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VINICIUS GUERRA BATISTA

Vocalizações e atividade acústica de anuros brasileiros: conhecimento atual, novas descrições e variação temporal em múltiplas escalas

Maringá
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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ciências Ambientais.
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Vocalizações e atividade acústica de anuros brasileiros: conhecimento atual, novas descrições e variação temporal em múltiplas escalas

RESUMO

Os sinais acústicos dos anuros são importantes mecanismos de isolamento reprodutivo e, portanto, são caracteres informativos para identificação e delimitação das espécies, sendo utilizados em estudos de comportamento, ecologia, conservação, taxonomia, inventários de biodiversidade e influência de impactos ambientais sobre as espécies. Revisou-se e mensurou-se o conhecimento sobre os cantos de anúncio dos anfíbios anuros do Brasil, contribuindo com esse conhecimento ao fazer a descrição de novos cantos, e também analisou-se a variação temporal da atividade acústica de diferentes espécies e comunidades, avaliando a influência de distintos fatores ambientais e sociais. Identificou-se uma grande lacuna de conhecimento em relação a descrição dos cantos de anúncio. Foram descritos cantos de *Rhinella abei*, *Limnomedusa macroglossa* e *Boana jaguariaivensis*. Adicionalmente, avaliou-se a variação dos parâmetros acústicos em *Physalaemus centralis*. Em relação as três espécies de *Dendropsophus* simpátricas estudadas (*D. cruzi*, *D. minutus* e *D. rubicundulus*), houve partição do espaço acústico, bem como dos sítios de canto. Além disso, os machos de *Dendropsophus* modificaram seus parâmetros acústicos durante a noite, o que pode estar associado a preferências das fêmeas ou para diminuir os custos energéticos para manutenção da atividade de vocalização ao longo da estação reprodutiva. A nível de comunidade, os anuros apresentaram um padrão circadiano, com sobreposição de nicho temporal da atividade acústica durante a noite, embora quando avaliadas separadamente, as espécies apresentaram diferentes períodos de máxima atividade de vocalização. As variáveis climáticas atuam como fatores impulsionadores para determinar a atividade acústica e as espécies podem usar diferentes estratégias para evitar a sobreposição no nicho acústico e facilitar a coexistência.

Palavras-chave: Bioacústica. Canto de anúncio. Coeficiente de variação. Sobreposição de nicho. Partição acústica. Estratégia reprodutiva. Fenologia.

Vocalizations and acoustic activity of Brazilian anurans: current knowledge, new descriptions and temporal variation at multiple scales

ABSTRACT

Acoustic signals of anurans are important mechanisms of reproductive isolation and provide informative characters for identification and delimitation of the species, being used in studies of behavioral, ecology, conservation, taxonomy, biodiversity inventories and influence of environmental impacts on species. In this thesis, we reviewed and measured the knowledge about the advertisement calls of Brazilian anurans, contributing with this knowledge when describing new calls, and also analyzing the temporal variation of the acoustic activity of different species and communities, evaluating the influence of different environmental and social factors. We identified a large knowledge gap regarding call descriptions. Calls of *Rhinella abei*, *Limnomedusa macroglossa* e *Boana jaguariaivensis* were described. Additionally, we evaluated the variation of acoustic parameters in *Physalaemus centralis*. In relation to the three sympatric *Dendropsophus* species studied (*D. cruzi*, *D. minutus* and *D. rubicundulus*), there was partition of the acoustic space, as well as of the calling sites. Besides, the *Dendropsophus* modified their acoustic parameters throughout the night, which may be associated with female preferences or to decrease energetic costs to maintain vocalization activity throughout the reproductive season. At the community level, the anurans presented a circadian pattern, with temporal niche overlap in the acoustic activity during the night, although when evaluated separately the species presented different periods of maximum vocal activity. Climatic variables act as driving factors to determine acoustic activity and the species may use different strategies to avoid overlap in the acoustic niche and facilitate coexistence.

Keywords: Bioacoustics. Advertisement call. Coefficient of variation. Niche overlap. Acoustic partition. Reproductive strategy. Phenology.

