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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E CONSERVAÇÃO

PADRÃO DE VARIAÇÃO SAZONAL E MULTINÍVEL NO CANTO DE ANÚNCIO DE *SCINAX FUSCOMARGINATUS* (LUTZ, 1925), BRASIL CENTRAL

Autor: Antonio Olímpio de Souza
Orientador: Dr. Alessandro Ribeiro de Morais

Rio Verde - GO

Fevereiro - 2018

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Sou natural de Salgueiro (Pernambuco), Brasil. Concluí o Ensino Médio na escola estadual José Vitorino de Barros em 2008, Salgueiro (Pernambuco), Brasil. Eu obtive o licenciamento em Biologia pelo Instituto Federal de Educação, Ciência e Tecnologia Goiano (IF Goiano Campus Rio Verde) em 2015, Rio Verde (Goiás), Brasil. Já atuei como professor contratado pelo estado de Pernambuco na escola em que concluí o Ensino Médio. Além disso, já atuei como monitor no Laboratório de Biologia Animal (IF Goiano de Rio Verde) e no setor de logística da International Paper (Rio Verde, Goiás, Brasil) como conferente. Atualmente sou assistente administrativo (condição de servidor público sob estágio probatório) na Universidade de Rio Verde (Rio Verde, Goiás, Brasil).

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LISTA DE SÍMBOLOS, SIGLAS, ABREVIAÇÕES E UNIDADES

CV	Coefficient of variation
CV_a	Coefficient of variation among males
CV_w	Coefficient of variation within males
ADR	Automated Digital Recorder
h	Hour
PCA	Principal Component Analysis
SD	Standard deviation

RESUMO

SOUZA, ANTONIO OLÍMPIO. Instituto Federal Goiano - Campus Rio Verde - GO, Fevereiro de 2018. **Padrão de variação sazonal e multinível no canto de anúncio de *Scinax fuscomarginatus* (Lutz, 1925), Brasil Central**.

Orientador: Dr. Alessandro Ribeiro de Morais.

Coorientadora: Drª. Lia Raquel de Souza Santos.

No primeiro capítulo desta dissertação, nós (incluo coautores) usamos o método de registro automático de cantos (ADR) para registrar a atividade de vocalização dos machos de *S. fuscomarginatus* entre novembro de 2013 e outubro de 2014 no sudoeste do estado de Goiás, Brasil Central. Analisamos um total de 43.2 h e obtivemos a temperatura do ar e precipitação em estação meteorológica. Os registros por ADR mostraram que os episódios de vocalização de machos de *S. fuscomarginatus* ocorrem ao longo da noite, com o pico de vocalização entre 18:00 e 00:00 h. ADR detectou atividade de vocalização em agosto de 2014, porém a estação de chuva local ocorre tipicamente de outubro a março. Assim, argumentamos que ADR é um método potencial para implementar iniciativas de conservação em espécies relacionadas e útil para fornecer informação sobre história natural de espécies de anuros. Além disso, nossos resultados indicam a temperatura como um dos fatores relacionados à atividade de vocalização. No segundo capítulo, registramos o canto de anúncio de 2008 a 2016 em doze municípios do estado de Goiás, Brasil. Nós analisamos 655 cantos de anúncio de 131 machos de *S. fuscomarginatus*. O canto de anúncio apresentou estrutura com uma nota composta por múltiplos pulsos, em que as propriedades acústicas analisadas são predominantemente estereotipadas em nível individual. Apesar disso, as

propriedades acústicas analisadas estão potencialmente envolvidas no processo de discriminação entre machos ($CV_a/CV_w>1$), principalmente frequência mínima, frequência máxima e duração do canto. Neste caso não contradizemos a ideia de que a discriminação individual é mediada por um subconjunto de propriedades acústicas, pois a frequência dominante e a taxa de pulso (não medida no presente estudo) foram relevantes em outras descrições do canto de *S. fuscomarginatus*. Isto indica que um subconjunto de propriedades acústicas está envolvido na discriminação, quando comparamos com prévios estudos. Finalmente, os padrões de variação do canto de anúncio em nível populacional envolvem propriedades temporais e espectrais, sugerindo que espécies com muitas propriedades acústicas estereotipadas tendem a fornecer evidências relevantes sobre a variação acústica em diferentes níveis de organização.

PALAVRAS-CHAVE: Padrão noturno, padrão anual, canto de anúncio, *Scinax fuscomarginatus*, Brasil Central

ABSTRACT

SOUZA, ANTONIO OLÍMPIO. Instituto Federal Goiano - Campus Rio Verde - GO, February 2018. **Seazonal and multilevel pattern of variation in the advertisement call of *Scinax fuscomarginatus* (Lutz, 1925), Central Brazil**.

Adviser: Dr. Alessandro Ribeiro de Morais.

Co-adviser: Dr[a]. Lia Raquel de Souza Santos.

In the first chapter of this dissertation, we (I include coauthors) used the Automated Digital Record (ADR) method to record the vocalization activity of *S. fuscomarginatus* males between November 2013 and October 2014 in the southwest of the Goiás state, Central Brazil. We analyzed a total of 43.2 h, and obtained the air temperature and rainfall in meteorologic station. Registers by ADR showed that calling episodes of *Scinax fuscomarginatus* males occur along the night, with the peak of vocalization between 18:00 and 00:00 h. ADR detected vocalization activity in August 2014, however the local rainy season typically occurs from October to March. Thus, we argue that ADR is a potential method for implementing conservation initiatives in related species and useful for providing information on the natural history of anuran species. In addition, our results indicate temperature as one of the factors related to vocalization activity. In the second chapter, we recorded the advertisement call from 2008 to 2016 in twelve municipalities in the state of Goiás, Brazil. We analyzed 655 advertisement call of 131 males of *S. fuscomarginatus*. The advertisement call exhibited the structure with a note composed by multiple pulses, in which the acoustic properties analyzed are predominantly stereotyped at the individual level. Nevertheless, the analyzed acoustic properties are potentially involved in the discrimination process between males (CV_a /

$CV_w > 1$), mainly minimum frequency, maximum frequency and singing duration. In this case, we do not contradict the idea that individual discrimination is mediated by a subset of acoustic properties, since the dominant frequency and the pulse rate (not measured in the present study) were relevant in other descriptions of *Scinax fuscomarginatus*' advertisement call. Such results indicate that a subset of acoustic properties is involved in discrimination, when we compared with previous studies. Finally, the patterns of advertisement call variation at the population level involve temporal and spectral properties, suggesting that species with many stereotyped acoustic properties tend to provide relevant evidence on acoustic variation at different organization levels.

KEYWORDS: Nightly pattern, annual pattern, advertisement call, *Scinax fuscomarginatus*, Central Brazil.

1. INTRODUÇÃO

Muitos anuros neotropicais apresentam o período reprodutivo em estação de chuva (Aichinger 1987, Bertoluci 1998, Bertoluci & Rodriguez 2002, Colli et al. 2002, Kopp et al. 2010). Em estação de chuva há frequentemente disponibilidade de corpos de água e poças. Estes ambientes são favoráveis à reprodução de anuros, pois o ciclo reprodutivo destes animais está associado tipicamente à presença de água (Wells 2007). Além disso, a umidade tende a favorecer estes animais, uma vez que a pele deles é permeável e, portanto, eles são sensíveis à dessecação (Shoemaker & Nagy 1977, Duellman & Trueb 1986).

Durante estação reprodutiva, espécies de anuros geralmente formam agregados reprodutivos. Em espécies nas quais o período reprodutivo ocorre apenas durante poucas semanas ou dias, o padrão reprodutivo é dito explosivo, enquanto espécies em que o período reprodutivo se estende por mais de um mês o padrão reprodutivo é classificado como prolongado (Wells 1977). A atividade de canto é comumente observada em espécies com padrão reprodutivo prolongado ou em espécies com padrão reprodutivo explosivo em agregados com baixa densidade de indivíduos (Wells 1977). De acordo com Wells & Schwartz (2007) os cantos emitidos podem ser de diferentes tipos, por exemplo: (1) canto de anúncio, o qual anuncia diferentes informações ao receptor, tal como posição e identidade do macho vocalizador; (2) canto de soltura, emitido por fêmeas ou machos não-receptivos quando agarrado por um macho; (3) canto de corte, emitido por fêmeas na presença do canto de machos ou por machos na presença de fêmeas; (4) canto agressivo, presente geralmente em interações agonísticas entre machos; (5) canto de agonia, emitido geralmente quando o anuro é agarrado por um predador. Dentre estes tipos, o canto de anúncio é o mais comum. Machos coespecíficos, fêmeas coespecíficas e predadores podem localizar machos vocalizadores por meio do canto de anúncio (Gerhartd & Huber 2002, Ryan & Kime 2003). Em particular, os estudos sobre comunicação em anuros apontam que fêmeas se aproximam de machos que emitem o canto de anúncio, bem como indicam que tais fêmeas tendem a preferir o canto de indivíduos coespecíficos em vez de indivíduos heteroespecíficos (Gerhartd & Huber 2002). Dada a relevância do canto de anúncio no sistema de comunicação destes animais, o registro deste tipo de canto é usado por pesquisadores para indicar a presença de machos vocalizadores, bem como para indicação de período reprodutivo (Walpole et al. 2012, Klaus & Loughreed 2013, Ximenez & Tozetti 2015).

