0.90, $P < 0.05$, $n = 6$), but *n* oocytes and oocyte diameter were not correlated (r_{s4} = -0.54, P = 0.26, $n = 6$), neither were female SVL and *n* eggs (r_{s4} = 0.49, P = 0.32, $n = 6$).

Courtship behaviour, male territoriality, and satellite males

We observed three sequences of courtship behaviour: courtship 1 and 2 with the same female, on 18 January, 2011 (air temperature = 21°C), and courtship 3 on 28 February, 2011 (air temperature = 22°C). The male calls, attracting the female; when she approaches, they stand face to face and he slaps the female at her side, once or twice, guiding her to the nest, where she enters to inspect. The basic events of *B. ibitiguara* courtship are summarized in fig. 3; however, there were some differences among the three courtships (table 2).

During courtship number 2, a satellite male followed the pair quietly. Although he was noticed by the pair, the courtship occurred normally. After the courtship, they all entered the oviposition site, inside a cavity amidst the rocks, and the resident male kept calling for about five more minutes. Then, the resident stopped calling for another 5 min, and this should be the total time of oviposition (see discussion below). Afterwards, both the resident male and female left the oviposition site, and the satellite male remained inside. After a while, the satellite male began to call inside the cavity and the resident male approached and called toward the satellite male. Following, the resident male entered the cavity and different sounds were emitted, probably fight calls (Chapter 2). After that, both males jumped off the cavity into the water stream, and it was possible to observe the fight (fig. 4A). They remained clasped chest to chest, using their prepollical spines to injure the opponent’s head region (e.g., eyes and the tympanum). The fight lasted about 18 min, but at the final 6 min, the satellite male simply subdued the resident male, until they released themselves. The satellite male won the fight and remained calling inside the cavity. He was larger (SVL = 41.6 mm) and heavier (body mass = 5.44 g) than the calling male (38.75 mm, 4.5 g). Many scars became visible on the resident's head (fig. 4B).

Satellite behaviour was observed many times during the study (*n* = 20 satellite males), where non-calling males remained next to males that were calling. In two occasions we observed two satellite males next to one calling male. Average distance between satellite and calling male was 54 ± 29 cm (range 15 – 100 cm; *n* = 13). Mean SVL of satellite males was 39.2 mm (*n* = 9) and mean mass was 4.32 g (*n* = 8); mean SVL of calling males was 39.3 mm (*n* = 8) and mean mass was 4.50 g (*n* = 7). Satellite and calling males did not differ significantly neither in SVL (t_{15} = 2.13, *P* = 0.95) nor in mass (t_{13} = 2.16, *P* = 0.76).

DISCUSSION

Bokermannohyla ibitiguara males used a great variety of microhabitats for calling. Eterovick et al. (2010) presented some data about microhabitat use for other *Bokermannohyla* species [(*B. martinsi*, *B. nanuzae, B. saxicola*, and *Bokemannohyla* sp. (gr. *circumdata*)], allowing comparison. Adults of *B. ibitiguara* showed the greatest variation in microhabitat use, followed by *B. nanuzae*; *B. saxicola*, both species in the *B. pseudopseudis* group, which used only three types of microhabitats (rocks, leaves, and branches). *Bokermannohyla saxicola* and *B. nanuzae* showed the shortest distance from the water (average 0.18 and 0.32 m, respectively), followed by *B. ibitiguara* (average 0.67 m). In terms of height (above ground or water), all species remain closer to the substrate compared to *B. ibitiguara*, except for *Bokermannohyla* sp. (gr. *circumdata*), which showed an average height varying from 1.38 to 2.67 m.

During this study, adults of *B. ibitiguara* were always in the interior of the gallery forests, associated with the streams. Considering species in the *B. pseudopseudis* group, which occur predominantly in open formations, *B. oxente* and *B. itapoty* also have association with riparian vegetation (Lugli and Haddad, 2006a,b), but *B. alvarengai* and *B. saxicola* seem to breed in streams without vegetation (Eterovick and Sazima, 2000). The dependence on forested areas and streams is also known for other species of *Bokermannohyla* (*B. circumdata, B. hylax, B. izecksohni,* and *B. luctuosa*), as well as species in the related genus *Aplastodiscus* (*A. albosignatus, A. arildae, A. callipygius, A. ehrhardti, A. leucopygius*) (Haddad, Toledo and Prado, 2008), all species typical of the Brazilian Atlantic Forest domain. At our study site, even with the riparian vegetation being fragmented in many portions and presenting roads nearby, males were able to use a great variety of microhabitats as calling sites, suggesting that *B. ibitiguara* is, at some extant, resistant to human alterations. However, as the species is strongly associated to gallery forests and streams, it is mandatory to preserve these habitats properly.

Calling males of *B. ibitiguara* tended to be closer to each other during the rainy season compared to the dry season. This suggests that calling males seem to spread themselves along the water bodies as much as possible, but in the rainy season, when male density is higher, they end up being closer to each other. The organization of calling males in choruses in specific parts of the water bodies has advantages, for example by increasing female attraction efficiency and decreasing individual exposure and predation risk (Ryan, Tuttle and Taft, 1981; Wells, 2007). However, for *B. ibitiguara*, as males are extremely territorial, it is probably more profitable to remain as far as possible from each other, avoiding costs associated to territorial conflicts (e.g. male wounds; this study).

Calling activity of *B. ibitiguara* males was positively correlated with air humidity, air temperature, and total rainfall. This result was expected, since high temperatures and rains are known to strongly influence the reproductive activity of anurans in the neotropics (Eterovick and Sazima, 2000; Bertoluci and Rodrigues, 2002; Prado, Uetanabaro and Haddad, 2005). Also, air humidity is another factor that undoubtedly limits breeding activity of frogs, although few studies have actually tested this prediction (e.g. Cree, 1989). *B. ibitiguara* is a typical prolonged breeder (sensu Wells, 1977a), because (i) males called during several months (Oct – Jun), (ii) we did not observe females arriving synchronously to the reproductive site, and (iii) breeding activity did not increase significantly after heavy rains, as observed for some explosive breeders at the study site (e.g. *Scinax fuscovarius* and *Elachistocleis* sp.; pers. obs.). Furthermore, other explosive breeder traits were not observed, such as active search for females or males intercepting females.

Contrary to most anurans, where females are larger than males (Shine 1979), *B. ibitiguara* showed no SSD in SVL, as previously observed for other species in the *B. pseudopseudis* group: *B. oxente* (Lugli and Haddad, 2006a), *B. saxicola* (Eterovick and Sazima, 2004), and *B. alvarengai* (Lugli and Haddad, 2006b). Actually, in this last case, Sazima and Bokermann (1977) observed that males are larger than females. Differently, females of *B. itapoty* are larger than males (Lugli and Haddad, 2006b). However, *B. ibitiguara* males were significantly heavier than females without eggs, probably because females showed a high reproductive investment (RI) (average 20.26%), as already observed for other anurans, such as *Leptodactylus chaquensis* (RI = 16%; Prado and Haddad, 2005), *Physalaemus albonotatus* (RI = 16.6%; Prado and Haddad, 2005) and *P. crombiei* (RI = 32.06%; Pupin et al., 2010). Since we found a positive correlation between female body mass vs. ovary mass, we suggest that there is a selective pressure towards female fecundity increase and, therefore, female body size increase (the fecundity advantage hypothesis; Darwin, 1874). This hypothesis has constantly been proposed to explain larger females in different animal groups (e.g. Head, 1995; Brana, 1996), including anurans (e.g. Shine, 1979; Kupfer, 2007).

The size-fecundity relationship was not significant between the variables *n* oocytes vs. female SVL, which could be explained by the small sample size, but also because oocyte size was highly variable among females. Intraspecific variation in egg size has been reported for other anuran species (e. g. Rafińska, 1991; Kaplan and King, 1997; Camargo, Sarroca and Maneyro, 2008). However, in *B. ibitiguara* this variation was not explained as a tradeoff between oocyte number and oocyte size, which could also be due to the small number of gravid females analyzed here.

The RI of males was low (average 0.29%), compared to some other frogs (e.g. *Rhacophorus arboreus*: 5.15%; *R. schlegelii*: 1.06%; Kusano, Toda and Fukuyama, 1991; *Leptodactylus chaquensis*: 4.13%; *L. podicipinus* 0.75%; Prado and Haddad, 2003; *Scinax fuscovarius*: 1.21%; Rodrigues, Uetanabaro and Lopes, 2005). High RI measured as relative testes size in anuran males are generally associated to the occurrence of multi-male spawning and sperm competition (Emerson, 1997; Prado and Haddad, 2003; Rodrigues, Uetanabaro and Lopes, 2005), term defined by Parker (1970). The basic idea of this concept is that a male with larger testes would increase the probability of fertilizing female's oocytes when breeding simultaneously with other males. On the other hand, low RI for males, as we found in *B. ibitiguara*, might occur when there is low risk of sperm competition, for example: (i) in species in which the female interacts with only one male during a complex courtship and deposit eggs in a hidden place, as observed here and previously reported for species in the genus *Leptodactylus* (Prado and Haddad, 2003), and/or (ii) in species in which males actively defend their territories, engaging in fights, reducing the probability of multi-male spawning, as observed for *B. ibitiguara*. As a result, we hypothesize that although it seems to exist a strong selective pressure towards female fecundity increase, which could lead to female body size increase, the presence of male-male fights tends to select larger males as well (see Shine, 1989; Katsikaros and Shine, 1997). Consequently, *B. ibitiguara* males and females do not differ in SVL.

Many species of the genus *Bokermannohyla* deposit their eggs in flowing waters, which is described as reproductive mode number 2 (e.g. *Bokermannohyla alvarengai*, *B. nanuzae* e *B. sazimai*; Haddad and Prado, 2005). However, other species are known to deposit their egss in standing water, as *B. luctuosa* (Haddad and Sawaya, 2000), or in natural or constructed basins in both lotic or lentic environments (modes 1 and 4, respectively; Haddad e Prado, 2005). From our observations, we suggest that *B. ibitiguara* exhibits a variation of reproductive mode number 4, depositing eggs in lotic environments, probably in basins in hidden places, such as rock crevices or amidst fallen leaves or trunks.