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SUMÁRIO

1 INTRODUÇÃO GERAL

A comunicação animal pode ser definida como o processo de transmissão de informação através do uso de morfologia ou comportamento especializado, típico das espécies, entre um emissor e um receptor mediante um código de sinais conhecido, para influenciar o comportamento presente e futuro do indivíduo receptor (BRADBURY; VEHRENCAMP, 2011; GERHARDT; HUBER, 2002). O uso e troca de informação através da comunicação animal proporciona ferramentas importantes para que o investigador possa avaliar a energia despendida durante a transmissão dos sinais (WELLS, 2007), o modo de reprodução (RYAN, 2001), o funcionamento das interações sociais para o estabelecimento e manutenção de padrões espaciais (MORAIS *et al.*, 2012), as estratégias anti-predatórias (TOLEDO, LUIS FELIPE; FERNANDO; HADDAD, 2009), e até mesmo a forma de localização e movimento dos indivíduos (THOMAS; MOSS; VATER, 2004). Os animais podem se comunicar através de sinais químicos, visuais, auditivos ou táteis (BRADBURY; VEHRENCAMP, 2011). Nesta tese vamos focar nos sinais acústicos (vocalizações e atividade acústica) de anfíbios anuros brasileiros.

O estudo dos sinais acústicos se dá através da bioacústica, que pode ser definida como uma ciência multidisciplinar que estuda o som dos animais, sua função, incluindo a sua produção, emissão e recepção, a dispersão através do meio em que é emitido, assim como as técnicas usadas para detecção e reconhecimento (GERHARDT; HUBER, 2002; RYAN, 2001). Portanto, as aplicações da bioacústica dependem, em grande parte, do conhecimento científico das descrições dos cantos das espécies. Embora os estudos sobre os sinais acústicos tenham aumentado nos últimos anos, muitas espécies ainda não possuem o canto descrito, principalmente nas regiões tropicais, que possuem uma alta diversidade (RICKLEFS, 2004). No Brasil, por exemplo, país com a maior diversidade de anfíbios anuros do mundo (SEGALLA *et al.*, 2016), o canto de muitas espécies ainda não é conhecido. Quantificar, analisar e conhecer os estudos sobre os cantos permite não só que possamos melhorar nossa compreensão sobre as espécies, mas também entender os padrões ecológicos de coocorrência das espécies e da formação das comunidades nos diferentes habitats e ecossistemas.

A comunicação acústica surgiu no início da história evolutiva dos anuros, e exerce um papel fundamental no reconhecimento específico e na discriminação dos indivíduos coespecíficos, refletindo também as relações evolutivas entre os táxons (GERHARDT; HUBER, 2002; RYAN, 2001). Para a maioria das espécies ela é a principal forma de comunicação, sendo associada principalmente à seleção sexual (RYAN, 2001). Consequentemente, o comportamento acústico está sujeito a forças seletivas e evolutivas (WELLS, 2007). Uma vez que os cantos são um forte mecanismo de isolamento reprodutivo, eles têm sido usados em estudos de taxonomia, auxiliando na delimitação de espécies crípticas (KÖHLER *et al.*, 2017). Além disso, o conhecimento sobre os sinais acústicos das espécies tem sido particularmente relevante em estudos de fisiologia (POUGH *et al.*, 1992), comportamento (BASTOS *et al.*, 2011), ecologia (OSEEN; WASSERSUG, 2002), conservação (LAIOLO, 2010), mudanças climáticas (LLUSIA *et al.*, 2013), evolução (COCROFT; RYAN, 1995), e inventários de biodiversidade (MADALOZZO *et al.*, 2017).