Os registros do canto de anúncio mostram que os episódios de vocalização ocorrem principalmente durante a noite, mas que existe variação interespecífica na distribuição destes episódios de vocalizão ao longo de 24 horas (Bridges & Dorcas 2000, Gerhardt & Huber 2002). Por exemplo, os episódios de vocalização (i.e., a emissão de cantos de anúncio em um dado intervalo de tempo) de machos de *Hyla gratiosa* ocorrem entre o início e término da noite, enquanto os episódios de vocalização de *Acris gryllus* estendem-se ao longo de 24 horas (Bridges & Dorcas 2000). De acordo com Gerhardt & Huber (2002) a explicação mais parcimoniosa para a atividade de vocalização durante a noite é temperatura e umidade favoráveis, bem como baixa possibilidade de machos vocalizando serem encontrados por predadores que se guiam pela visão.

Condiserando a ênfase no declínio populacional de anuros, os métodos de registro e detecção do canto de anuros ganharam enfoque (Bridges & Dorcas 2000, Solla et al. 2006, Lemckert & Mahony 2008, Schalk & Saenz 2016, Madalozzo et al. 2017). Neste caso, a atividade de vocalização é eficaz para indicar a ocorrência de espécies de anuros (Bridges & Dorcas 2000, Steelman & Dorcas 2010). Por exemplo, o registro dos episódios de vocalização com gravadores automáticos (e.g. Sistema de Registro Automático, ARS) pode identificar períodos em que a presença de machos vocalizando é imprecisa (Bridges & Dorcas 2000). Adicionalmente, os registros extensivos da atividade de vocalização podem determinar o período em que esta atividade é mais provável de ocorrer (Lemckert & Mahony 2008), bem como a relação de variáveis ambientais tais como temperatura do ar, umidade do ar e precipitação com a atividade de vocalização (Yoo & Jang 2012, Walpole et al. 2012).

Por outro lado, o canto de anúncio em anuros também é enfatizado nos estudos sobre o sistema de comunicação entre indivíduos (revisão em Gerhardt 1994, Gerhardt & Huber 2002). Por exemplo, Gerhardt (1991) encontrou padrões de variação em propriedades acústicas do canto de anúncio. Ele mostrou que algumas propriedades como a frequência dominante e taxa de pulso do canto são menos variáveis que outras propriedades como taxa de canto. Esta diferença em variação das propriedades acústicas foi classificada de acordo com o coeficiente de variação (CV) em nível individual como estáticas (CV<5%) e dinâmicas (CV>12%), sendo que ambas as categorias são extremos de um contínuo (Gerhardt 1991). Adicionalmente, a percepção de que propriedades estáticas estão sob influências morfológicas ou que propriedades dinâmicas são influenciadas por condições fisiológicas do indivíduo vocalizador levou Gerhardt (1994) a sugerir que esta diferença de variação está associada a diferentes informações transmitidas no canto de anúncio (veja também Castellano & Giacoma 1998). Por exemplo, em espécies nas quais há correlação negativa entre frequência dominante e tamanho do corpo do macho vocalizador, a frequência é comumente considerada como informação sobre o tamanho deste macho para o receptor do canto (Bee & Gerhardt 2001, Morais et al. 2012). Desta forma, a investigação da variação nas propriedades do canto de anúncio mostra as propriedades importantes para reconhecimento e discriminação entre indivíduos coespecíficos, bem como o modo com que tais propriedades variam em diferentes níveis de organização (Gerhardt 1991, Castellano et al. 2002, Bee et al. 2010, Morais et al. 2012).

Os estudos sobre padrões de variação em propriedades do canto de anúncio também mostram que tais propriedades variam em nível interindividual, intrapopulacional, interpopulacional (Gerhardt 1991, 1994, Castellano & Giacoma 1998, Bee et al. 2010) e em escala geográfica (Castellano et al. 2002, Baraquet et al. 2015, Tessarolo et al. 2016). Algumas das hipóteses para a variação em propriedades acústicas incluem as diferentes forças que atuam no canto de anúncio, tais como seleção sexual exercida por fêmeas em machos vocalizadores (Castellano et al. 1998), variação ambiental (Ryan et al.1990), bem como restrições morfológicas e fisiológicas (Castellano et al. 1998, Castellano et al. 2002) e ação de predadores guiados pelo canto (Tuttle & Ryan 1981). Assim, em espécies de anuros com ampla distribuição, o canto de anúncio pode apresentar drástica variabilidade por causa das grandes variações em tais forças (Ryan & Kime 2003).

Neste contexto, *Scinax fuscomarginatus* (Lutz, 1925) é uma espécie amplamente distribuída na América do Sul. Considerando a classificação taxonômica recente (Brusquetti et al. 2014), esta espécie ocorre do norte da Argentina ao norte, nordeste e noroeste do Brasil. *Scinax fuscomarginatus* é típica de formações abertas ao longo de sua distribuição (Leite et al. 2008, Brusquetti et al. 2014, Toledo & Haddad 2005a, 2005b). Toledo & Haddad (2005a) classificaram o padrão reprodutivo da espécie como prolongado, no qual o pico de atividade de vocalização ocorre na primeira parte da noite. Os machos desta espécie emitem o canto de anúncio empoleirados sobre a vegetação ao redor de corpos de água e a sequência de emissão tende a evitar sobreposição de cantos em agregados reprodutivos (Toledo & Haddad, 2005a). O canto de anúncio da espécie é formado por uma nota com múltiplos pulsos justapostos cujo número está positivamente correlacionado com a duração do canto (Toledo & Haddad 2005a). Em recente estudo, Brusquetti et al. (2014) detectou sobreposição em parâmetros do canto, como frequência dominante e duração do canto, para indivíduos de diferentes localidades. Dada a ampla distribuição desta espécie e o intervalo de condições ao qual ela está submetida, tais observações tornam o canto de anúncio de *S. fuscomarginatus* compatível com a investigação da variação de propriedades acústicas em múltiplos níveis de organização (Castellano et al. 2002). Além disso, os estudos prévios descreveram a ocorrência da atividade de vocalização desde o início da noite até no máximo meia noite (Pombal 1997, Toledo & Haddad 2005a). Assim, o padrão noturno de vocalização dos machos desta espécie ainda é impreciso.

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OBJETIVOS

No contexto descrito acima, nós (incluo coautores) dividimos os objetivos em dois blocos. No primeiro capítulo desta dissertação nossos objetivos foram: (1) descrever a atividade de vocalização dos machos de *S. fuscomarginatus* ao longo da estação reprodutiva, bem como (2) descrever o padrão de vocalização destes machos ao longo da noite e (3) testar se a atividade de vocalização destes machos é influenciada por fatores ambientais, tais como temperatura do ar e precipitação. No segundo capítulo, nossos objetivos foram: (1) testar se as propriedades do canto de anúncio de *S. fuscomarginatus* diferem do nível intraindividual ao interpopulacional e se tais propriedades estão potencialmente envolvidas na discriminação individual; (2) descrever os possíveis padrões de variação nas propriedades do canto de anúncio em nível intraindividual, intrapopulacional e entre populações.

CAPÍTULO I: **Annual and daily pattern of calling activity in males of *Scinax fuscomarginatus* (Lutz, 1925) (Anura; Hylidae) from Southwest of Goiás State, Central Brazil**

(Normas de acordo com a revista North-Western Journal of Zoology)

Abstract

Scinax fuscomarginatus is a wide-range South American species with unclear night calling pattern. Herein we used the adaptation of automated digital recorder (ADR) to describe the calling pattern of *S. fuscomarginatus* males along the night and throughout reproductive season. In addition, we tested whether temperature and rainfall influence calling activity. We registered the calling activity of *S. fuscomarginatus* males between November 2013 and October 2014 at Southwest of Goiás State, Central Brazil. Overall, we analyzed 43.2 h. Only minimum temperature influenced calling activity ($P < 0.03$). *Scinax fuscomarginatus* males exhibited a prolonged breeder pattern in which monthly calling peak occurred from November 2013 to February 2014, and along October 2014. Although the nightly calling peak occurred between sunset and midnight, ADR showed that *S. fuscomarginatus* males vocalize between 1800 and 600 h. ADR also detected the calling males in August 2014, but the local rainy season typically occurs from October to March. We argue that ADR is a potential method to implement conservation initiative in related species, and useful to provide information about natural history of anuran species. We also discuss the role of temperature in calling activity during breeding season.

Keywords: Seasonal pattern, calling activity, automated digital recorder, Central Brazil.