The courtship in *B. ibitiguara* is relatively complex, involving accoustic and tactile stimuli, during which the male guides the female toward his nest and the female can inspect it. This is the first description of courtship for the genus *Bokermannohyla*. A similar courtship is known for other species in the Cophomantini tribe, such as some species of *Aplastodicus* (Haddad and Sawaya, 2000; Hartmann, Hartmann and Haddad, 2004; Zina and Haddad, 2007). However, the process from female arrival until her entrance in the nest lasted much less time in *B. ibitiguara* than compared to *Aplastodiscus* species: in *B. ibitiguara*, the first courtship lasted about one hour, and the other two, about 30 minutes, whereas in *Aplastodiscus* the courtship can last many hours (Haddad and Sawaya, 2000; Hartmann, Hartmann and Haddad, 2004; Zina and Haddad, 2007). Another difference is that the tactile stimulus in *B. ibitiguara*, when the male reaches the female, is similar to a slap and it is somewhat aggressive, while in *Aplastodiscus* there is only one ore more soft touches. Considering aggressiveness itself, the stimulus in *B. ibitiguara* is more similar to the one reported for the eleutherodactylid *Diasporus diastema*, where the male bumps the female, almost making her fall off the perch (Ovaska and Rand, 2001).

Female inspection of male nest has been previously reported for other species in the Cophomantini tribe (e.g. species of *Aplastodiscus*: Zina and Haddad, 2007; *Hypsiboas faber*: Martins and Haddad, 1988). In *B. ibitiguara*, it is possible that inspection can be decisive for male rejection by the female, as observed in courtship 1 (this study). However, she can also reject the male prior to nest inspection, as in courtship 3 (this study), when the male remained calling perched on a branch, there was no nest inspection, and the female moved towards another male. The importance of female inspection of nests is an evidence that males with better territories can achieve higher reproductive success (e.g. Wells, 1977b), which can explain why males of this species should risk themselves in fights. Scars were commonly found in males of *B. ibitiguara*, which could have been caused by male fights, as observed once in the present study.

We did not observe any amplexus, but it probably occurred after courtship 2, inside the groove cavity. After the female has left the cavity, she was collected and had almost no mature oocytes in her ovaries. Analyzing the abdominal cavity, we observed that the right ovary still had two mature oocytes and the oviduct was dilated, although the left ovary had only atretic oocytes and the oviduct was not dilated. These evidences support the occurrence of amplexus and spawning in the cavity. In this case, the amplexus could have lasted at most 10 minutes, which was the total time the calling male and the female remained inside the nest. Short amplexus and spawning have already been reported for other anuran species (e.g. *Guibemantis liber:* Blommers-Schlösser, 1975; *Crossodactylus gaudichaudii*, 1 to 2 minutes: Weygoldt and Carvalho-e-Silva, 1992; *Hyla cinerea*,15 to 35 minutes under lab conditions: Garton and Brandon, 1975; *Crinia georgiana*, 23 minutes: Roberts et al., 1999).

Satellite male behaviour is common in anurans with prolonged breeding season (Wells, 2007). We sometimes observed that when manipulating calling males, they stopped calling and satellites started calling. This suggests that males of *B. ibitiguara* show plasticity in the reproductive strategies, alternating between calling and satellite behaviour, as already observed for other hylids (Perril, Gerhardt and Daniel, 1978; Haddad, 1991; Zina and Haddad, 2006). In one occasion, a smaller satellite male approached a calling male (distance 15 cm) and remained still. At first, the calling male emitted an agressive call composed by short notes facing the satellite male, but after that the resident male soon turned the other way around and kept calling normally. It was also observed that satellite males were less agressive then calling males when manipulated; calling males tended to puncture our fingers with their spines. These observations fit the concept of a more submissive behaviour of satellite male compared to the calling male (Emlen, 1976; Toledo and Haddad, 2005). However, during courtship 2, the satellite male was agressive, larger and heavier, being able to defeat and take the calling male territory. Also, on average, satellite males were the same size of calling males, as commonly observed for hylids (e.g. Perril and Magier, 1988; Lance and Wells, 1993, Toledo and Haddad, 2005; Alcantara, Lima and Bastos, 2007). Thus, satellite behaviour in *B. ibitiguara* males seems to depend on ecological factors rather than male size itself.

Bokermannohyla ibitiguara reproduced for several months at the study site and individuals were abundant in many streams. Moreover, the species occur in Southeastern Brazil, the most developed region of the country. However, besides the original description (Cardoso, 1983), little information had been previously published on the species' natural history. This highlights how poorly is our knowledge on anuran biology in Brazil, the world richest country in anuran species (SBH, 2011). Natural history data is essential for comprehending the reproductive strategies and social interactions in anurans, also raising questions for future studies. Therefore, researchers should concentrate efforts in studying and publishing data on basic biology, mainly for Data Deficient species (IUCN), which will be useful for studies focusing on other aspects, such as ethology, ecology, genetics, and phylogeny. Also, natural history data might support conservation strategies, producing more effective results.

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Table 1. Body size and reproductive traits for individuals of *Bokermannohyla ibitiguara* in the studied population. Gravid females were weighed without oocytes for female body mass. Number of eggs per clutch, clutch mass, and egg diameter were estimated by analysis of gravid females ovaries. Results are shown as mean ± SD (range; number of observations).

	Females	Males
SVL (mm)	39.96 ± 2.72	39.90 ± 3.75
	(36.7 – 43.9; *n* = 9)	(32.6 – 48.4; *n* = 67)
Body mass (g)	3.27 ± 1.02	4.45 ± 1.23
	(1.79 – 4.58; *n* = 8)	(2.65 – 7.86; *n* = 67)
Reproductive investment (RI)	20.26% ± 4.53%	0.29% ± 0.10%
	(13.98% – 26.73% ; *n* = 6)	(0.15% – 0.55% ; *n* = 21)
n eggs per clutch	202 ± 75	-
	(128 – 347; *n* = 6)	
Clutch mass (g)	0.97 ± 0.36	-
	(0.65 – 1.62; *n* = 6)	
Egg/oocyte diameter (mm)	1.96 ± 0.16	-
	(1.69 – 2.41; *n* = 61)	

Table 2. Characteristics of the three sequences of courtship behaviour of *B. ibitiguara* observed in this study.

	Courtship 1	Courtship 2	Courtship 3
Distance between calling male at first sight and nest	About 40 cm	About 150 cm	Unknown
Presence of satellite male	No	Yes	No
Beginning time and duration	20h30; approximately 60 minutes	22h10; approximately 30 minutes	21h; approximately 30 minutes
Number of male slaps towards the female	1	2	2
Female inspection of oviposition site	Yes	Unknown	No
Amplexus	No	Probably; not possible to observe	No
Other observations	Female entered oviposition site twice, remaining out for 5 minutes	At the beginning, female bumped the male, making him jump to a lower leaf, from where he kept calling	-

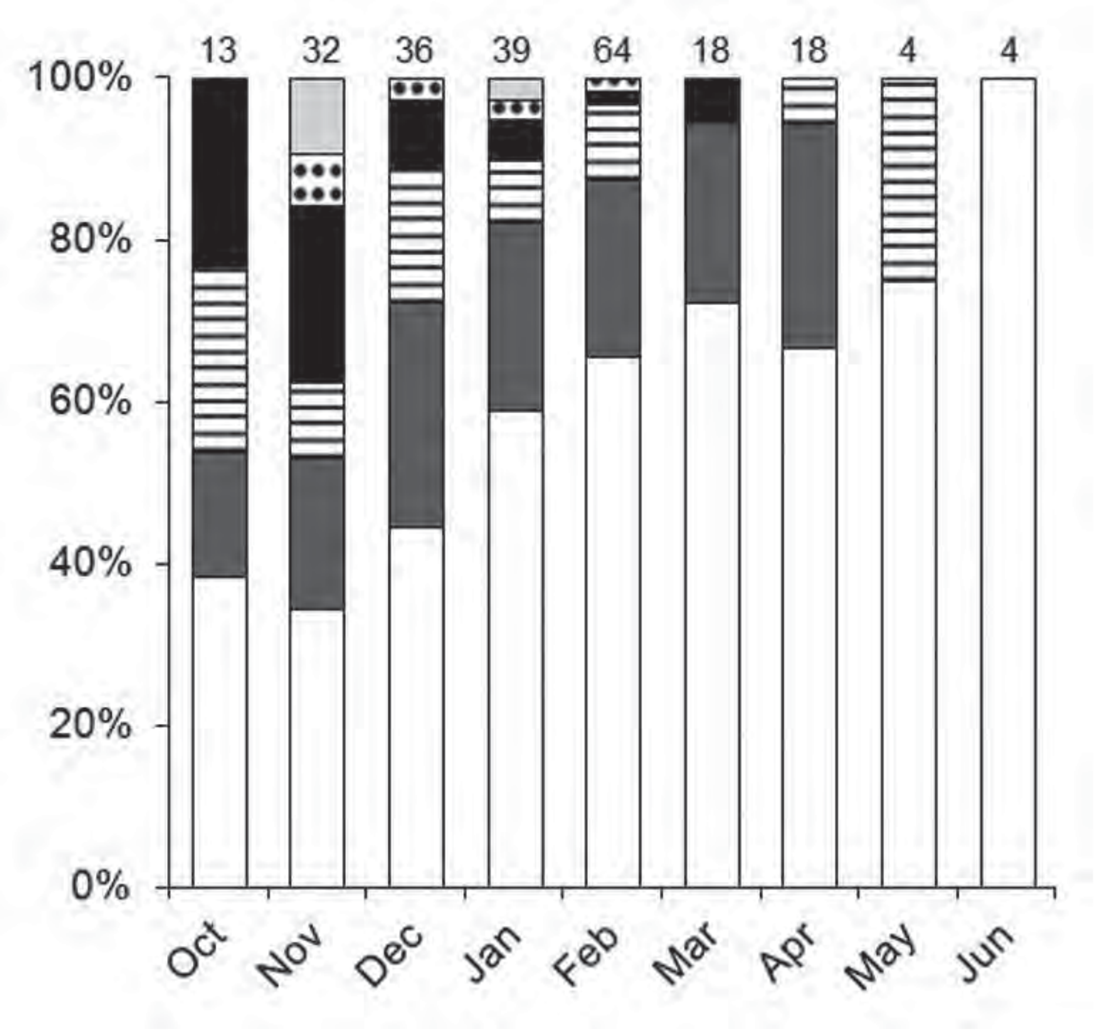

Figure 1. Monthly variation in calling sites of *B. ibitiguara* males during this study (Oct/2010 to Jun/2011). Number of observations per month is above each column. White = leaf; dark gray = trunk; striped = branch; black = hidden; dotted = on the ground; light gray = partially submersed in water.