Além de serem considerados espécie específicos, os cantos podem fornecer informações importantes sobre o indivíduo emissor, como por exemplo o estado físico (condições energéticas) e o tamanho corporal (GERHARDT; HUBER, 2002; MORAIS *et al.*, 2012). Como a atividade acústica requer uma grande quantidade de energia metabólica, os machos devem fazer um balanço entre quando cantar e a quantidade de cantos emitidos, enquanto minimizam os custos energéticos ao longo do período reprodutivo (POUGH *et al.*, 1992; REICHERT, 2010). Dado que as fêmeas podem escolher seus parceiros baseando-se nos sinais acústicos, a estratégia usada pelo macho durante a defesa e estabelecimento do território também é muito importante para determinar o sucesso de acasalamento (GERHARDT; DYSON; TANNER, 1996). Por exemplo, os machos devem se estabelecer nos sítios de canto no começo da noite, defendendo seus territórios e anunciando sua posição para as fêmeas disponíveis (BEVIER, 1997). A atividade acústica dos machos durante a noite é importante para determinar o sucesso reprodutivo (RUNKLE *et al.*, 1994).

Além disso, os cantos podem ter diferentes funções e são usados não só para atrair parceiros de acasalamento, mas também em interações agressivas (por ex. disputas territoriais), e para defesa contra potenciais predadores (TOLEDO, LUIS FELIPE; FERNANDO; HADDAD, 2009). O tipo de canto emitido depende, portanto, do contexto social em que o indivíduo está inserido, e fatores como o tamanho do coro (número de indivíduos) e a distância entre os machos coespecíficos podem determinar o repertório vocal (GAMBALE; BASTOS, 2014; MORAIS *et al.*, 2012). TOLEDO *et al.*, (2015) fizeram uma revisão dos tipos de cantos que existem em anuros, fornecendo uma descrição dos tipos de chamadas e do contexto social em que cada tipo é emitido. Em geral, os cantos de anúncio emitidos pelos machos são as vocalizações mais comumente ouvidas e mais conhecidas para os anuros, sendo também as que possuem maior valor para a taxonomia (KÖHLER *et al.*, 2017). Os cantos de anúncio geralmente são os mais descritos porque (1) são os mais frequentemente emitidos pelos machos na maioria das espécies, sendo, portanto, mais fáceis de gravar, e (2) são emitidos durante a reprodução, transmitindo informações das espécies (KÖHLER *et al.*, 2017). No entanto, a descrição de novos tipos de cantos tem se tornado cada vez mais comum e com isso o repertório vocal das espécies tem se tornado mais conhecido (GAMBALE, PRISCILLA G.; BASTOS, 2014; LEMES *et al.*, 2012; TOLEDO, LUÍS F. *et al.*, 2015).

Uma vez que os anuros são animais ectotérmicos, os sinais acústicos podem variar de acordo com fatores ambientais, como a temperatura (LEMES *et al.*, 2012; MORAIS *et al.*, 2012) e a umidade (GAMBALE, PRISCILLA G.; BASTOS, 2014). Muitos estudos têm demonstrado essa associação, que ocorre principalmente para os parâmetros do canto que são dependentes das contrações musculares, como taxa de repetição e duração do canto (GERHARDT; HUBER, 2002). Outros fatores como as características do habitat também podem influenciar nos parâmetros do canto (RÖHR; JUNCÁ, 2013). Embora muitos desses fatores ambientais possam determinar o padrão de vocalização e atividade acústica de algumas espécies, pode existir uma variação entre os táxons e cada espécie pode responder de forma diferente, de acordo com o ambiente em que está inserida.

A variação dos sinais acústicos em anuros é geralmente estudada em quatro níveis: (1) em indivíduos, (2) entre indivíduos da mesma população, (3) entre populações da mesma espécie (geograficamente separadas) e (4) entre linhagens evolutivas independentes (espécies diferentes) (KÖHLER *et al.*, 2017). Em geral, os padrões de variação dos cantos estão relacionados com a preferência das fêmeas e, portanto, diferentes parâmetros acústicos estão relacionados a diferentes tipos de funções nas interações a níveis intra e interespecíficos (GERHARDT; HUBER, 2002; WELLS, 2007). Existem os parâmetros que são mais variáveis (parâmetros temporais), como por exemplo a duração do canto e a taxa de repetição, que estão mais relacionados a fatores sociais e climáticos, e aqueles mais estáveis (parâmetros espectrais), como a frequência dominante, que estão mais relacionados às características morfológicas (GERHARDT, 1991; GERHARDT; HUBER, 2002).