1. Introduction

There are two basic temporal patterns in anuran breeding period: prolonged breeding and explosive breeding (Wells 1977). The former extends for more than one month, whereas the latter occurs during few weeks or days (Wells 1977). These two patterns have implications in terms of mate-locating behavior. The vocal communication is not common in high-density males' aggregation in explosive breeding species, whereas this locating strategy is typical in prolonged breeding species (Wells 1977). The advertisement call is the most typical call type used in vocal communication of prolonged breeding species (Wells 1977; Wells & Schwartz 2007). Conspecific females, conspecific males and predators can use advertisement call to locate calling males (Gerhardt & Huber 2002). Consequently, this call type is important to spatial organization and mate-locating strategy (Wells 1977; Gerhardt & Huber 2002), as well as to indicate the occurrence of calling males to researchers (Bridges & Dorcas 2000; Steelman & Dorcas 2010).

The recordings of advertisement call indicate that calling episodes of most species occur during the night but there is an interspecific variation in distribution of calling episodes along 24 h (Duellman & Trueb 1986; Bridges & Dorcas 2000; Steelman & Dorcas 2010). Based on advertisement call recording, the use of automated recording techniques (e.g. automated recording system, ARS) is effective to indicate the occurrence of anuran species in a given area or along the breeding season (Bridges & Dorcas 2000; Steelman & Dorcas 2010). Additionally, the extensive calling survey by using these techniques can identify periods in which the register of calling males is unclear, as observed in *Rana sphenocephala* (Bridges & Dorcas 2000). Extensive calling survey also determine the period when calling activity is most likely to be heard

(Lemckert & Mahony 2008), and the relationship between environmental variables and variation in calling activity (Walpole et al. 2012; Yoo & Jang 2012).

Year-round investigations of calling activity also show that the presence of calling males is usually associated with temperature and rainfall (Bertoluci & Rodrigues 2002; Prado et al. 2005; Wells 2007); therefore, the reproductive period of some anuran species can be restrict to rainy season (Wells 2007). For anuran species from Cerrado biome, this is particularly true (Colli et al. 2002) and, in this sense, Kopp et al. (2010) identified three temporal patterns: (1) species in which large abundance is registered during extended period at rainy season; (2) species in which large abundance is registered only at rainy season; and (3) species in which large abundance is registered only one month at rainy season.

In this context, *Scinax fuscomarginatus* is a species allocated in the *Scinax ruber* clade and it is typical of open formations of South America, occurring from north of Argentina to Venezuela, including Paraguay, Bolivia and Brazil (Brusquetti et al. 2014). Recently, Brusquetti et al. (2014) considered *Scinax parkeri*, *S. lutzorum*, *S. pusillus* and *S. trilineatus* as synonym of *S. fuscomarginatus*. Therefore, this species is widely distributed in Brazil, occurring in the Amazon, Cerrado and Pantanal biomes (Leite et al. 2008, Brusquetti et al. 2014, Frost 2017).

Previous studies on *S. fuscomarginatus* populations described the occurrence of calling males from start of the sunset to just before midnight or until midnight (Pombal 1997, Toledo & Haddad 2005). In this context, the nighttime period of *Scinax fuscomarginatus* calling males is unclear; therefore, we studied the calling activity of this species in ponds located in the Southwest of Goiás State to describe 1) the calling pattern of the males along a reproductive season; 2) the calling pattern of the males along of the night and 3) to test if calling activity of the males is influenced by environmental factors, such as air temperature and rainfall.

2. Material and Methods

We conducted fieldworks at five ponds in the municipality of Rio Verde (17°47’52” S and 50°55’40” W), southwest of Goiás State, Central Brazil. In these ponds, the Cerrado is the main vegetation form, in which can be observed different Cerrado physiognomies and also a diversity of land uses, such as: pasture and plantations of soybean. In this region the climate is seasonal, with rainy season between October and March. According to Köppen’s climate classification, the local climate is Aw with annual rainfall 1600-1900mm and annual mean air temperature 22-24°C (Alvares et al. 2014).

From November 2013 to October 2014, we registered the calling activity of *Scinax fuscomarginatus* males in the ponds described above. To register the calling activity of this species, we used a technique based on automated digital recorder (ADR) with adaptations (Fig 1), as proposed by Madalozzo et al. (2017), in which we installed a Sony ICD-PX312 (48kbps and MP3 format). We estimated the detection distance of this recorder, as described by Llusia et al. (2011); therefore, for these ponds, the maximum detection distance of this recorder was about 50 m. In each pond, these recorders registered uninterruptly 24 h during three consecutive days per month, totaling 864 h (51,840 minutes) of sample effort. These recorders were installed in tree or shrubs located in the edges of the ponds, where they were positioned about 50 cm above the ground and protected against the rain using a plastic shelter. We used Audacity software version 2.1.3 (Audacity Team 2017) to cut off portion referent to nighttime period (*sensu* Madalozzo et al. 2017) and quantify the number of advertisement call registered in six minutes (10%) of each hour along the night (from 1800 to 0600 h); therefore, we analyzed 43.2 h (2,592 minutes) of recording obtained between November 2013 and October 2014. The mean of advertisement call/minute was calculated based on each 6 minutes analyzed for each hour. Call terminology followed Toledo and Haddad (2005).

For each sampled day, we obtained minimum temperature (°C), maximum temperature (°C) and rainfall (mm) from a meteorological station, located approximately 15 km from the ponds. To evaluate the relationship between environmental factors (independent variables) and calling activity (dependent variable), we performed multiple regression. We tested the assumptions of multiple regression, and we considered $P \leq 0.05$ as criteria of significant result (Zar 2010).

3. Results

Scinax fuscomarginatus males' calling episodes occurred from November 2013 to April 2014 and from August 2014 to October 2014 (Table 1). The monthly calling peaks occurred between November 2013 and February 2014, and along October 2014. We did not observe any calling activity between May 2014 and July 2014. *Scinax fuscomarginatus* males vocalized between 1800 and 0600 h. However, the nightly calling peak occurred from 1800 to 2200 h (Fig. 2). From November 2013 to October 2014, the mean values of rainfall, minimum temperature and maximum temperature were 4.51 mm (0–73 mm), 17.94°C (11.1–20.8 °C) and 30.73°C (24.3–34.6 °C), respectively. The rainfall varied irregularly during study period and did not exhibit significant relation with calling activity (Fig. 3, Table 2). Conversely, only minimum temperature influenced calling activity (R^2_{adj}= 0.34; $F_{3,32}$; P<0.03).

4. Discussion

In this study, we used a sampling device based on automated digital recorder system (Acevedo & Villanueva-Rivera 2006), which allowed us to expand the previous observations about calling activity of *Scinax fuscomarginatus*, since we registered the calling episodes of the males during 12 consecutive months (from November 2013 to October 2014) and also along of the night. This species had seasonal calling activity, since the calling episodes occur along the rainy season. So, as reported in other areas from Cerrado and Pantanal biomes (Prado et al. 2005, Toledo & Haddad 2005, Kopp et al. 2010), our results reinforce that males of *S. fuscomarginatus* exhibited prolonged breeder pattern (*sensu* Wells 1977).

Additionally, the males called from 1800 to 0600 h, with nightly calling peak occurring from 1800 to 2200 h and consequent reduction between 2200 and 0600 h. In contrast to populations from Atlantic Rainforest and Cerrado (e.g., Pombal 1997, Toledo & Haddad 2005, Kopp et al. 2010), *Scinax fuscomarginatus* males called between 1800 and 0600 h. In this sense, Toledo & Haddad (2005) and Kopp et al. (2010) reported the calling activity from just before the sunset to considerable decrease or stopped and from 1900 to 0000 h, respectively. According to Wells (1977), even in a limited geographic area, temporal pattern in a single population of a given species might be different in relation to this species as a whole. Although the clear explanation for the night calling peak at early evening is unknown (Pombal 1997), the males of *Scinax fuscomarginatus* vocalized between 1800 and 0600 h. Therefore, as observed in *Rana sphenocephala* (Bridges & Dorcas 2000), the register of calling activity only at first part of the night would not detect the presence of calling males during some days. For example, we observed calling males during August 2014, but the rainy season at Rio Verde Municipality occurs typically from October to March.

Prolonged breeder pattern is frequently observed in species from *Scinax* genus, in which it coincides with rainy season (Toledo & Haddad 2005, Prado et al. 2005, Kopp et al. 2010). However, our result based on multiple regression analysis shows no significant relation between rainfall and calling activity of *Scinax fuscomarginatus*. Despite no correlation has been found in other study (Pombal 1997), the calling activity might response to a set of seasonal conditions, instead of one single environmental variable (Pombal 1997, Canavero et al. 2008).