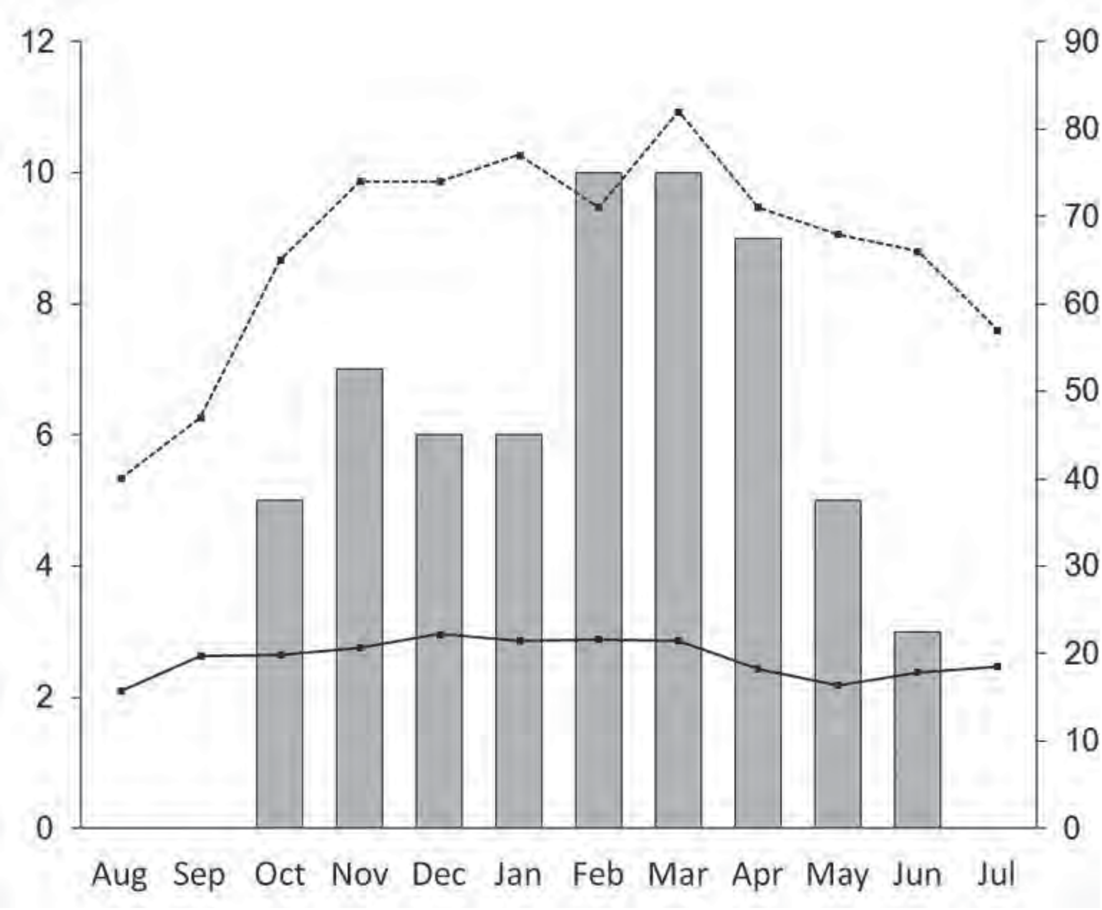

Figure 2. Maximum number of males of *B. ibitiguara* observed calling at each month (left vertical axis: bars), from Aug/2010 through Jul/2011, and variation on abiotic factors. Right vertical axis: solid line = temperature (ºC); dashed line = air humidity (%).

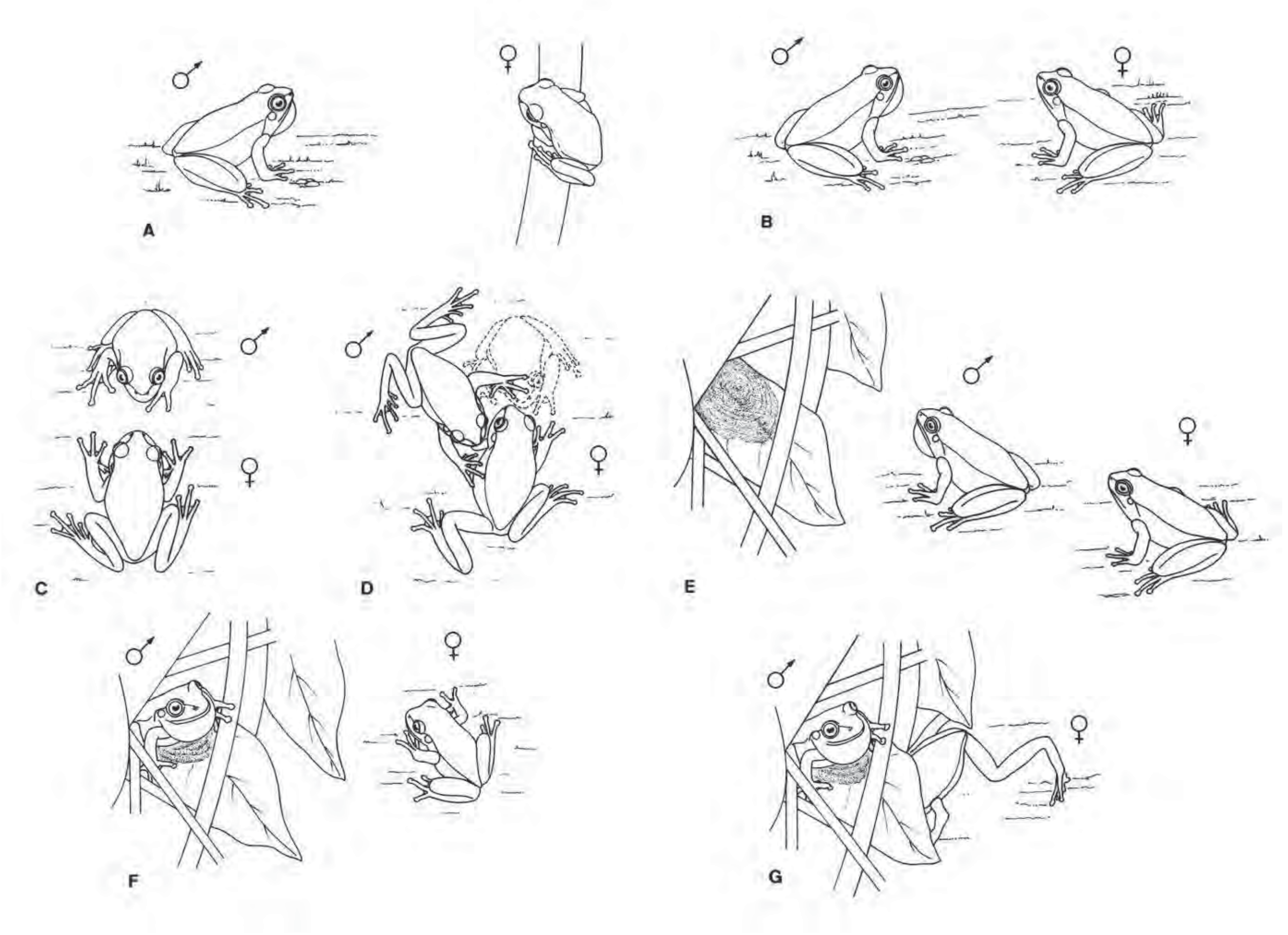

Figure 3. Courtship behaviour in *Bokermannohyla ibitiguara*. (A) ♂ calling and ♀ observing him; (B) ♀ approaches the ♂ gradually; (C) ♂ and ♀ stand face to face, and (D) the ♂ slaps the ♀ at her side, once or twice, with or without walking between them. (E) ♂ starts to approach his nest; (F) the ♂ enters the nest and keep calling to attract the ♀, which (G) enters probably to inspect the nest. During all the process, male called regularly, except for the moments C and D. We observed the male emitting courtship call (not recorded) between moments B and C, when the female approached and faced the male.

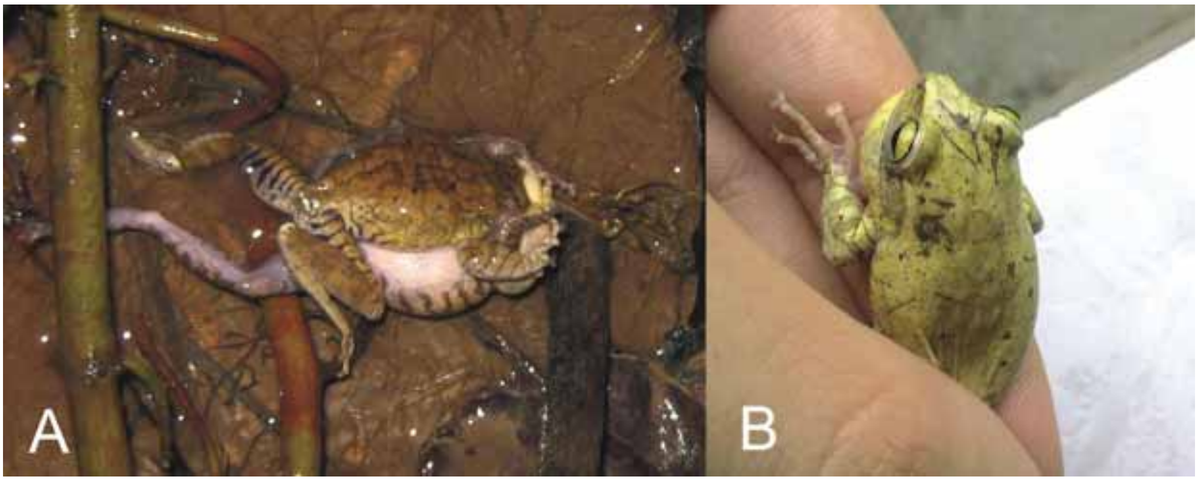

Figure 4. Male-male fight in *B. ibitiguara* and the scars on the back of a male after the fight. A) Note that both males use their prepollical spines to cause injury, mainly in the eyes and tympanum area of the opponent. B) The defeated male shows several scars, mainly on its head.