GERHARDT (1991) propôs que, a nível individual, os parâmetros dos sinais acústicos mais variáveis (dinâmicos) podem transmitir informações sobre a qualidade do parceiro, enquanto aqueles menos variáveis (estáticos ou estereotipados) podem codificar o reconhecimento de espécies e a identidade populacional ou individual. A classificação entre parâmetros dinâmicos e estáticos é baseada nos limiares do coeficiente de variação (CV = SD * 100 / média): os traços estáticos são aqueles com valores CV inferiores a 5%, enquanto que os traços dinâmicos são aqueles tendo valores CV superiores a 10% (GERHARDT, 1991). Os coeficientes de variação podem ser usados a nível intraindividual (*within-individual*; CVw) ou interindividual (*between-individual*; CVb) e muitas vezes a relação entre variação intraindividual e interindividual (CVw / CVb) é usada testar se os indivíduos diferem uns dos outros por seus sinais acústicos (GERHARDT, 1991), tornando-se um método padrão em estudos de variação de cantos em anuros (BEE; REICHERT; TUMULTY, 2016). Distinguir os traços estáticos versus dinâmicos pode ser muito importante em estudos taxonômicos, uma vez que as diferenças (entre indivíduos ou populações) em caracteres estáticos podem ser mais taxonomicamente relevantes do que as diferenças em caracteres dinâmicos (KÖHLER *et al.*, 2017). Em geral, para que as espécies sejam consideradas distintas deve haver uma variação interespecífica suficientemente grande em relação a variação intraespecífica (KÖHLER *et al.*, 2017).

Enquanto que a variação entre indivíduos pode fornecer pistas para escolha de parceiros para acasalamento (seleção sexual), ao nível das espécies, a variação nos sinais acústicos pode ser investigada a partir de uma perspectiva evolutiva em relação a coros (por exemplo, na partição de nicho). Ainda, uma vez que os indivíduos estão expostos a mudanças ambientais e genéticas (WELLS, 2007), os sinais acústicos podem mudar ao longo do tempo (GAMBALE; SIGNORELLI; BASTOS, 2014; SMITH; HUNTER, 2005). Assim, entender o intervalo de variação entre espécies e indivíduos é importante para fornecer informações sobre identidade e especificidade sexual, além de entender os padrões de formação das comunidades através da partição do espaço acústico (DUELLMAN; PYLES, 1983).

Os anfíbios anuros podem cantar durante todas as horas do dia, no entanto a maioria das espécies das zonas tropicais realizam a atividade acústica durante a noite, pois é o período onde a temperatura é mais amena e a umidade é mais alta (WELLS, 2007). Neste período muitas espécies podem vocalizar ao mesmo tempo (DUELLMAN; PYLES, 1983). Uma vez que as fêmeas selecionam os machos mais atrativos através dos parâmetros acústicos, o ruído produzido por coespecíficos ou espécies que coocorrem no mesmo ambiente pode representar uma barreira para a escolha de parceiros (VÉLEZ; SCHWARTZ; BEE, 2013). Nesse contexto, a partilha acústica é considerada um importante fator afetando a permanência das espécies nos sítios reprodutivos e pode, portanto, influenciar na estruturação das comunidades de anuros (DUELLMAN; PYLES, 1983; PROTÁZIO *et al.*, 2015). Assim, as espécies em atividade de vocalização devem emitir mais sinais acústicos em períodos distintos ou apresentar diferenças nos parâmetros do canto, reduzindo ou deixando inexistente a interferência acústica entre elas (PROTÁZIO *et al.*, 2015). O sítio de canto também é um fator importante na segregação espacial das espécies, uma vez que pode ocorrer competição interespecífica por sítios de canto similares (BORZÉE; KIM; JANG, 2016). Portanto, dados sobre as dimensões do nicho espaço-temporal e acústico podem permitir uma visão mais completa das interações entre as espécies e de suas posições e funções nas comunidades.