Previous studies showed a relation between calling activity and temperature at reproductive season in species from *Scinax* genus (Prado et al. 2005, Toledo & Haddad 2005, Borges & Juliano 2007, Kopp et al. 2010). The body temperature regulation of anurans is usually associated with environmental temperature (Wells 2007), i.e. these animals are ectothermic. Despite the positive relation supports the idea that low temperatures constrain anuran activity (Bertoluci 1998, Bertoluci & Rodrigues 2002), different anuran species response asymmetrically to temperature variation (Bertoluci 1998, Walpole et al. 2012).

Finally, to provide information about natural history of the species can be important to support conservation actions (Hortal et al. 2015); particularly this is true for anuran species, because these animals are seriously threatened (IUCN 2017). In this sense, our results expand the knowledge about calling activity and, consequently, breeding period of *S. fuscomarginatus*. Despite of this species to be classified as Least Concern (MMA 2014, IUCN 2017), the results described in the present study can be useful to implement conservation initiative for species closely related. Additionally, we reinforce that sampling methodology (e.g., use of these automated digital recorders) used in this study could be important to provide information about natural history of anuran species classified as threatened or data deficient, because it has relatively low cost and also allows us to maximize the sample effort in field.

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Table 1. Number of advertisement call per minute emitted by males of *S. fuscomarginatus* along of the night in different months, Rio Verde Municipality, Goiás state, Brazil.

Month	1800 – 1900h	1900 – 2000h	2000 – 2100h	2100 – 2200h	2200 – 2300h	2300 – 0000h	0000 – 0100h	0100 – 0200h	0200 – 0300h	0300 – 0400h	0400 – 0500h	0500 – 0600h
November/2013	16.71	39.37	22.04	13.24	12.26	17.71	16.78	18.07	13.22	16.31	6.73	2.88
December/2013	18.69	35.79	26.97	23.28	13.22	14.68	12.38	11.15	7.28	5.63	2.97	2.51
January/2014	28.38	38.99	22.77	10.05	6.93	7.36	7.06	5.82	3.94	3.82	4.05	1.04
February/2014	15.97	35.09	22.59	7.17	5.66	5.10	6.92	5.68	4.38	3.32	2.78	1.02
March/2014	12.14	7.36	4.63	2.14	1.72	1.53	0.72	1.25	0.41	0.18	0.64	0.94
April/2014	0.06	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
May/2014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
June/2014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
July/2014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
August/2014	8.84	3.03	1.69	0.94	1.14	1.60	1.18	0.78	1.22	0.39	0.52	0.00
September/2014	9.38	4.82	2.81	1.83	2.29	1.59	1.51	1.07	1.09	1.02	0.76	0.00
October/2014	22.59	30.96	17.48	10.78	14.50	16.03	14.82	11.25	9.25	9.53	4.49	0.59

Table 2. Result of multiple regression between environmental factors and calling activity. (R^2_{adj}= 0.34; $F_{3,32}$=5.6; p<0.03). Tmin= minimum temperature. Tmax= Maximum temperature. Significant values are in bold.

Variable	β	SE	t(32)	p-level
Tmin (°C)	**0.497**	0.145	3.41	**0.001**
Tmax (°C)	0.112	0.146	0.76	0.45
Rainfall (mm)	0.229	0.147	1.55	0.129

Figure 1. (A) Study site at municipality of Rio Verde, Goiás state, Brazil. (B) Adaptation of ADR using Sony ICD-PX312.

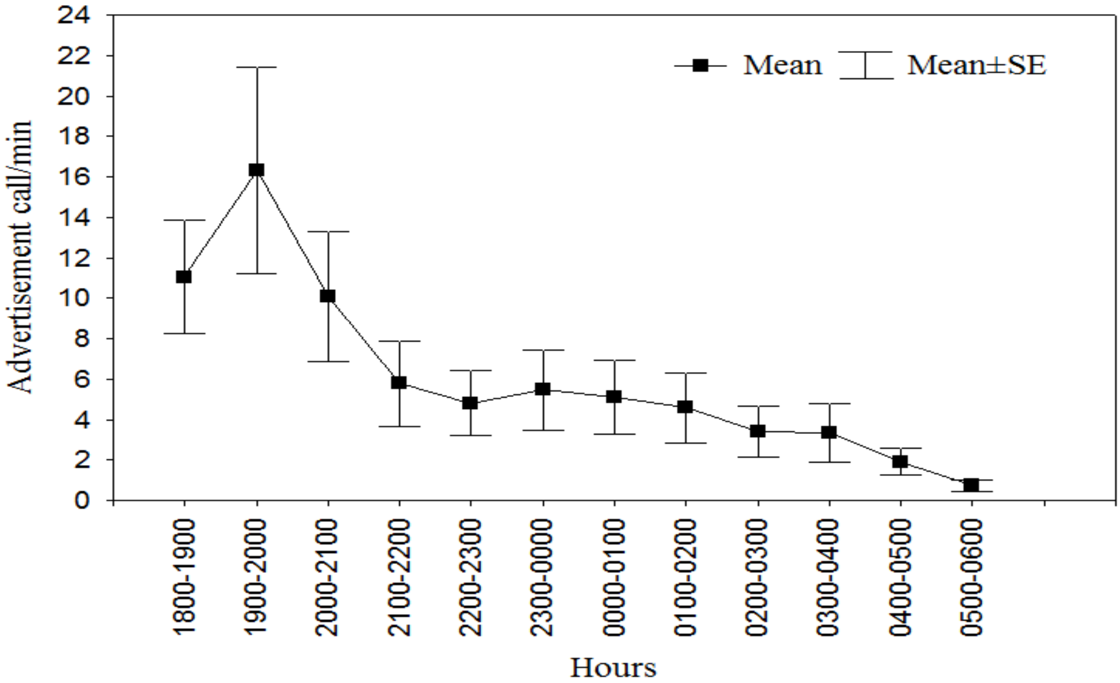

Figure 2. Nightly calling activity in five populations of *S. fuscomarginatus* at Rio Verde municipality, Goiás State, Brazil. Mean values was estimated between 2013 and 2014 considering all sampled ponds.

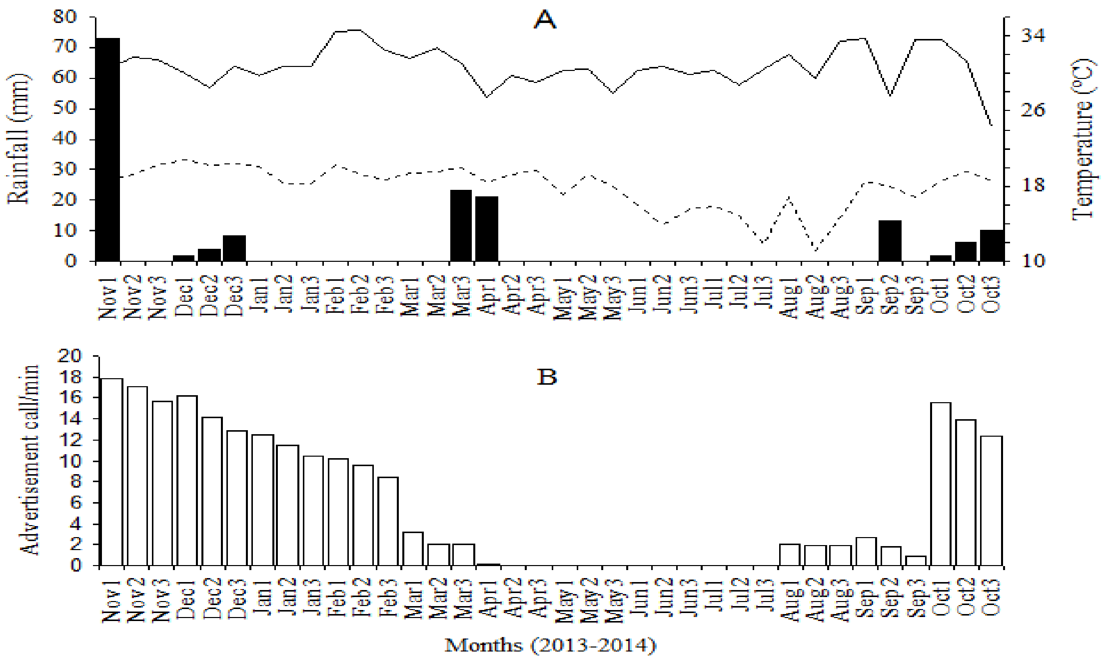

Figure 3. (A) Monthly rainfall (black bars), minimum temperature (dashed line), maximum temperature (solid line) between November 2013 and October 2014, Rio Verde municipality, Goiás State, Brazil. (B) Advertisement call registered per minute (white bars) in the same period, three days per month for each of five ponds.