Chapter 2

CALL PROPERTIES, PLAYBACK EXPERIMENTS, AND FIGHT CALL IN A GLADIATOR TREEFROG ENDEMIC TO THE BRAZILIAN CERRADO

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(In preparation for submission to the Journal of Herpetology)

ABSTRACT

Studies on sexual selection and male territoriality using playback experiments are much more common for temperate frogs, although the tropics harbor the greatest anuran diversity. The gladiator frog, *Bokermannohyla ibitiguara*, is a hylid endemic to the Brazilian Cerrado and has a mixed call, composed by long and short notes. Vocalizations and social contexts of their emissions have never been studied before. Thus, herein, we describe the influence of male size and temperature on call properties, and investigate the social contexts of the different notes of the call through direct observations and playback experiments conducted in the field. Dominant frequency correlated negatively with male body size, whereas long note duration correlated positively, and there was a weak correlation between pulse rate and male body size. Air temperature correlated negatively with long note duration and positively with pulse rate. Playback experiments with males showed that that they did not change the proportion of long to short notes in the presence of an intruder (= playback), but rather they exhibited a graded aggressive call, where short notes become longer, with lower dominant frequency and more pulsated. We interpreted this call as an aggressive call. Our results suggest that dominant frequency and long note duration might be under sexual selection by females, as they could potentially choose larger males with better territories relying on such parameters. On the other hand, pulse rate variation seems to be best explained by temperature. Short notes seem to be the aggressive component of the mixed call, and the aggressive call is a variation of such component. We also describe the fight call emitted by males engaged on a combat, each one emitting one type of note. This is the first description of a fight call for the genus *Bokermannohyla*.

Keywords: *Bokermannohyla ibitiguara*, Hylidae, sexual selection, fight call, calling behavior.

INTRODUCTION

Sexual selection was defined as being the selection force acting differently in each sex or sexual function, due to the different strategies adopted by each sex, favoring reproductive sex-specific adaptations (Darwin, 1859, 1871; Carranza, 2009). It drives the ability of individuals of one sex (generally males) in competing for mates (intra-sexual selection) and also affects reproductive traits evaluated during mate choice (inter-sexual selection) (Krebs & Davies, 1993). In anurans, some of these traits associated with “best” quality males could be body size, reproductive site fidelity, territory quality, and call properties, each one indicating stronger or healthier individuals (see Lips, 2005).

Studies on male calling behavior in anurans is a good way to access sexual selection in this group (e.g. Murphy, 1994; Gerhardt et al., 2000; Lea et al., 2000; Murphy & Gerhardt, 2002; Murphy, 2003; Giasson & Haddad, 2007; Wogel & Pombal-Jr, 2007). Most species emit more than one type of call, used in different social contexts (Duellman & Trueb, 1994). In general, male calls have two main purposes: to attract females (advertisement call) and to repel other males (aggressive call). The advertisement call is considered a pre-zygotic isolating mechanism, preventing hybridization (Haddad et al., 1994). Aggressive call is part of the male’s territorial behavior, which can also include chases and fights (Martins et al., 1998). Despite these benefits, the emission of calls throughout the breeding season may impose costs because of the higher energy expenditure and higher predation risk (Wells, 2007). However, intraspecific call variation has been described, in which males emit different parts of the call in the presence of the predators or the females (e.g. Ryan, 1985). This may represent an adaptive behavior to solve the conflict between predation risks and reproductive success.

In general, the most commonly heard sound emitted by anuran males is the advertisement call (Wells, 1977a), consequently it is also the most studied one (e.g. Ryan & Wilczynski, 1990; Martins & Jim, 2003, 2004; Lourenço et al., 2010). For many species there is a negative correlation between male body size and call dominant frequency (e.g. Sullivan, 1982; Howard & Young, 1998; Giasson, 2003). Additionally, male success in obtaining mates can be related to its larger body size (Lee & Crump, 1981; Lee, 1986; Morris, 1989; Bastos & Haddad, 1996; Bernard, 2007), probably because females are capable of choosing males evaluating their call properties (e.g. Howard & Young, 1998; Lea et al., 2000; Murphy & Gerhardt, 2002; Gerhardt et al., 2007). In short, males' calling behavior is essential to elucidate sexual selection and territoriality in anurans.

Besides direct observations in the field, playback experiments with frogs have been conducted either in the laboratory or in the field (Gerhardt et al. 2000; Murphy & Gerhardt, 2002; Gerhardt et al., 2007; Wogel & Pombal-Jr, 2007). The advantages of lab-based experiments are that one can effectively control for abiotic factors (e.g. temperature, rainfall, wind) and there is little or no noise interference, such as water sound or other conspecific or heterospecific males' vocalizations. However, any degree of captivity is disturbing and unnatural (Warwick, 1990), which certainly interferes in the animals' responses. Furthermore, when the aim of the study is the male vocal responses to playback, lab work is completely ineffective, as males, in general, tend to stop calling when manipulated and kept in captivity (e.g. Taigen & Wells, 1985). In this case, playback experiments in the field are recommended, despite the lack of control for many variables and the difficulty in reaching the animals. (e.g. Bee et al., 2000; Lüddecke, 2002).

Bokermannohyla ibitiguara (Cardoso, 1983) is an endemic hylid frog from the Brazilian Cerrado. Despite being very common in its area of occurrence, its basic biology is poorly known (see Chapter 1). Males have a developed prepollex spine, establish territories along the stream, and engage in physical combats with other males (Chapter 1). According to the original description, the advertisement call is composed by four to six primary long notes, followed by a sequence of about 10 secondary short notes (Cardoso, 1983). When two males call next to each other, commonly one emits only long notes while the other one emits only short notes (Cardoso, 1983). The occurrence of mixed call plus the fact that males can emit different parts of the call depending on the social context, led us to hypothesize that long and short notes should have different social functions. As *B. ibitiguara* males are territorial, we tested, through direct observation and playback experiments in the field, the hypothesis that the first part of the call, with long notes, have the function of female attraction, while the second part, composed by short notes, have the function of aggressive call. We also checked if male body size and air temperature are correlated with acoustic characteristics of the call, and tested whether male response to playback is associated with male body size. Moreover, we describe a fight call recorded during a male-male fight, which had never been previously reported for the genus *Bokermannohyla*.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in private farms in the municipality of Sacramento (19°51’S; 47°26’W), Minas Gerais state, southeastern Brazil. Three streams were sampled: (i) permanent stream 1 (20º16’21.9”S; 47º04’24.5”W; 677 m elevation); (ii) temporary stream (20º13’09.9”S; 47º06’21”W; 880 m elevation), and (iii) permanent stream 2 (20º12’20.1”S; 47º07’53.6”W, 839 m elevation).

The study area is located at about 60 km from the Serra da Canastra National Park.The climate is markedly seasonal, with a hot and rainy summer (October to March) and a dry winter (April to September), with total annual precipitation between 1300-1700 mm (Queirolo and Motta-Junior, 2007). The topography is mountainous, with flat or rounded hills, reaching up to 1500 m elevation, with deep valleys and many streams (Dietz, 1984). The landscape consists of savannas, locally named as Cerrado vegetation, grassland fields at higher elevations, gallery forests in the valleys along streams, and patches of semideciduous forest (Dietz, 1984; Araujo et al., 2009).

Data collection

Observations on male calling behavior occurred throughout the species' calling season (Oct – Jun). Individuals were located by search at breeding sites, guided by male calling activity, and they were observed with headlamps. To reduce light interference on males' behavior, red lights were used, as suggested for different nocturnal animals (e.g. Sazima & Pombal-Jr., 1986; Miranda et al., 2008; Mott & Sparling, 2009). Whenever possible, we avoided to point the light focus directly to the animals (Toledo, 2004).

Vocalization analyses, male body size and temperature

Calls were recorded using a Marantz Professional PMD-660 digital recorder, with unidirectional microphone Sennheiser ME66 (19 cm/s speed), at a 16-bit resolution and 44100 Hz sampling rate. Air temperature and time of recording were registered, as well as social context assumed as relevant. We described the fight call emitted by males during a male-male fight. The analyses were performed using the software Raven Pro 1.3 (Cornell Lab of Ornithology). We mostly used the 512 points option (Fast Fourier Transformation, FFT), brightness = 70, and contrast = 70.

For bioacoustic analyses and interpretations, we used the following terms (adapted from McLister et al., 1995; Martins & Jim, 2003):

- ✓ *Call/vocalization:* sequence of sounds produced and emitted in a temporal pattern; in this species it consisted of more than one type of note (mixed or composite call).
- ✓ *Note:* the total amount of sound energy generated by the male during an air flow cycle, observed through trunk and vocal sac inflation and deflation.
- ✓ *Pulses:* subdivision of the note in smaller temporal units; in this species, pulses are generally distributed in groups of pulses.
- ✓ *Short pulses:* pulses with extremely short durations (< 5 ms), not well-structured and difficult to measure; in *B. ibitiguara*, such pulses are present in the short notes and sometimes at the very beginning of long notes.