Embora existam muitos trabalhos sobre as variações dos cantos das espécies e dos fatores que influenciam a emissão dos sinais acústicos, ainda existem muitas perguntas para serem respondidas em relação a influência de espécies congêneres (simpátricas) vocalizando no mesmo sítio reprodutivo, a susceptibilidade das espécies a interferência de sons intra e interespecíficos, e a partição do nicho espaço-temporal e acústico entre as espécies nas comunidades. Além disso, muitas espécies ainda não possuem o repertório vocal descrito e somente recentemente foi publicado um trabalho com a revisão de metodologias padronizadas para a descrição dos cantos dos anuros (ver KÖHLER *et al.*, 2017). Com base nisso, nesta tese apresento uma revisão bibliográfica sobre a descrição dos cantos de anúncio das espécies de anuros com registro de distribuição para o Brasil, avaliando o que foi publicado nas últimas seis décadas para verificar as lacunas de conhecimento e assim propor novos estudos. Para complementar esse conhecimento, fiz a descrição das vocalizações e sítios de canto de quatro espécies de anuros brasileiros, e para algumas (*Physalaemus centralis* e *Boana jaguariaivensis*) foram avaliados os padrões de variação intra e interindividual e a influência de fatores climáticos sobre as vocalizações. Além disso, foram realizados dois estudos em campo, um a nível de espécie e outro a nível de comunidades, a fim de analisar a atividade de vocalização ao longo da noite sob a ótica da sobreposição de nicho espacial, temporal e acústico, determinando também a influência de fatores ambientais sobre essa atividade. Os termos bioacústicos que usamos neste trabalho estão de acordo com o trabalho de Köhler et al. (2017).

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2 THE ADVERTISEMENT CALLS OF BRAZILIAN ANURANS: HISTORICAL REVIEW, CURRENT KNOWLEDGE AND FUTURE DIRECTIONS

RESUMO

Os cantos de anúncio são frequentemente usados como uma informação básica essencial em estudos de comportamento animal, ecologia, conservação, taxonomia ou inventários de biodiversidade. No entanto, a descrição destes sinais acústicos está longe de ser concluída, especialmente em regiões tropicais, e é frequentemente não padronizada ou limitada em informações, restringindo a aplicação da bioacústica na ciência. Neste estudo realizamos uma revisão ciênciométrica dos cantos de anúncio das espécies de anuros do Brasil, o país mais rico em espécies de anuros do mundo, para avaliar a quantidade, padrões e tendências do conhecimento sobre esse importante traço e identificar lacunas para direcionar futuras estratégias de pesquisa. Com base em nossa revisão, 607 estudos foram publicados entre 1960 e 2016, descrevendo o canto de 719 espécies de anuros do Brasil (68,8% de todas as espécies), a uma taxa de publicação de 10,6 descrições por ano. De cada um desses estudos, trinta e uma variáveis (metadados) foram registradas e examinadas com estatísticas descritivas e inferenciais. Apesar de um aumento exponencial ao longo das últimas seis décadas no número de estudos, de cantos descritos e da quantidade de metadados publicados, conforme revelado pelos modelos de regressão, foram identificadas claras lacunas de conhecimento em relação as famílias, aos biomas e as categorias de ameaça. Mais de 55% dessas espécies pertencem às duas famílias mais ricas, Hylidae ou Leptodactylidae. A porcentagem mais baixa de espécies com cantos descritos corresponde aos biomas mais diversos, nomeadamente a Mata Atlântica (65,1%) e a Amazônia (71,5%), e as categorias de ameaça da UICN (56,8%), em relação às categorias menos ameaçadas (74,3%). Além disso, apenas 52,3% das espécies possuem algum de seus cantos depositados nas principais coleções de sons científicos. Nosso estudo evidencia notáveis lacunas de conhecimento nos cantos de anúncio das espécies de anuros brasileiras, enfatizando a necessidade de novos esforços para padronizar e aumentar a descrição dos cantos dos anuros para sua melhor aplicação em estudos de comportamento, ecologia, biogeografia ou taxonomia das espécies.

Palavras-chave: Bioacoustica, Biomas brasileiros, lacuna de Raunkiaer, redes de pesquisa, cienciometria, espécies ameaçadas.