CAPÍTULO 2: Patterns of variation in the advertisement call of *Scinax fuscomarginatus* (Lutz, 1925) males from Central Brazil: from individual to population

(Normas de acordo com a revista North-Western Journal of Zoology)

Abstract

Advertisement call varies in multiple levels. It is expected that wide-range species tend to exhibit some pattern of variation in this levels because underlying forces influencing call variation. *Scinax fuscomarginatus* is a wide-range species in which exist high degree of overlap in call properties. Therefore, we investigated the advertisement call of this species in the following levels: within males, within population and among populations. We registered the advertisement call during eight breeding season from 2008 to 2016 in twelve municipalities from Goiás state, Central Brazil. In total, we analyzed 655 advertisement call from 131 males. Advertisement call was composed by a multipulsed note, in which all acoustic properties were predominantly stereotyped. Nevertheless, this acoustic properties are potentially involved in individual discrimination, mainly maximum frequency, minimum frequency and call duration ($CV_a/CV_w>1$). This observation agrees with the idea of the subset of call properties involved in individual distinctiveness. Additionally, PCA results indicated that temporal and spectral parameters are important in distinctiveness at population level. Finally, the patterns of advertisement call variation at the population level involve temporal and spectral properties, suggesting that species with many stereotyped acoustic properties tend to provide relevant evidence on acoustic variation at different organization levels.

Keywords: Multilevel acoustic variation, Advertisement call, *Scinax fuscomarginatus*, Central Brazil.

1. Introduction

The advertisement call is the most common acoustic signal emitted by male frogs (Gerhardt 1994, Gerhardt & Huber 2002). By emitting this call type, males can attract females, indicate their position to other males and maintain the distance between them (Wells 1977, Bee & Gerhardt 2001). Advertisement call exhibits patterns of variation in call properties (Gerhardt 1991, Bee et al. 2010, Morais et al. 2012). Based on within male coefficient of variation (CV) patterns, Gerhardt (1991) classified the call properties as static (stereotyped, CV<5%), and dynamic (CV>12%). Studies also showed that call properties are under different forces, such as sexual selection, male body length and temperature constraints (Gerhardt 1994, Castellano & Giacoma 1998). As an example of sexual selection, females exert stabilizing preference on dominant frequency that tend to be more stereotyped than properties under directional preferences, such as call rate (Gerhardt 1991, Bee et al. 2010).

Moreover, stereotyped properties are usually constrained by morphological characters (e.g. body length), whereas dynamic properties tend to be under physiological constraint, such as temperature (Castellano & Giacoma 1998). Therefore, Gerhardt (1994) suggested that these patterns of variation in call properties could represent different biological information in advertisement call. This information is important to recognition of conspecific individuals, as well as to female discrimination between conspecific males (Gerhardt 1994, Gerhardt & Huber 2002).

Despite the importance of recognition and discrimination in frog communication system, variation in call properties is present in different organization level, such as within male, between males, within population and among populations (Gerhardt 1991, Castellano & Giacoma 1998, Castellano et al. 2002, Grenat et al. 2013). For example, European green toad (*Bufo viridis*) showed that pulse rate and dominant frequency increase the coefficient of variation from individual to population level, whereas call duration and intercall duration showed an irregular decreasing pattern in the same direction (Castellano & Giacoma 1998). In *Litoria booroolongencis*, Smith & Hunter (2005) showed that call properties with low variation within male also exhibited low variation between males. These observations indicate the distinct patterns of variation in call properties along organization levels. These patterns, in turn, are related to sexual selection force as well as morphological and physiological constraints (Castellano et al. 2002). Consequently, wide-ranging species should exhibit dramatically variation in call properties because the different environmental condition influencing these constraints throughout the distribution range (Ryan & Kime 2003).

Scinax fuscomarginatus (Lutz, 1925) is a widespread frog species in South America. Considering the recent taxonomic changes (Brusquetti et al. 2014), this species ranges from north of Argentina to north, northwest and northeast of Brazil. *S. fuscomarginatus* is typical from open formations (Brusquetti et al. 2014; Leite et al. 2008; Toledo & Haddad 2005a, 2005b). The males call mainly perched on vegetation surrounding temporary ponds (Toledo and Haddad, 2005a). A multipulsed composes the advertisement call note (Toledo & Haddad 2005a). In recent study, Brusquetti et al. (2014) detected high degree of overlap in call properties variation of *S. fuscomarginatus* males from different localities along the distribution range. These authors also found highly variable dominant frequency, even at intrapopulation level. Therefore, *S. fuscomarginatus* males are potential model to evaluate the possible patterns of variation in call properties at different organization levels.

In the present study, we analyzed the advertisement call of *S. fuscomarginatus* males from twelve populations to: (1) test whether call properties differ within and among populations and whether these properties are potentially involved in individual discrimination; (2) describe the possible patterns of variation in call properties at the following levels: within males, within population and among populations.

2. Material and Methods

We conducted the fieldwork from 2008 to 2016 at twelve municipalities from Goiás state, Central Brazil (Fig 1). The observations were carried out during active choruses, from 1800 to 2400 h, over eight breeding season. In the twelve studied sites, open vegetation formation from Cerrado was dominant. This biome has a seasonal tropical climate in which the rainy season extends from October to March.

For each locality, we registered the advertisement call from 3 to 25 males. To register these calls, we used a Marantz PMD222 digital recorder equipped with a Sennheiser ME66 external microphone positioned approximately 50 cm from calling males. We registered the calls of each male during at least 2 minutes. After each register, we measured snout-vent length (to the nearest 0.05) and mass (to the nearest 0.01 g) of males with caliper and digital scale, respectively. We also measured the air temperature (to the nearest 0.1°C) with digital thermometer.

We recorded the calls with a sample rate of 48 kHz in .wav file types with 16 bits of resolution. We randomly selected five calls per male and analyzed them using Raven pro version 1.4 (Bioacoustics Research Program, 2011). The following call properties were measured: call duration (s), minimum frequency (Hz), maximum frequency (Hz), dominant frequency (Hz), pulse duration (s) and pulse number (pulses/call). The acoustic terminology followed Toledo & Haddad (2005a) and Wells (2007).

To measure the variation of each call property, we calculated the coefficient of variation (CV=SD.100/mean) at two levels: (1) within males (CV_w); and (2) among males of the same population. We classified the call properties as static (CV_w <5%), intermediate (5%< CV_w <12%) and dynamic (CV_w > 12%) according to Gerhardt (1991). In our approach, we used the CV mean of immediately lower level to estimate the CV of immediately higher level (Castellano & Giacoma 1998). In addition, we computed the ratio between coefficient of variation among males of the same population and coefficient of variation within males (CV_a/CV_w). If the acoustic parameter exhibits $CV_a/CV_w > 1$, then its variation among males is higher than within males (Morais et al. 2012). We also used Kruskal-Wallis non-parametric test to evaluate whether acoustic parameters differ among males of the same population. To describe the acoustic variation among population level, we z-transformed (standardized) the values of acoustic properties. We then used Principal Component Analysis (PCA) to analyze the variation of call properties among-population level (Gotelli & Ellison 2012, Jansen et al. 2016). We considered significant values for $p<0.05$ (Zar, 1999).

3. Results

We analyzed 655 advertisement calls of 131 *S. fuscomarginatus* males. Overall, the males exhibited the call structure formed by one multipulsed note (Fig 2) with the mean call duration 0.56±0.11s and the mean pulse number 102.62±18.18 (Table 1). The mean dominant frequency was 3898.88±737.83 Hz, whereas maximum and minimum frequency were 3163.57±489.98 Hz and 4726.33±704.82 Hz, respectively.

Based on CV_w, the call properties were predominantly static, i.e. CV_w<5% (Table2). Despite some call properties showed intermediate values (e.g. dominant frequency and pulse duration), there were not dynamic properties (CV_w>12%). For spectral and temporal parameters, the CV_a was higher than the CV_w in most populations, that is, CV_a/CV_w were higher than 1 in most cases (Table 3).

The results of Kruskal-wallis test were significant to most acoustic parameters within population level (table3). The variation of call parameters overlapped among males of the twelve populations, even when considering CV_w. In the table 4 are described the results of the PCA, in which two first axis explained variation in acoustic properties at population level. PC 1 axis had eigenvalue=2.3 and explained 38.38% of total variance, while PC 2 axis had eigenvalue=1.67 and explained 27.96% of total variance. Spectral and temporal acoustic properties highly loaded PC 1 axis (call duration, maximum frequency) and PC 2 axis (pulse number, call duration).

4. Discussion

Herein, we showed that acoustic properties of *S. fuscomarginatus* males vary significantly in three levels: within-males, among-males and among-populations. The acoustic properties are predominantly stereotyped within-male level. They are also potentially involved in among-male discrimination process. Furthermore, the most important call properties explaining the variation at among-population level were maximum frequency, call duration and pulse number.