To evaluate if parameters of males' advertisement call were influenced by male body size and temperature (e.g. Howard & Young, 1998; Toledo & Haddad, 2005; Giasson & Haddad, 2006), we performed linear regression analyses (Zar, 1999) between these variables and the call parameters. Advertisement call acoustic parameters evaluated were: (1) average dominant frequency (Hz) – about 5 long notes and 5 sequences of short notes of each male, (2) long note duration (s) – 10 long notes for each male, and (3) pulse rate, counting the number of pulses and short pulses in 0.5 s from the onset of the 5 long notes for each male. We chose these parameters because they are known to be preferred by females of anurans (Gerhardt, 1994). Snout-vent length (SVL) of males was measured in the field with an analogic caliper (0.05 mm) and male mass was measured with a dynamometer (0.1 g). Males were individualized through natural marks (spots, scars, etc.; Bradfield, 2004).

Social functions of notes: Direct observations and playback experiments

During our study, we observed any male behavior that could yield conclusions about social functions of both parts of the advertisement call. We also recorded focal male calls in natural conditions, checking if males emitted different parts of the call depending on the number of males attending the chorus and/or whether their calling activity was influenced by the distance of the nearest calling neighbor (DNN). We expected that in larger choruses and at short DNN, emission of short notes – assumed to have a territorial function – would increase. Thus, we used Student's t-test to compare: (1) the average number of short notes emitted by males calling alone or with another male and (2) the average number of short notes emitted by males in groups of 3 to 5 individuals. ANOVA was used to compare the number of short notes emitted in three different groups of DNN: 101 to 500 cm, 501 to 1000 cm and above 1000 cm. We expected that males would emit more short notes in the group of 3 to 5 calling males, and within the group of DNN from 101 to 500 cm.

For the playback construction, we used advertisement calls recorded from October to December 2010, from which we analyzed the following parameters: (1) average dominant frequency (Hz), (2) average duration of long notes (s), (3) average duration of the short notes' sequences (s), (4) average time interval between notes (s), and (5) average number of long notes emitted between short notes' sequences. For parameters 1, 2 and 3, we analyzed about 5 notes and 5 sequences of short notes for each individual (N = 15 males); for number 4, we analyzed 15 intervals for each individual (N = 5 males) and for number 5 we also used the latter individuals (N = 32 sections). After determining mean values for each parameter (table 1), we chose, from our recordings, specific notes that showed the closest values from these averages. We then constructed playbacks of 1 minute each, according to each treatment, using the free software Audacity 1.3 Beta.

Table 1. Mean ± SD of the acoustic parameters of the advertisement call of *Bokermannohyla ibitiguara* analyzed to construct the playbacks. Range and sample size are shown in parentheses.

Parameter	Value
1) Dominant frequency (Hz)	1860 ± 211 (1378 – 2240; N = 145)
2) Duration of long note (s)	0.95 ± 0.21 (0.53 – 1.52; N = 75)
3) Duration of short note's sequence (s)	1.45 ± 0.35 (0.72 – 2.28; N = 70)
4) Time interval between notes (s)	0.64 ± 0.30 (0.31 – 2.34; N = 75)
5) Number of long notes emitted between short notes' sequence	6 ± 4 (1 – 17; N = 32)

We calculated the average sound pressure level (SPL) for 7 calls at a distance of 50 cm (average 82 ± 7 dB) using a Brüel & Kjaer type 2232 SPL meter. For each test, we used a mini system Philips AZ-302S with an audio CR-ROM, containing the constructed playback in original WAV format, adjusting the volume to emit the same SPL at 50 cm, thus simulating at most the natural condition of male's call (e.g. Rosso et al., 2006). Although the ideal condition for playback experiments is to use potent amplifiers and fixed speakers (e.g. Gerhardt et al. 2000; Murphy & Gerhardt, 2002), such thing is difficult to do with this species in the field, because individuals are commonly found perched on the vegetation and inside the gallery forests (Chapter 1). Nonetheless, our speakers were good enough to evaluate males' responses.

The playback experimental procedure was as follows: the sound source was held at about 50 cm from the male, recording male’s call without playback (control treatment; 1 minute). After that, we began the test period, playing the playback for 1 minute and recording male’s response during this minute (Giasson, 2003). This procedure was performed for three different treatments, each one with its own playback track: T1) a call with only long notes, T2) a call with only sequences of short notes, and T3) a mixed call, similar to the common advertisement call, with both long and short notes. For both control and test periods we counted the number of short and long notes emitted by the male. Each individual was tested only once per treatment (N = 41 males); in a few cases, two tests using the same male occurred, but those were separated by at least a period of non-calling activity (e.g. Luna et al. 2010). We managed to do 16 replicas for T1, 16 for T2, and 17 for T3. However, as many males practically stopped calling during the playback – which was assumed as lack of response – we were able to consider 7 replicas for T1, 13 replicas for T2, and 11 replicas for T3.

Differences in the mean number of short and long notes before and during the playback were tested using a t-test for dependent samples (Zar, 1999). We assumed that, if short notes had the function of agressive call, the number of short notes should increase when simulating an intruder, i. e., during the playback; similarly, if long notes had the main function of female attraction, the number of long notes should decrease.

Since we had a number of replicas without male response to the playbacks, we investigated whether males of the non-responding group were significantly smaller than males that responded to the playbacks. Mean SVL and body mass of responding and non-responding males were compared through a Student t-test (Zar, 1999). All data set was checked for normality with Shapiro-Wilk test. Tests were considered statistically significant when $p < 0.05$. All analyses were performed in the program Statistica v.10 (Statsoft).

RESULTS

Advertisement call properties, body size, and temperature

Although the call of *B. ibitiguara* has already been described in the original description of the species (Cardoso, 1983), table 1 shows complementary measures of the advertisement call. Dominant frequency of calls were negatively correlated with both male body mass ($R^2 = 0.65$; $N = 49$; $p < 0.001$; figure 1A) and male SVL ($R^2 = 0.74$; $N=49$; $p < 0.001$; figure 1B). As expected, larger and heavier males showed calls with lower dominant frequency, whereas smaller and lighter males showed calls with higher dominant frequency. Long note duration (s) correlated positively with male body mass ($R^2 = 0.55$; $N = 40$; $p < 0.001$; figure 1C) and at a marginally significant level with male SVL ($R^2 = 0.51$; $N = 40$; $p = 0.052$; figure 1D): larger and heavier males emitted longer notes. Although the correlations between male body mass and SVL versus pulse rate were statistically significant ($p < 0.05$), they were very weak ($R^2 = 0.03$ and $R^2 = 0.006$, respectively). Long note duration (s) correlated negatively with air temperature (ºC) ($R^2 = 0.24$; $N = 40$; $p < 0.001$; figure 2A), whereas pulse rate showed a positive correlation ($R^2 = 0.13$; $N =38$; $p < 0.001$; figure 2B).

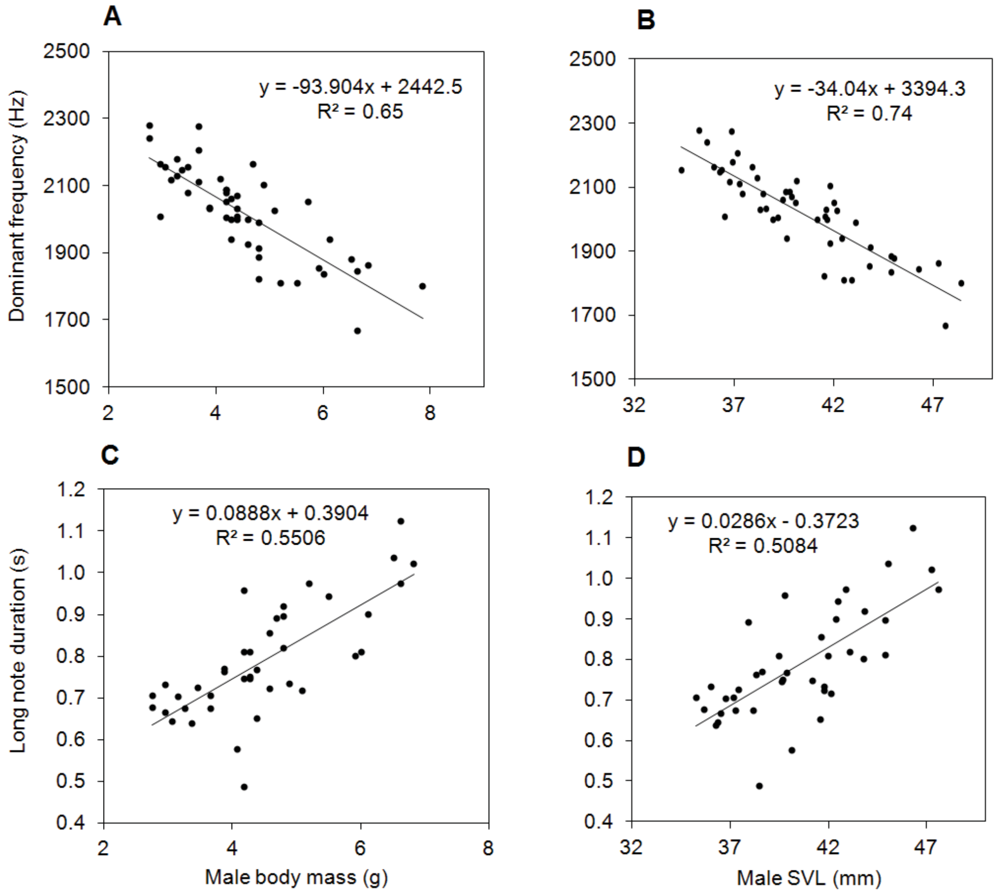

Figure 1. Regression analyses between acoustic parameters of the advertisement call and male body size in *Bokermannohyla ibitiguara*, municipality of Sacramento, Minas Gerais, Brazil.