ABSTRACT

Advertisement calls are often used as essential basic information in studies of animal behaviour, ecology, evolution, conservation, taxonomy or biodiversity inventories. Yet the description of this type of acoustic signals is far to be completed, especially in tropical regions, and is frequently non-standardized or limited in information, restricting the application of bioacoustics in science. Here we conducted a scientometric review of the described adverstisement calls of anuran species of Brazil, the world richest territory in anurans, to evaluate the amount, standard and trends of the knowledge on this key life-history trait and to identify gaps and directions for future research strategies. Based on our review, 607 studies have been published between 1960 to 2016 describing the calls of 719 Brazilian anuran species (68.8% of all species), a publication rate of 10.6 descriptions per year. From each of these studies, thirty-one variables were recorded and examined with descriptive and inferential statistics. In spite of an exponential rise over the last six decades in the number of studies, described calls, and quantity of published metadata, as revealed by regression models, clear shortfalls were identified with regard to anuran families, biomes, and categories of threat. More than 55% of these species belong to the two richest families, Hylidae or Leptodactylidae. The lowest percentage of species with described calls corresponds to the most diverse biomes, namely Atlantic Forest (65.1%) and Amazon (71.5%), and to the IUCN categories of threat (56.8%), relative to the less-than-threatened categories (74.3%). Moreover, only 52.3% of the species have some of its calls deposited in the main scientific sound collections. Our findings evidence remarkable knowledge gaps on advertisement calls of Brazilian anuran species, emphasizing the need of further efforts in standardizing and increasing the description of anuran calls for their application in studies of the behaviour, ecology, biogeography or taxonomy of the species.

Key words: Bioacoustic, Brazilian biomes, Raunkiaeran shortfall, research networks, scientometric, threatened species.

2.1 INTRODUCTION

Although the role of natural history in current science is subjected to a long debate [1–3], hypothesis testing, experimental design, observational studies or modelling are strongly based on information of the general biology of living beings [4–6]. Basic knowledge about the species traits, such as those involved in behaviour, ecology or distribution, are key to assist multiple research disciplines. The lack of knowledge on the traits ecologically relevant for the species is known as Raunkiaeran shortfall [7,8]. This shortfall can be related to the ecological functions played by each trait, to the trait variation both within and between species, and to the interaction among traits acting together to perform specific ecosystem functions [8,9]. This lack is further aggravated by the gap of knowledge about the taxonomic diversity of species and the accelerated rate of habitat loss and species extinction [10–12].

In adult anuran amphibians, acoustic signals are the predominant form of communication of almost all species, being a key life-history trait mainly associated with sexual selection and reproduction [13,14]. Frog calls may have different functions and are used to attract mating patterns, in disputes over territory and in other aggressive interactions, and thus acting in social organization [15,16]. Emerging early in the evolutionary history of Anura and by their crucial role in the recognition and discrimination of conspecific individuals, the vocalizations can reflect evolutionary relations among taxa [13]. Moreover, acoustic signals are subject to changes through the influence of biotic and abiotic factors [17], resulting in inter-individual and inter-populational differences [18,19].

Thereby, bioacoustic information has been particularly relevant in studies of behaviour [17], ecology [20], evolution [21,22], conservation [23], thermal biology in light of climate change [24], biodiversity inventory (passive acoustic monitoring; [25]), and becoming a suitable tool for studies on taxonomy (integrative taxonomy; see [14,26]). Since call is a particularly powerful premating isolation mechanism, it has been used as an undisputed means for species delimitation [14]. Comparative bioacoustical analyses have also resulted in the discovery of many morphologically cryptic anuran species [26–28].

The advertisement call can also be considered as a crucial functional trait of most anuran amphibians. Functional traits are any phenotypic attribute that affects a species' individual fitness and population dynamics and/or their influence on other organisms and ecosystem functions [8,29]. Besides the advertisement call being an honest indication of position, body size, body temperature or energetic condition of the calling male [13], it incorporates characteristics for species recognition, serving as an essential criterion in female choice [13,14], although acoustic signals can also attract the attention of predators [30,31]. Moreover, species may compete for calling sites or acoustic space during breeding season, leading to competitive exclusion [32] or changes in acoustic parameters of their advertisement calls [33]. Thus, such acoustic signals may be a prominent trait in studies on community ecology and functional diversity.