Additionally, our results demonstrated that the same characteristics of the advertisement call of *S. fuscomarginatus* may be recognized among populations, since the values of the acoustic properties are within the known range for this species (Toledo & Haddad 2005a, Brusquetti et al. 2014, Jansen et al. 2016). Most call properties were classified as static in the present study, showing high degree of within-male stereotypy. This dominance of static call parameters was also found in *Physalaemus enesefae* (Tárano 2001) and *Allobates femoralis* (Gasser et al. 2009). In a previous description, only minimum frequency and call rate (not measured in the present study) of the advertisement call of *S. fuscomarginatus* males in 16 localities from Brazil and Bolivia were classified as dynamic properties (Jansen et al. 2016). The acoustic properties that we classified as static are within the CV_w range of *S. fuscomarginatus* males reported by Jansen et al. (2016). Therefore, *S. fuscomarginatus* males tend to exhibit overlap in call properties (Brusquetti et al. 2014) and consistent pattern of stereotypy.

Even when most acoustic properties are stereotyped, some properties are potentially involved in individual distinctiveness (Tárano 2001, Gasser et al. 2009). Our results showed that acoustic properties are potentially involved in individual distinctiveness of *S. fuscomarginatus* males, as proposed by other anuran species (Bee & Gerhardt 2001, Gasser et al. 2009, Morais et al. 2012, Gambale et al. 2014). Futhermore, maximum frequency, minimum frequency and call duration tended to exhibit highest values of CV_a/CV_w and H (from Kruskal-Wallis test) within the same population. The acoustic properties with highest variation among males are mainly involved in individual recognition (Bee & Gerhardt 2001, Morais et al. 2012). Hence, maximum frequency, minimum frequency and call duration may be fundamental in *S. fuscomarginatus* male discrimination. Jansen et al. (2016) also suggested the pulse rate (a highly stereotyped acoustic property) as an important property in *Scinax fuscomarginatus* males discrimination. These observations do not contrast with the idea of the subset of significant variable call features being important in among-male discrimination (Bee & Gerhardt 2001, Morais 2012). We also notice that call duration is classified as dynamic property, as observed in other species (e.g. *Hyla versicolor*, Gerhardt 1991, *Scinax constrictus*, Gambale et al. 2014). This latter observation may represent lowest CV_a/CV_w of call duration in relation to CV_a/CV_w of static call properties (Gambale et al. 2014). Finally, Toledo & Haddad (2005a) observed that body length of *S.*

fuscomarginatus males correlates negatively with dominant frequency. The dominant frequency was classified as static property (Jansen et al. 2016, the present study) and thus may be important by conveying information of male body size (Gerhardt & Huber 2002, Wells 2007).

The stereotyped acoustic properties within-male level also vary at among-population level (Gerhardt 1991, Castellano & Giacoma 1998, Castellano et al. 2002, Pröhl et al. 2007, Tessarolo et al. 2016). Properties such as maximum frequency, call duration and pulse number were important call properties by explaining most variation at among-population level. By using adjusted data (for air temperature and body length) and non-adjusted data, Jansen et al. (2016) reported that minimum frequency and call duration represent important loadings on the PCA axis from populations of *S. fuscomarginatus*. Because we did not adjust call variation for air temperature and male body length, we cautiously argue that temporal and spectral call properties explain call variation at among-population level. For instance, pulse number and call duration are useful to discrimination of different population of the gender *Eleutherodactylus* (Padial et al. 2008). In *Hyla arborea*, only stereotyped properties explain significantly the differences at between-population level (Castellano et al. 2002), but, in this case, acoustic data were air temperature- and male body length-corrected. Nevertheless, Jansen et al. (2016) showed that such a correction did not cancel the loadings of temporal acoustic properties in populations of *Scinax fuscomarginatus.* The studies of anuran populations with such a correction has been showed the relevance of static acoustic properties in discrimination of anuran populations (e.g. Castellano et al. 2002, Pröhl et al. 2007, Tessarolo et al. 2016).

Our description of the advertisement call variation in *S. fuscomarginatus* populations indicates potential acoustic properties involved in individual discrimination for this species, as well as a remarkable stereotypy in lowest organization levels. We also showed the stability in the ranges of acoustic properties in the continuum static-dynamic by comparing with populations of *S. fuscomarginatus* from other localities. This stability is fundamental in anuran communication system (Gerhardt 1991; Gerhardt & Huber 2002). Additionally, species with high degree of stereotypy tend to provide hints on the acoustic property variation at different organization level (see Castellano & Giacoma 1998, Castellano et al. 2002, Smith & Hunter 2005 for comparison). On the same hand, playback experiment can provide additional evidence by testing the individual discrimination in *S. fuscomarginatus* males with the acoustic properties cited in our study. Playback experiment can also test whether the predominance of stereotyped acoustic properties is related to female preferences.

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Table 1. Descriptive statistics [Mean±SD (min-max)] of six call properties of *S. fuscomarginatus* males, Goiás, Brazil.

Municipality	Acoustic parameters					
	Call duration (s)	Min. frequency (Hz)	Max. frequency (Hz)	Dom. frequency (Hz)	Pulse duration (s)	Pulse number
Itapaci (80 calls/16 males)	0.56±0.03 (0.49-0.68)	3234.37±67.43 (2812.50-3562.50)	4696.87±173.70 (4312.50-5062.50)	3860.15±279.37 (3375.00-4687.50)	0.0043±0.0003 (0.0035-0.0052)	100.27±7.25 (89.00-119.00)
Jataí (75calls/15males)	0.63±0.04 (0.50-0.74)	3335.00±169.65 (2812.50-3750.00)	4805.00±191.94 (4500.00-5437.50)	4005.00±355.00 (3562.50-5625.00)	0.0042±0.0006 (0.0020-0.0064)	109.23±9.39 (87.00-131.00)
Mineiros (50calls/10males)	0.52±0.04 (0.40-0.64)	3135.00±177.83 (2812.50-3562.50)	4620.00±124.25 (4500.00-4875.00)	3783.75±269.69 (3375.00-4500.00)	0.0047±0.0002 (0.0039-0.0052)	100.50±7.72 (87.00-123.00)
Aporé (55calls/11males)	0.58±0.03 (0.51-0.66)	3306.81±145.96 (3000.00-3562.50)	4950.00±679.49 (4500.00-6937.50)	4251.13±924.24 (3562.50-6937.50)	0.0037±0.0008 (0.0020-0.0050)	113.16±6.99 (99.00-128.00)
Pirenópolis (55calls/11males)	0.66±0.06 (0.55-0.80)	3354.47±205.31 (2842.40-3703.70)	4735.73±121.23 (4478.90-4995.70)	3866.57±250.11 (3531.40-4737.30)	0.0044±0.0005 (0.0029-0.0050)	109.51±11.39(91.00-131.00)
Palmeiras (85calls/17males)	0.62±0.09 (0.42-0.83)	3151.42±212.35 (2584.00-3617.60)	5015.96±739.94 (4134.40-6890.60)	4092.82±1017.98 (3186.90-6632.20)	0.0048±0.0005 (0.0031-0.0069)	110.00±16.94 (76.00-146.00)
Piracanjuba (125calls/25males)	0.57±0.08 (0.31-0.74)	3153.31±526.04 (516.80-3562.50)	4656.43±179.05 (4312.50-4875.00)	3834.13±255.43 (3445.30-4687.50)	0.0042±0.0006 (0.0020-0.0050)	102.82±16.23 (54.00-132.00)
Caiapônia (15calls/3males)	0.53±0.07 (0.42-0.62)	3158.20±259.77 (2756.20-3445.30)	4944.00±217.90 (4565.00-5168.00)	4266.42±455.61 (3703.70-4823.40)	0.0042±0.0002 (0.0037-0.0046)	103.86±13.60 (82.00-122.00)
Catalão (25calls/5males)	0.59±0.04 (0.51-0.67)	2942.27±139.95 (2756.20-3186.90)	4437.56±147.35 (4220.50-4651.20)	3562.46±126.48 (3359.20-3703.70)	0.0040±0.0001 (0.0045-0.0050)	101.00±6.95 (90.00-114.00)
Guapó (50calls/10males)	0.50±0.08 (0.35-0.68)	3271.31±141.88 (3014.60-3531.40)	5006.03±396.67 (4565.00-6546.10)	4024.11±739.51 (3359.20-6632.20)	0.0045±0.0003 (0.0037-0.0051)	95.22±16.00 (67.00-120.00)
Piranhas (20calls/4males)	0.59±0.05 (0.48-0.67)	3027.55±175.14 (2670.10-3273.00)	4659.78±114.87 (4478.90-4823.40)	3845.81±334.51 (3531.40-4651.20)	0.0050±0.0002 (0.0048-0.0050)	96.65±6.97 (84.00-109.00)
Serranópolis (20calls/4males)	0.39±0.03 (0.30-0.43)	3376.40±320.58 (2842.40-3703.70)	5120.59±244.38 (4737.30-5426.40)	3906.11±234.27 (3531.40-4220.50)	0.0037±0.0002 (0.0034-0.0043)	86.30±9.37 (65.00-100.00)
Total	0.57±0.11 (0.04-0.83)	3163.57±489.98 (139.95-3750.00)	4726.33±704.82 (114.87-6937.50)	3898.88±737.83 (126.48-6937.50)	0.0043±0.0008 (0.0001-0.0069)	102.62±18.18 (6.95-146.00)

Table 2. Coefficient of variation within males [CV_w (min-max)] and among males (CV_a) of six call properties of *S. fuscomarginatus* populations from Central Brazil.