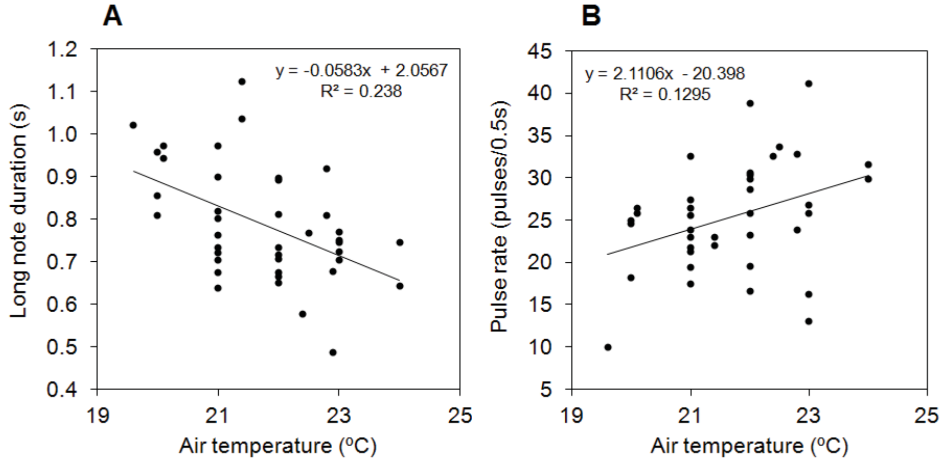

Figure 2. Regression analyses between temporal acoustic parameters of the advertisement call of *Bokermannohyla ibitiguara* and air temperature, municipality of Sacramento, Minas Gerais, Brazil.

Direct observations and natural choruses' analyses

The most common call emitted by *B. ibitiguara* males was the mixed advertisement call, but they could also emit only long notes for a long period of time. During the peaks of calling activity, males never emitted only short notes; this only happened during periods of low calling activity, when males emitted mostly sequences of short notes but at a low repetition rate.

On 21 January, 2010, we observed two males approximately 60 cm of distance between each other, and there was a female nearby, which was noticed by the males. In this case, both males emitted mostly long notes, in antiphony. On 25 January, 2011, we observed a calling male, a satellite male, and a female nearby. In this case, the calling male also emitted mostly long notes.

On 20 November, 2010, we observed two males, with no females nearby. Both males emitted mostly short notes and few long notes. In two occasions, we observed a calling male turning to a satellite male and emitting short notes toward him; after that, the calling male turned around and restarted the emission of mixed calls. There was a female nearby in one of these occasions. On 18 January, 2011, before engaging on a fight, a resident male emitted short notes towards the satellite male. Also, when satellite male started to call, both males emitted short notes, although long notes were still present.

The number of short notes emitted by individuals alone or with another calling male was not different from those emitted by males in groups of 3 to 5 individuals ($t = -0.54$; $p = 0.59$). Also, there was no difference in short notes' emission among any of the groups of DNN ($F = 1.31$, $p = 0.29$).

Playback experiments and aggressive call

During the playback, nearly all males would instantaneously turn around to face the sound source. This happened even in cases of total lack of response (N = 18). Some abandoned the calling site after a while (N = 3), and some interacted not aggressively, but rather in antiphony (N = 5). Males responded better to calls from T1 (long notes) and T3 (mixed notes), but after T2 (only short notes) they were often inhibited (N = 9). This behavior was conspicuous because, even when they remained calling, the call was usually less intense and at a lower repetition rate.

There was no difference in average SVL (mm) between males that responded and males that did not respond to T1 ($t = 1.72$; $p = 0.12$), T2 ($t = -0.09$; $p = 0.93$) and T3 ($t = 0.02$; $p = 0.98$). Considering average male body mass (g), there was no difference for T2 ($t = 0.28$; $p = 0.78$) and T3 ($t = 0.11$; $p = 0.91$), but for T1, responding males were significantly heavier than non-responding ones ($t = 2.22$; $p < 0.05$). Considering the treatments all-together, there was no difference neither in SVL ($t = 0.32$; $p = 0.75$) nor in mass ($t = 0.61$; $p = 0.55$) between responding and non-responding males.

Inside each treatment, there was no difference between the number of short and long notes emitted before and during the playback, except for T3 (normal advertisement call), in which males significantly reduced the emission of long notes during the playback ($t = 3.75$, $p < 0.05$). Moreover, on average, males tended to emit less long notes and more short notes during T1 and T3 playback experiments, whereas during the T2 playback males emitted more long notes and practically maintained the number of short notes (figure 3).

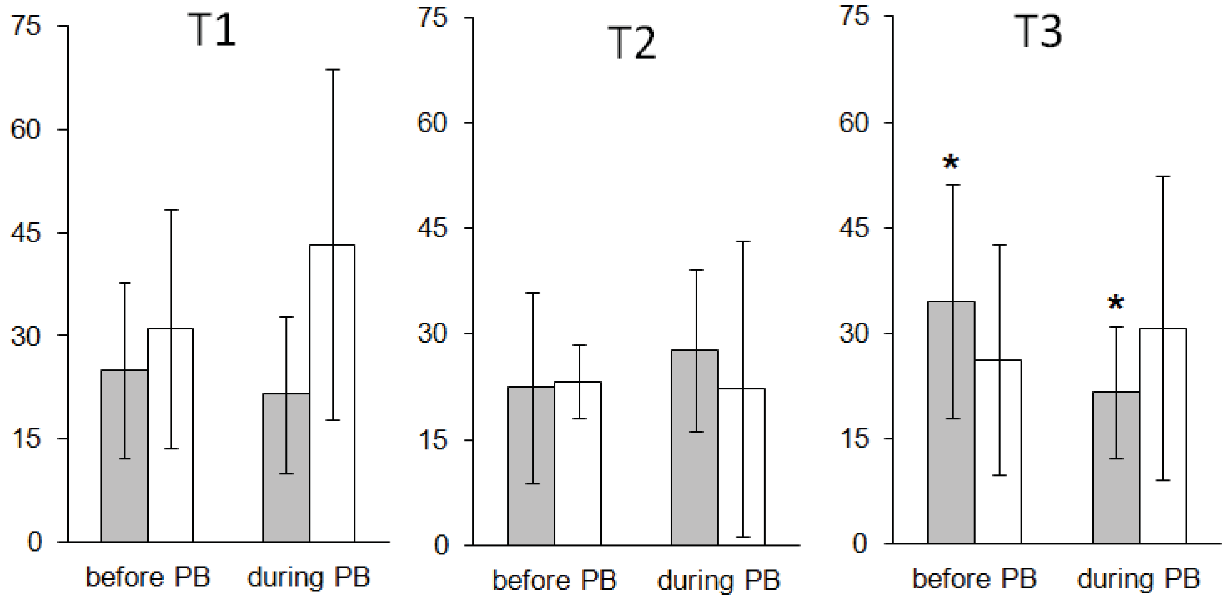

Figure 3. Average number of notes (vertical axis) emitted by males before and during each playback (PB) treatment (T1, T2, T3). The * indicates significant statistic difference ($p < 0.05$). Gray bars are long notes and white bars are short notes (see text for details).

We observed that dominant frequency and note duration of the short notes emitted in response to the playbacks were slightly different from that of the short notes emitted in the common mixed advertisement call before the playback, but the number of short notes per sequence was similar (table 2; figure 4). We propose that the call emitted during the playback is the aggressive call itself, which was heard sometimes in natural conditions without playback. The short notes commonly emitted in the mixed advertisement call could be considered as an aggressive component of the mixed call (see discussion).

Table 2. Mean ± SD of acoustic parameters of the short notes recorded before and during the playback experiments. Results for the t-test for dependent samples are shown (N = 8 individuals, 10 notes before and 10 notes during playbacks). Range in parentheses.

Parameter	Before playback	During playback	t-test
Dominant frequency (Hz)	2026.3 ± 127.9 (1877.7 – 2239.5)	1953.1 ± 115.0 (1826.0 – 2187.8)	t = 6.06; p < 0.001
N notes per sequence	5.1 ± 0.9 (4.0 – 6.8)	4.5 ± 0.9 (3.2 – 6.1)	t = 1.42; p = 0.20
Note duration (s)	0.053 ± 0.008 (0.042 – 0.065)	0.077 ± 0.014 (0.059 – 1.000)	t = -5.79; p < 0.001
Pulse structure	One group of short pulses	Groups of pulses and short pulses	-
Call type	Aggressive component of mixed call	Aggressive call	-

Fight call

On 18 January, 2011, we registered a male-male fight (Chapter 1) and recorded the calls emitted for both males simultaneously. Although the recording presents water noise on the background, we were able to recognize two types of notes, A and B, each one emitted by one of the males (figure 5). Note A is to some extent similar to a common long note, but note B is different from any other notes previously described for this species. Note B shows two well-defined harmonics, and possibly a third harmonic after the middle of the note (figure 5). It was possible to individualize four notes A and four notes B in a segment of 10 seconds, which were used here to measure and describe the call (table 3).

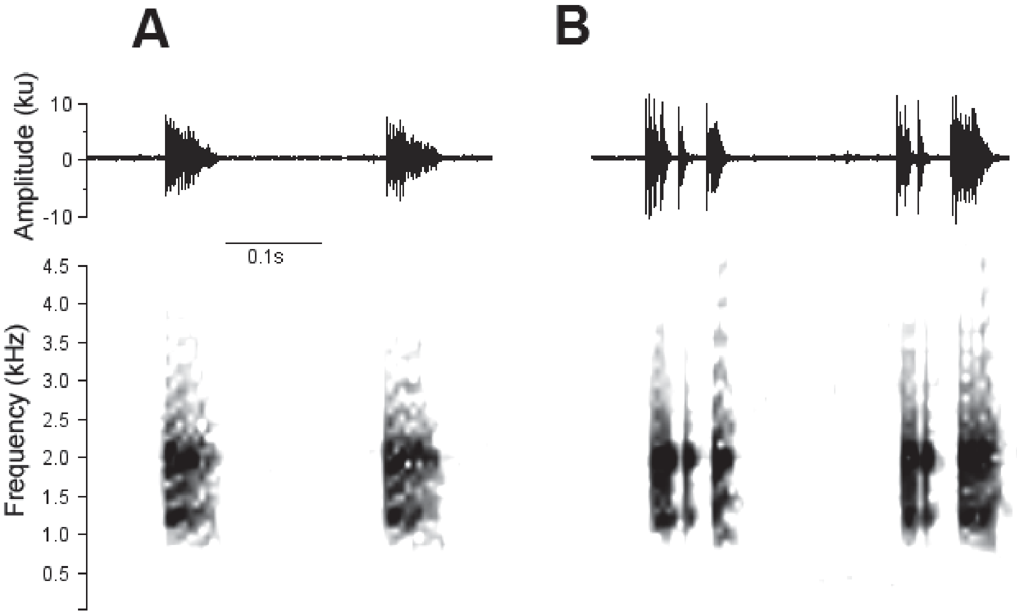

Figure 4. (A) Two short notes commonly emitted by *B. ibitiguara* males in natural conditions and (B) two short aggressive notes emitted during the playback. Oscillogram above and spectrogram below, with the exact same contrast and brightness and FFT=512. In B, notes seem to be more intense and composed by separate groups of pulses and short pulses, while in A, notes are composed by one group of short pulses.