The variety of applications of bioacoustics relies on the scientific knowledge of the signals, and basically, as a first step, on the quantitative and qualitative descriptions of the species calls. Yet this task is far to be completed, especially in tropical regions, which hold most of the planet's biodiversity [10–12]. Moreover, call descriptions are often limited in information and usually restricted to comparisons between closely related taxa [34]. In this sense, metadata related to environmental conditions, behavioural context and/or methodological procedures can be succinct or absent in many studies of call descriptions [14,35]. Only recently a comprehensive guideline for the description of anuran calls has been published [14].

Brazil is the country with the richest diversity of anuran amphibians in the world [36]. Brazilian anurans are distributed throughout various biomes and may also be found in transition areas [37,38], representing different climate types and a characteristic fauna of each region. Brazil also houses a high endemism rate of amphibians (for example, in the Cerrado biome the endemism rate is around 51.7%; [37]). The wide variety of habitats and the changes of the climate throughout evolutionary time-scale were likely responsible for the generation of such a high diversity of endemic species [39,40]. Nevertheless, many taxonomic problems associated with sympatric and cryptic species still remain, especially in larger taxa, such as the *Terrarana* species [41], being necessary to increase the knowledge in biology, distribution and phylogenetic relationships of these Neotropical anurans.

Examining the amount, standard and trends of the study of species traits allows to identify knowledge gaps and to guide future research strategies. In this study, we used a quantitative analysis of research literature (scientometric review; [42]) to understand the state of the art of the call description of Brazilian anuran species, addressing a series of essential aspects: (i) historical revision of the description of species calls in the last six decades, (ii) number and percentage of species with described calls across families, biomes, and categories of threat, (iii) available metadata in the call descriptions, and (iv) publication of descriptions in relation to journals, number of authors and countries. In addition, we searched for voucher recordings of each taxon deposited in scientific sound archives, and compared the description of anuran calls with the original description of the species, concluding with general directions for future studies.

2.2 MATERIALS AND METHODS

2.2.1 Bibliographical survey

Scientific literature about the description of anuran calls of Brazil was search in the databases of published articles of the Institute for Scientific Information (ISI; www.isiknowledge. com), Scientific Electronic Library online (Scielo; www.scielo.org), Google Scholar (www.scholar.google.com), and Amphibiaweb (http://www.amphibiaweb.org/). For this survey, we considered only anuran species occurring, exclusively or not, within the territory of the Brazilian federation and which were described until December 2016. These species were determined based on the list of amphibians from Brazil provided by Segalla et al. [36], completed with recent species descriptions published in the site Amphibian Species of the World [43]. As a result, 1,045 Brazilian anuran species were identified and used for further analysis. Among them, three species classified as "Incertae sedis" (*Eleutherodactylus bilineatus*, *Hyla imitator*, and *Calamita melanorabdotus*) were kept since they are on the list of the Brazilian Society of Herpetology [36]. The species names and year of species description were registered according to the taxonomic classification proposed by Frost [43]. The Brazilian biomes where each taxon occurs were also recorded using the species distribution range which was based on the manuscript of the species description, on the calls description and according to Frost [43]. Moreover, the threatened category of each species was obtained from the IUCN Red List of Threatened Species [44] and the Brazil Red Book of Threatened Species of Fauna (BRB) [45]. We considered both categories because they are evaluated differently. While the IUCN list is developed to be applied on a global scale, the BRB list is applied on a smaller scale, giving a complementary picture about the species' risk of extinction [46,47]. Accordingly, significant differences between these two lists in the categorization of several amphibian species were found [48].

The bibliographical review was focused on the description of advertisement calls since this call type is the most prevalent, biologically relevant and easiest to record acoustic signal in anuran species [14,16]. In the search for articles, two sets of terms were used. The first set encompassed the combination of the specific name of each taxon and all their synonyms (for species with taxonomic revisions). The second set included the terms *advertisement call*, *call*, *vocalization*, *canto*, and *vocalização*. Scientific articles containing at least one term of each set, and published before 2017, were retrieved and added to the study database. Additionally, we searched for references cited by collected studies that were not recovered by the bibliographic search, which included articles not available from on-line databases. Only works that presented numerical data for the acoustic parameters of the calls (e.g., temporal and spectral parameters) were considered. Grey literature (theses and conference abstracts) and sound guides (CD Audio) were not included, as well as many papers, particularly older, having subjective descriptions of the calls (e.g., [49,50]). No other filter was used during the search.