Municipality	Acoustic parameters					
	Call duration (s)	Min. frequency (Hz)	Max. frequency (Hz)	Dom. frequency (Hz)	Pulse duration (s)	Pulse number
Itapaci	3.25 (1.09-6.31) 6.38	2.19 (00.00-5.08) 4.68	1.52 (00.00-5.28) 3.30	4.17 (00.00-14.31) 5.30	5.51 (2.15-9.59) 5.33	3.52 (1.65-6.38) 6.53
Jataí	3.93 (1.09-9.69) 6.89	1.71 (0.00-6.02) 4.63	1.24 (0.00-3.37) 3.76	4.33 (0.00-20.24) 6.20	10.37 (1.49-47.68) 9.83	4.37 (1.60-9.83) 7.56
Mineiros	2.74 (1.00-5.45) 8.60	1.68 (0.00-5.88) 5.38	0.59 (0.00-2.24) 2.62	4.52 (0.00-10.14) 4.99	3.56 (1.22-6.66) 4.03	3.22 (1.27-6.81) 7.27
Aporé	3.21 (1.61-7.22) 6.04	1.52 (0.00-3.34) 4.17	7.26 (0.00-20.60) 9.06	11.36 (0.00-29.97) 15.21	10.96 (3.15-19.48) 19.57	3.33 (2.22-7.34) 5.46
Pirenópolis	3.29 (1.49-8.79) 9.49	1.86 (0.00-4.30) 5.99	0.64 (0.00-1.56) 2.54	2.81 (1.20-8.46) 5.77	7.75 (2.15-19.78) 10.15	3.18 (0.97-8.22) 10.25
Palmeiras	4.43 (1.82-10.75) 15.04	2.27 (0.00-7.40) 6.34	5.25 (0.00-21.55) 12.97	9.58 (0.00-32.83) 20.57	4.77 (0.62-25.64) 10.32	7.65 (1.17-21.29) 15.06
Piracanjuba	3.58 (0.36-14.46) 15.42	2.41 (0.00-31.04) 16.82	0.32 (0.00-2.34) 3.85	2.59 (0.00-9.02) 5.78	5.39 (1.45-14.91) 14.04	3.50 (0.55-15.10) 15.65
Caiapônia	3.69 (2.78-4.34) 15.36	3.93 (1.64-8.33) 7.95	2.16 (0.00-5.53) 3.96	3.55 (0.00-9.48) 11.31	2.77 (2.18-3.67) 6.27	4.19 (3.61-4.88) 14.82
Catalão	3.83 (1.85-8.64) 6.79	1.35 (0.00-2.66) 4.96	0.33 (0.00-0.85) 3.59	1.09 (0.00-3.27) 3.56	1.51 (0.88-2.63) 3.36	3.83 (1.72-8.37) 6.08
Guapó	4.18 (2.06-6.51) 17.84	1.53 (0.00-4.56) 4.04	2.70 (0.73-15.16) 6.36	7.66 (1.14-30.02) 13.31	4.36 (1.51-6.85) 5.31	4.42 (3.11-5.68) 17.02
Piranhas	3.96 (2.53-6.26) 9.85	1.24 (0.00-2.21) 6.34	0.71 (0.00-1.00) 2.64	2.08 (0.00-7.10) 8.85	2.88 (1.77-4.28) 5.27	4.23 (2.58-5.96) 6.69
Serranópolis	5.47 (2.67-12.59) 5.57	2.11 (0.00-7.25) 10.14	2.04 (0.00-4.46) 4.62	3.77 (0.99-7.18) 5.01	4.66 (2.56-9.22) 3.40	6.57 (1.62-17.37) 8.76
Total	3.85 (0.36-14.47) 10.27	2.22 (0.00-31.04) 6.78	2.33 (0.00-21.55) 4.94	5.53 (0.00-32.83) 8.82	6.28 (0.62-47.68) 8.07	4.44 (0.55-21.29) 10.09

Table 3. CVinter/CVintra ratio and results of Kruskal-Wallis test for six call properties of *S. fuscomarginatus* males, Goiás, Brazil.

Municipality	Acoustic parameters					
	Call duration (s)	Min. frequency (Hz)	Max. frequency (Hz)	Dom. frequency (Hz)	Pulse duration (s)	Pulse number
Itapaci	1.96 $H_{(15)}$=64.06; p<0.01	2.14 $H_{(15)}$=61.48; p<0.01	2.18 $H_{(15)}$=60.61; p<0.01	1.27 $H_{(15)}$=48.28; p<0.01	0.97 $H_{(15)}$=41.38; p<0.01	1.85 $H_{(15)}$=61.14; p<0.01
Jataí	1.75 $H_{(14)}$=56.72; p<0.01	2.69 $H_{(14)}$=61.41; p<0.01	3.03 $H_{(14)}$=62.43; p=0.01	1.43 $H_{(14)}$=42.78; p<0.01	0.95 $H_{(14)}$=41.82; p<0.01	1.73 $H_{(14)}$=53.49; p<0.01
Mineiros	3.14 $H_{(9)}$=41.19; p<0.01	3.20 $H_{(9)}$=42.18; p=0.01	4.44 $H_{(9)}$=40.83; p<0.01	1.11 $H_{(9)}$=27.37; p<0.01	1.13 $H_{(9)}$=30.69; p<0.01	2.26 $H_{(9)}$=33.24; p<0.01
Aporé	1.88 $H_{(10)}$=41.89; p<0.01	2.75 $H_{(10)}$=45.35; p<0.01	1.25 $H_{(10)}$=39.23; p<0.01	1.34 $H_{(10)}$=38.13; p<0.01	1.78 $H_{(10)}$=41.70; p<0.01	1.64 $H_{(10)}$=37.88; p<0.01
Pirenópolis	2.88 $H_{(10)}$=46.91; p<0.01	3.22 $H_{(10)}$=49.08; p<0.01	3.96 $H_{(10)}$=49.53; p<0.01	2.05 $H_{(10)}$=39.09; p<0.01	1.31 $H_{(10)}$=32.48; p<0.01	3.23 $H_{(10)}$=48.89; p<0.01
Palmeiras	3.39 $H_{(16)}$=76.67; p<0.01	2.79 $H_{(16)}$=70.62; p<0.01	2.47 $H_{(16)}$=57.84; p<0.01	2.15 $H_{(16)}$=50.90; p<0.01	2.16 $H_{(16)}$=68.36; p<0.01	1.97 $H_{(16)}$=77.71; p<0.01
Piracanjuba	4.29 $H_{(24)}$=119.40; p<0.01	6.98 $H_{(24)}$=110.80; p<0.01	11.91 $H_{(24)}$=118.30; p<0.01	2.22 $H_{(24)}$=90.54; p<0.01	2.60 $H_{(24)}$=103.30; p<0.01	4.47 $H_{(24)}$=114.80; p<0.01
Caiapônia	4.16 $H_{(2)}$=11.60; p<0.01	2.02 $H_{(2)}$=7.29; p=0.02	1.83 $H_{(2)}$=5.08; p=0.07	3.18 $H_{(2)}$=10.04; p<0.01	2.26 $H_{(2)}$=9.50; p<0.01	3.53 $H_{(2)}$=12.32; p<0.01
Catalão	1.77 $H_{(4)}$=16.48; p<0.01	3.66 $H_{(4)}$=21.34; p<0.01	10.71 $H_{(4)}$=23.53; p<0.01	3.26 $H_{(4)}$=20.41; p<0.01	2.22 $H_{(4)}$=16.65; p<0.01	1.58 $H_{(4)}$=11.47; p=0.02
Guapó	4.27 $H_{(9)}$=46.10; p<0.01	2.63 $H_{(9)}$=40.13; p<0.01	2.35 $H_{(9)}$=42.67; p<0.01	1.73 $H_{(9)}$=39.80; p<0.01	1.21 $H_{(9)}$=31.56; p<0.01	3.85 $H_{(9)}$=44.02; p<0.01
Piranhas	2.48 $H_{(3)}$=14.71; p<0.01	5.11 $H_{(3)}$=17.70; p<0.01	3.71 $H_{(3)}$=16.93; p<0.01	4.25 $H_{(3)}$=18.51; p<0.01	1.83 $H_{(3)}$=12.10; p<0.01	1.58 $H_{(3)}$=12.96; p<0.01
Serranópolis	1.02 $H_{(3)}$=8.94; p=0.03	4.79 $H_{(3)}$=17.39; p<0.01	2.25 $H_{(3)}$=13.45; p<0.01	1.33 $H_{(3)}$=10.68; p=0.01	0.73 $H_{(3)}$=6.040; p=0.10	1.33 $H_{(3)}$=9.45; p=0.02

Table 4. PCA results for six acoustic properties of *S. fuscomarginatus* populations from Goiás state, Central Brazil. Acoustic data was z-transformed before PCA.