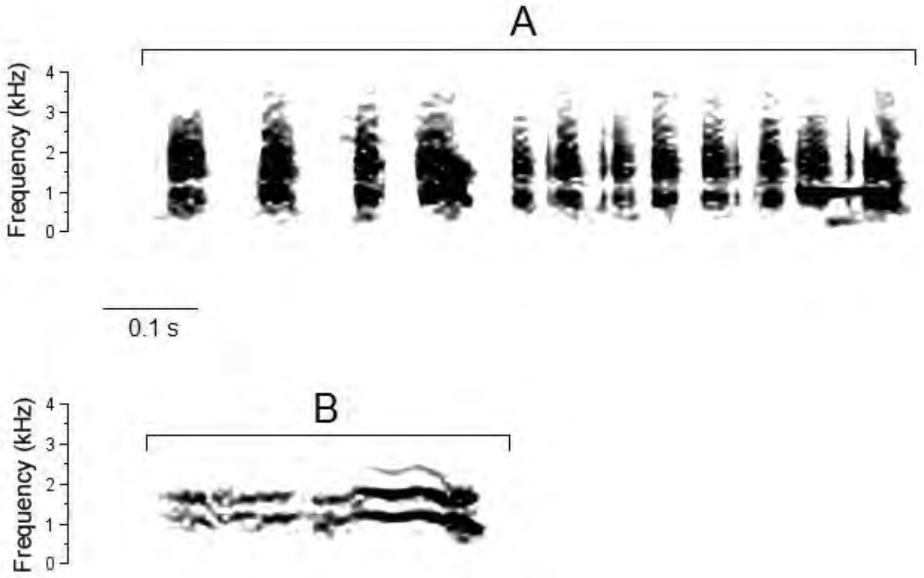

Figure 5. Spectrogram of the fight call notes of *B. ibitiguara* males. Notes A were recorded from one individual and notes B from the other one engaged in the same fight. FFT = 512, air temperature = 21ºC, and water temperature = 22ºC.

Table 3: Mean ± SD of the acoustic parameters of the fight call of *B. ibitiguara* males, recorded in the municipality of Sacramento, Minas Gerais state, southeastern Brazil. Range in parentheses.

	Individual 1 (note A)	Individual 2 (note B)
Note duration (s)	0.85 ± 0.18	0.35 ± 0.03
	(0.70 – 1.11)	(0.32 – 0.38)
Low frequency (Hz)	563.0 ± 80.2	676.8 ± 35.9
	(477.1 – 667.9)	(644.1 – 722.8)
High frequency (Hz)	2767.0 ± 190.2	1825.8 ± 57.4
	(2518.9 – 2957.8)	(1758.1 – 1888.7)
Dominant frequency (Hz)	1248.9 ± 469.2	1313.5 ± 325.1
	(861.3 – 1808.8)	(861.3 – 1636.5)
Note repetition rate (N notes/10s)	6	14
Pulse structure	Groups of pulses and short pulses	No pulses

DISCUSSION

We observed some differences from the original description of the advertisement call of *B. ibitiguara* (Cardoso, 1983), which was based on individuals from Alpinópolis, Minas Gerais state, distant ca. 100 km from our study site. We found that the mean interval between notes was 0.64 s (table 1), while in the original description it was 0.15 s (Cardoso, 1983). Cardoso (1983) stated that the pulses are similar for all the notes, but we observed that the pulses of the short notes are shorter and more difficult to measure than the pulses of the long notes. We also observed that the number of long notes between the sequences of short notes varied from 1 to 17, while Cardoso (1983) described a variation between 4 and 6. Herein, the number of pulses for long notes in a 0.5 s interval varied from 10 to 41 (see figure 2B), while in the original description the variation is from 15 to 18 pulses for the entire note. These differences could be explained by the smaller sample size in the original description, although this information is not clear there (Cardoso, 1983). On the other hand, in the original description the dominant frequency ranged from 1.5 to 2.5 kHz (Cardoso, 1983), which is similar to our results (1.4 – 2.3 kHz). Such slight differences in characteristics of the calls are commonly observed between populations of the same species (e.g. Snyder & Jameson, 1965; Ryan & Rand, 2003). Furthermore, it is important to mention that our results and Cardoso's results are separated by almost 30 years of bioacoustic analyses improvement.

Dominant frequency of *B. ibitiguara* calls was strongly correlated to male body size: as expected, larger and heavier males exhibited calls with lower frequency, whereas smaller and lighter males exhibited calls with higher frequency (e.g. Guimarães & Bastos, 2003; Toledo, 2004; Giasson & Haddad, 2006). Although this correlation is originated because of the difference in laryngeal components of individuals of different sizes (Martin, 1972), it may also indicate that this spectral trait of male call might be under sexual selection. It is possible that females of *B. ibitiguara* select males through the evaluation of the call frequency, as previously reported for other anurans (Ryan, 1980; Ryan et al., 1992; Márquez & Bosch, 1997). Females might have an advantage in choosing larger males, i.e., males with lower dominant frequencies, because since this species is territorial (Chapter 1), larger males are likely to monopolize better-quality territories for oviposition.

Long note duration was also correlated with male body size, a trait that may also be under sexual selection pressure (e.g. Klump & Gerhardt, 1987; Gerhardt, 1994). However, in the present study, this trait was explained by air temperature as well: at higher temperatures, males emitted shorter calls. Thus, the evaluation of this call trait by females will depend on the current air temperature. Pulse rate correlated very weakly with male body size ($R^2 < 0.05$), but more strongly with air temperature ($R^2 = 0.13$), which indicates that this trait might be more influenced by abiotic factors, but other abiotic variables should be investigated. The evolution of anuran call properties and female choice is very complex, given that calls are composed by a set of characters, which evolve at different rates (Cocroft & Ryan, 1995). Studies on male choice by *B. ibitiguara* females are needed to elucidate the role of call properties on the sexual selection mechanism in this species.

Bokermannohyla ibitiguara males exhibit a mixed call and our results indicate that one part of the call may be directed to females (advertisement) and another part directed to males (aggressive). The mixed call is advantageous to males because they can share the functions of female attraction and male aggressiveness, lowering energetic costs related to aggressive calls' emission (Wells, 1988; Lingnau et al., 2004). These calls may be less attractive to females (Wells, 1988; Lingnau et al., 2004), but the aggressive part seems to be important, as male territoriality in frogs is often related to higher reproductive success (e.g. Wells, 1977b; Ursprung et al., 2011). In *B. ibitiguara*, the long notes might constitute the advertisement part, as we observed in two occasions males emitting these notes with a female nearby. However, playback experiments with females are needed to confirm such assumption, as well as female preferences in general, which was not possible to conduct here because receptive females were very hard to find in the field during our study (Chapter 1).

Considering the short notes, we found that males can emit two types: one in natural conditions, and the other when simulating an intruder (e.g. playback). The latter call was sometimes also heard in natural conditions. These calls are similar, since the number of short notes emitted per sequence was not statistically different. However, they exhibited important differences; the call emitted during the playback had lower dominant frequency, was longer and presented groups of structured pulses. Based on these results, we suggest that the short notes of the mixed advertisement call may have territorial function, and the aggressive call itself, emitted when there is an intruder (during the playback), is a variation of the short notes.

If short notes have indeed a territorial function, why did not our playback experiments confirm such assumption? Males did not statistically reduce the emission of long notes, except for the T3 treatment, and did not increase the emission of short aggressive notes during the playbacks. Since we discovered a variation in the short notes, one possible explanation is that the aggressiveness of *B. ibitiguara* aggressive call is not related to the number of short notes emitted (e.g. Giasson & Haddad, 2006), but rather in the modulation of some parameters of the original short note. During the playbacks, short notes became longer, with lower dominant frequency, and exhibited more pulses and short pulses, a very much similar pattern to the African painted reed frog, *Hyperolius marmoratus* (Grafe, 1995). In *Pseudacris crucifer*, a North American hylid, for example, males increased aggressive call duration and the number of aggressive calls in response to increases in stimulus intensity (i.e. synthetic calls; Schwartz, 1989). Although the number of aggressive calls (short notes) in *B. ibitiguara* remained the same, aggressive notes were longer, which clearly shows energy investment of the male. Furthermore, the dominant frequency of the short notes emitted during the playbacks was significantly lower than that of the normal short notes. It is known that males may lower the dominant frequency when interacting vocally with nearby males (Wagner, 1989; Howard & Young, 1998; Bee et al., 2000), increasing its apparent fight ability and avoiding fights (see below). At last, the groups of pulses in the aggressive call were audibly more aggressive than the single group of short pulses from the original short notes of the mixed calls. Thus, it is possible that *B. ibitiguara* might show graded aggressive calls (*sensu* Wells, 1988; Martins et al., 1998), in which males may vary the aggressive and advertisement elements as males gradually approach. In this case, males can vary the aggressive element in terms of dominant frequency, duration, and pulse structure of the short notes when there is an intruder nearby.

It is worth mentioning that our replication with actual male response for each treatment was not high; maybe males do emit more short notes and less long notes in the presence of a real intruder. Since the males would instantaneously turn around to become "face to face" with the "intruder" (the sound source) during the playback, the visual component in territorial interactions might be important for this species. In some frog species, stereotyped behaviors, such as foot flagging, leg kicking, and body raising, are known to have aggressive function towards other males (e.g. Amézquita & Hödl, 2004; Giasson & Haddad, 2006; Preininger et al., 2009). We did not see any aggressive visual signal in this species during our direct observations, but they might have existed when running the playback, which we could not see because we turned off all the lights to avoid any interference. We did not use dummy frogs either, which could elicit different male responses (e.g. Luna et al., 2010). Future studies might focus on such aspects.

Males of *B. ibitiguara* emitted the same number of short notes regardless of the distance of the nearest neighbor and the number of males attending the chorus, which means that short notes are always present. These short notes were mostly the common short notes of the mixed call. As already observed (Chapter 1), males of *B. ibitiguara* tended to occur sparsely along the streams, establishing territories. Since fights are costly (Robertson, 1986; Martins & Haddad, 1988), physical combat is strongly avoided and disputes of males are generally resolved with signalizations (Robertson, 1986), which have been suggested as a way to evaluate each other's ability to fight without actually fighting (Parker, 1974). It seems to be true for *B. ibitiguara*, since we observed male-male fight only once during all field work (Chapter 1), and such fight only ocurred because the satellite male accompanied the entire calling male's courtship sequence and tried to steal the territory (Chapter 1). The conventional territorial signalization for this species is probably the aggressive part of the call (short notes), which might function as a constant signal that the male is willing to defend its territory if necessary.

Since males of *B. ibitiguara* tend to avoid fights, the capacity of *B. ibitiguara* to lower the dominant frequency of their aggressive calls in the presence of an intruder might be explained by the “bluff of size” hypothesis (Wagner, 1992): the signal sent to the opponent is deceptive, and by doing so, males seem larger than they actually are. The assessment of the opponent characteristics related to fight ability, such as male size, is an important part of the decision whether or not to engage on a fight, which fits the concept of the Game Theory (Myerson, 1991), a widespread concept used to explain conflicts between individuals. Also, the strategy of engaging on fights only in the last case can also be seen as an Evolutionary Stable Strategy (ESS; Maynard-Smith & Price, 1973), since *B. ibitiguara* males tend to exhibit this sequence of behaviors to prevent any unnecessary injuries, which might be adaptative. If all males are capable of behaving this way, it is probable that a mutant strategy will not succeed, as assumed by the EES theory.

We expected that smaller males would be more inhibited by the presence of an intruder (playbacks) than larger males, first because those are generally less likely to win male combats (Wells, 1978; Shine, 1989; Katsikaros & Shine 1997) and second because the winner of the fight observed for *B. ibitiguara* was indeed heavier and larger (Chapter 1). Still, body size of *B. ibitiguara* males did not interfere with male’s response to playback, except for body weight in T1. Although one could hyphotesize that males are not able to recognize intruder’s size through its call properties, this is probably not true, as males lowered the dominant frequency of their calls when exposed to an intruder (see discussion above). Moreover, by lowering the dominant frequency of their aggressive calls, even smaller males may increase their apparent fight ability, having the chance to defend their territories without actually fighting. Therefore, the lack of response to playbacks might be related with other factors, such as male condition, degree of male activity, social context, or even by the presence of the observer and artificial reproduction of the calls during the playback experiments.

Fight calls are common during male agonistic interactions in frogs, but this is the first description of this type of call for the genus *Bokermannohyla*. The two types of notes recorded during the fight between *B. ibitiguara* males had lower dominant frequencies than the advertisement call, as observed in *Centrolene lynchi* fight call (Dautel et al., 2011). However, in the latter case, only one of the males emitted the fight call, whereas in *B. ibitiguara* calls were emitted by both males. Other hylids in which both males emitted fight calls during the combat are *Hypsiboas faber* (Martins & Haddad, 1988), *Scinax fuscomarginatus* (Toledo & Haddad, 2005), *Dendropsophus elegans* (Bastos & Haddad, 1995), and *Scinax rizibilis* (Bastos & Haddad, 2002). *Hypsiboas faber* emitted two types of notes, similarly to *B. ibitiguara*, but *S. fuscomarginatus* emitted only one type. Like *B. ibitiguara*, males of *S. fuscomarginatus* and *S. rizibilis* emitted fight call notes at irregular intervals, while in *H. faber* such notes were emitted in an alternated way by the males. Brasileiro (1998) described a fight call for *Physalaemus centralis*, which has a similar structure compared to that of *B. ibitiguara*, with each type of note emitted by one male, one note similar to the advertisement call and the other very different. However, this fight call was not emitted when males were fighting, but rather when they were very close to each other (15 cm; Brasileiro, 1998).

Our study revealed that *B. ibitiguara* vocal repertoire is much more complex and further studies on calling behavior, call properties, and sexual selection are needed. However, this study provides important data on the species’ vocal behavior, which we expect will contribute and encourage future studies trying to understand the mechanisms of mate selection and male-male social interactions in this hylid frog endemic to the Brazilian Cerrado and included in category of Data Deficient (Caramaschi and Eterovick, 2004).

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CONCLUSÕES

- ✓ *Bokermannohyla ibitiguara* é uma espécie de reprodução prolongada; os machos vocalizaram de outubro a junho e o grau de atividade mensal foi positivamente correlacionado com a precipitação, umidade relativa do ar e temperatura do ar.

- ✓ Os seis sítios de vocalização mais utilizados pelos machos, em ordem de preferência, foram: folha, tronco, galho, esconderijo, no chão ou parcialmente submersos na água. Quando empoleirados, machos ficaram a uma altura média de 1,13 ± 0,89 m acima da água ou 0,99 ± 0,74 m acima do solo, neste caso distantes horizontalmente 0,67 ± 0,64 m em relação à água.

- ✓ Esta espécie não apresentou dimorfismo sexual em tamanho quanto ao comprimento rostro-cloacal (CRC), porém fêmeas sem ovos foram significativamente mais leves do que machos, provavelmente pelo fato de o investimento reprodutivo das fêmeas (20,26%) ser bem mais alto do que o dos machos (0,29%).

- ✓ Fêmeas grávidas continham uma média de 202 ± 75 ovócitos, com diâmetro médio de 1,96 ± 0,16. Os ovócitos apresentam polo animal preto e polo vegetativo bege. A partir da massa das gônadas ovadas, a massa da desova foi estimada em 0,97 ± 0,36 g.

- ✓ Em fêmeas grávidas, houve uma correlação positiva entre massa corpórea e massa do ovário. Porém, não houve relação entre CRC da fêmea e número de ovócitos. Isso pode estar relacionado com a variação individual do diâmetro dos ovócitos, apesar de não ter sido observado um *trade-off* entre número de ovócitos e diâmetro dos mesmos.

- ✓ A corte nesta espécie é complexa, apresentando além dos estímulos acústicos, uma ou duas investidas do macho na fêmea, semelhante a um tapa, o que parece ser importante para o processo de condução da fêmea até o sítio de oviposição. Os dois sítios observados foram locais escondidos, e em pelo menos um deles foi observada inspeção da fêmea. O modo reprodutivo da espécie é uma variação do modo reprodutivo 4, característico do gênero, com ovos e estágios larvais iniciais em bacias naturais ou construídas em riachos.

- ✓ O comportamento de macho satélite foi comum nesta espécie, sendo que um mesmo macho pode alternar entre satélite e vocalizador. Machos satélites ficaram a uma distância média de 0,54 ± 0,29 m dos machos vocalizadores. Machos satélites e vocalizadores não apresentaram diferenças quanto ao CRC e massa corporal.

- ✓ Machos vocalizadores tendem a se distribuir esparsamente ao longo dos corpos d’água, possivelmente evitando conflitos. Entretanto, os machos são territoriais, e foi observado um combate físico, cada um investindo seu espinho do pré-pólex, principalmente na região dos olhos e tímpanos do oponente; o canto de briga foi gravado e descrito, com duas notas distintas, cada qual emitida por um indivíduo, de forma temporalmente irregular.

- ✓ O canto da espécie é considerado misto, onde há uma parte direcionada às fêmeas (componente de anúncio) e outra aos machos (componente agressivo). Nossos resultados sugerem que o componente de anúncio são as notas longas, enquanto o agressivo, as notas curtas, emitidas em sequências. Este componente agressivo está sempre presente, independentemente do número de indivíduos no coro ou a distância do vizinho mais próximo.

- ✓ Machos maiores tendem a apresentar cantos com frequências mais baixas e notas mais longas. Em contrapartida, um aumento na temperatura do ar significou uma emissão de notas mais curtas, mas com uma taxa de pulsos maior. A taxa de pulsos apresentou uma correlação muito fraca com o tamanho corpóreo do macho. Esta combinação de fatores demonstra que a evolução da comunicação em *B. ibitiguara* é complexa, dependendo tanto de fatores possivelmente selecionados pela fêmea quanto de fatores abióticos, como a temperatura.

- ✓ Os experimentos de *playback* mostraram que o canto agressivo de *B. ibitiguara* é uma variação do componente agressivo do canto misto, pois é mantido o mesmo número de notas por sequência, mas modificam-se alguns parâmetros acústicos: a frequência dominante decresce, a nota se torna mais longa e mais pulsada, com grupos de pulsos e pulsos curtos.

- ✓ A capacidade de o macho emitir um canto agressivo com uma frequência mais baixa deve estar relacionada com um aumento da habilidade de luta aparente, já que isso indicaria um macho maior e mais provável de vencer combates e manter o território. Isto explica porque a resposta dos machos ao *playback* não dependeu do tamanho dos mesmos, já que através do canto agressivo os machos menores conseguem parecer maiores.

- ✓ Sendo esta espécie endêmica do Cerrado, provavelmente a savana mais ameaçada do mundo, e também fortemente associada com matas de galeria, acreditamos que nossos resultados de aspectos básicos e aplicados da história natural podem ser úteis em ações conservacionistas, além de encorajar futuros estudos com esta e outras espécies brasileiras.

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