	PC1	PC2
Call duration	0,736	0,632
Min. frequency	-0,250	0,390
Max. frequency	-0,795	0,400
Dominant frequency	-0,682	0,612
Pulse duration	0,472	-0,193
Pulse number	0,617	0,744

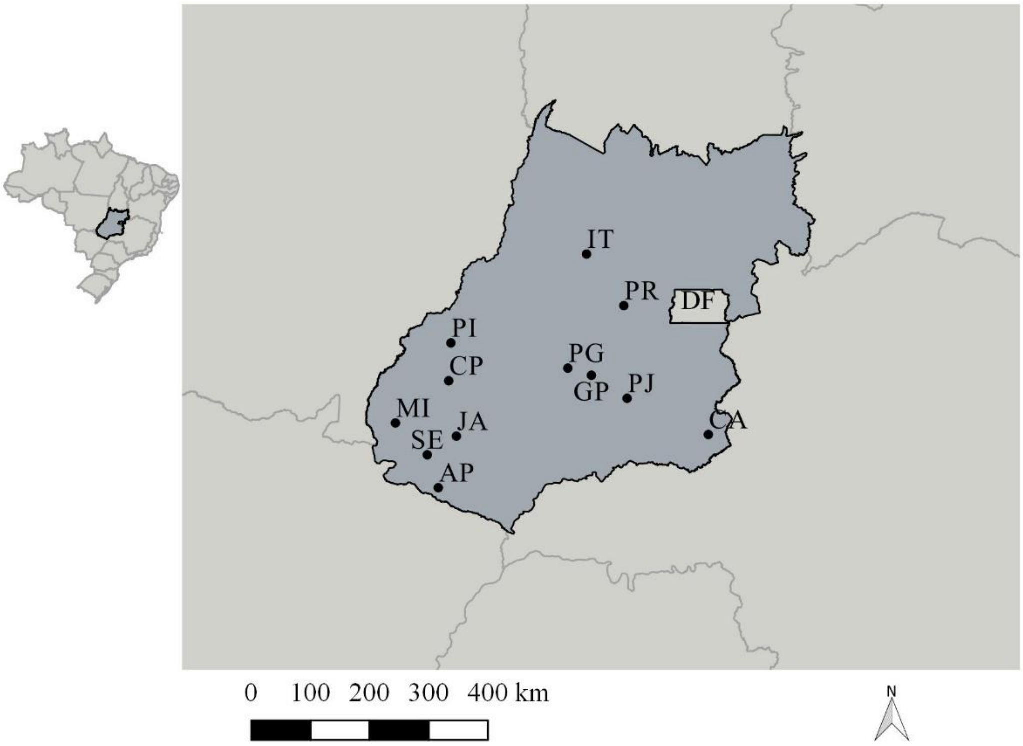

Figure 1. Map of Goiás state (central Brazil) showing the municipalities where the advertisement call of *S. fuscomarginatus* was registered. IT=Itapaci, PR=Pirenópolis, PI=Piranhas, PG=Palmeiras de Goiás, PJ=Piracanjuba, GP=Guapó, CP= Caiapônia, CA=Catalão, MI=Mineiros, JA=Jataí, SE=Serranópolis, AP=Aporé.

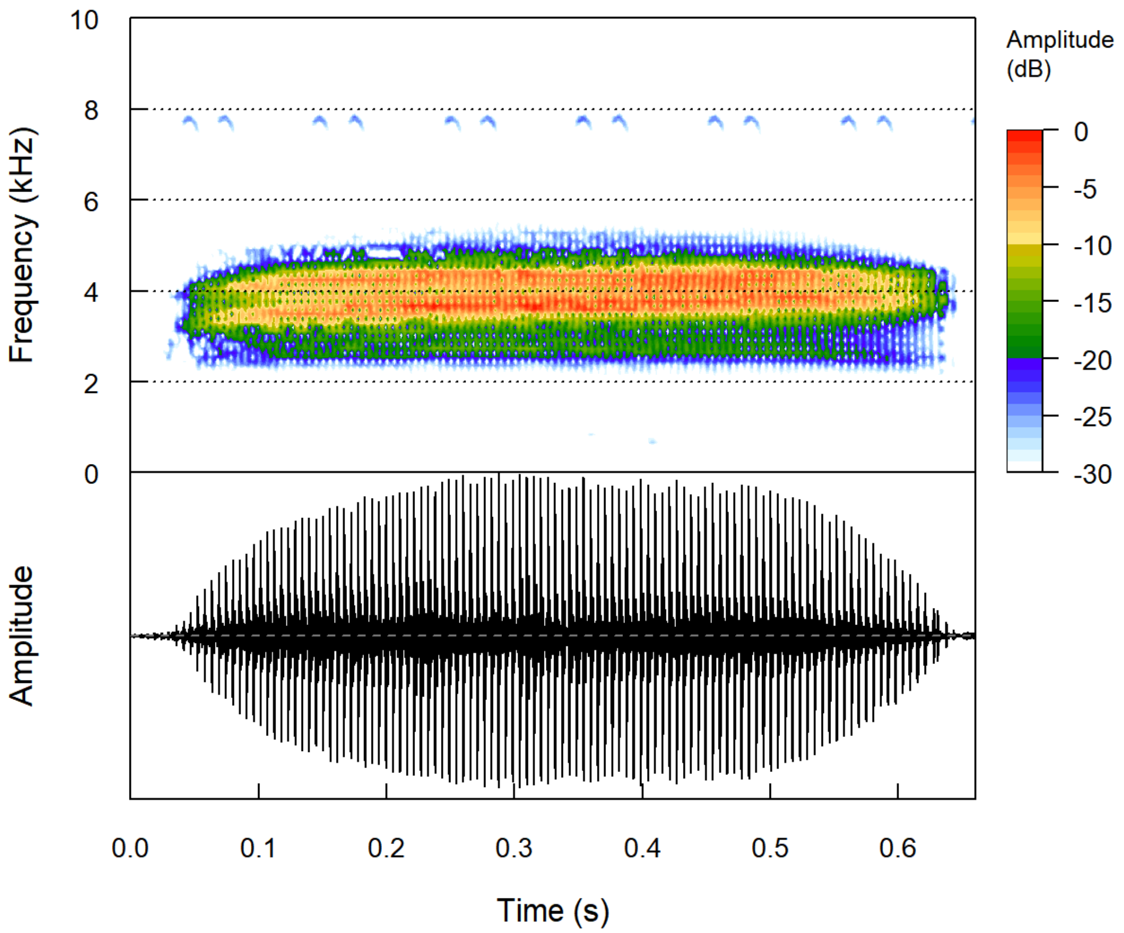

Figure 2. Spectogram (top) and waveform (bottom) showing the multipulsed structure of *S. fucscomarginatus* males' advertisement call from Piracanjuba, Goiás state, Brazil.

CONCLUSÃO GERAL

No primeiro capítulo da presente dissertação, nós (incluo coautores) descrevemos o padrão noturno e anual do canto de anúncio de machos de *S. fuscomarginatus* usando método automático de registro (ADR). Mostramos que apesar de o pico de atividade de vocalização ocorrer na primeira metade da noite, os episódios de vocalização dos machos desta espécie ocorrem ao longo da noite. Além disso, ADR também detectou machos vocalizando em um período anterior (agosto de 2014) ao período de estação de chuva local característico (outubro a março). Assim, argumentamos que ADR é um método potencial para implementar iniciativas de conservação em espécies correlatas, bem como é um método útil para fornecer informações sobre história natural de espécies de anuros. Ainda que a temperatura tenha influenciado a atividade de canto, a temperatura é parte do possível conjunto de preditores que influenciam a atividade de vocalização ao longo do ano na espécie estudada.

No segundo capítulo, descrevemos os padrões de variação do canto de anúncio dos machos de *S. fuscomarginatus* do nível individual ao populacional em doze municípios de Goiás, Brasil. Neste contexto, demonstramos que as propriedades acústicas estudadas são predominantemente estereotipadas em nível individual, bem como evidenciamos que este é um padrão consistente em relação a outros estudos da espécie. Inclusive, sugerimos que potenciais propriedades acústicas como frequência máxima, frequência mínima e duração do canto estão envolvidas na discriminação individual. Neste caso não contradizemos a ideia de que a discriminação individual é mediada por um subconjunto de propriedades acústicas, pois a frequência dominante e a taxa de pulso (não medida no presente estudo) foram relevantes em outras descrições do canto de *S. fuscomarginatus*. Finalmente, os padrões de variação do canto de anúncio em nível populacional envolvem propriedades temporais e espectrais, indicando que espécies com predominância de propriedades acústicas estereotipadas tendem a fornecer evidências relevantes sobre a variação acústica em diferentes níveis de organização.