In addition, six classical textbooks reviewing natural history of anurans [51–56] were also examined in order to complete the review of the call descriptions. We also searched for recordings of each taxon in the three largest scientific animal sound collections (Fonoteca Zoológica of the National Museum of Natural Sciences, Madrid ─ http://www.fonozoo.com/; Maculay Library of the Cornell Lab of Ornithology, New York ─ https://www.macaulaylibrary.org; and Fonoteca Neotropical Jacques Vielliard of the Campinas State University, São Paulo ─ www2.ib.unicamp.br/fnjv/). The data were collected from July 2016 through January 2017.

2.2.2 Data analysis

From each retrieved study, the following variables were obtained: (i) species name, (ii) year of publication, (iii) first author name, (iv) institution of the first author, (v) number of authors, (vi) journal name, and (vii) biome of ocurrence. We also registered the presence or absence of specific metadata associated with the sound recordings: (i) locality, (ii) date, (iii) water temperature, (iv) air temperature, (v) relative humidity, (vi) activity period, (vii) habitat, (viii) perch height, (ix) distance to the nearest calling male, (x) number of recorded males, (xi) recorder, (xii) software, (xiii) microphone distance, (xiv) voucher specimen, (xv) voucher recording, (xvi) sonogram, (xvii) oscillogram, (xviii) power spectrum, (xix) call duration, (xx) note duration, (xxi) pulse number, (xxii) call rate, (xxiii) call frequency, (xxiv) sound pressure level, and (xxv) harmonics presence (see Appendix A for the specific description of these variables).

Firstly, descriptive statistics of single variables or a combination of variables were calculated from this database. Secondly, to evaluate the annual progress in the descriptions of anuran calls, Generalized Linear Models with Poisson error structure and log link function [57] were used. Specifically, three GLMs were fitted to model the relationship between the year of publication and a series of response variables, such as (i) the annual number of described calls, (ii) the number of metadata variables provided per study, and (iii) the number of authors per study. Overall statistical assumptions were carefully checked for each of the three models. Leverage values as well as DFBeta values indicated no obviously influential cases [58,59]. Overdispersion was only identified in the first model (dispersion parameter=5.3, chi-2=289.9, df=55, $p<0.001$), but not in the other models (dispersion parameter=0.8, chi-2=1182.2, df=1401, $p=0.9$; dispersion parameter=0.7, chi-2=491.4, df=717, $p=1$, respectively). To correct for overdispersion, estimated regression coefficients of the first GLM were multiplied with the square root of the overdispersion parameter and then recalculated the statistics and the p-value. All models were fitted in R (version 3.2.1, [60]) using the function *glm*. We assume a criterion of significance of $p \leq 0.05$.

2.3 RESULTS

2.3.1 Historical perspective

Based on our literature review, a total of 607 studies including descriptions of advertisement calls of Brazilian anuran species have been published between 1960 and 2016, resulting in a publication rate of 10.6 descriptions per year. The first description was for the calls of three species of the family Leptodactylidae (*Leptodactylus bolivianus*, *L. mystacinus* and *L. pentadactylus*), published in 1960 in the Texas Journal of Science [61]. Since then, the annual number of call descriptions has increased with an exponential progress (estimate±SE= 0.036±0.006, z= 14.5, $p<0.001$, df= 55), as shown in Fig 1. The year 2012 had the greatest number of calls described (43 calls), followed by 2013 (41 calls), 2010 (31 calls), and 2011 (30 calls). On the other hand, the first description of a Brazilian anuran species that also included the call description was for the species *Scinax berthae,* published in 1962 in the journal Physis [62]. According to our dataset, the description of the species calls that did not have the call described in the original description occurred on average 60.17 ± 56.72 (0—238) years after the species was described.

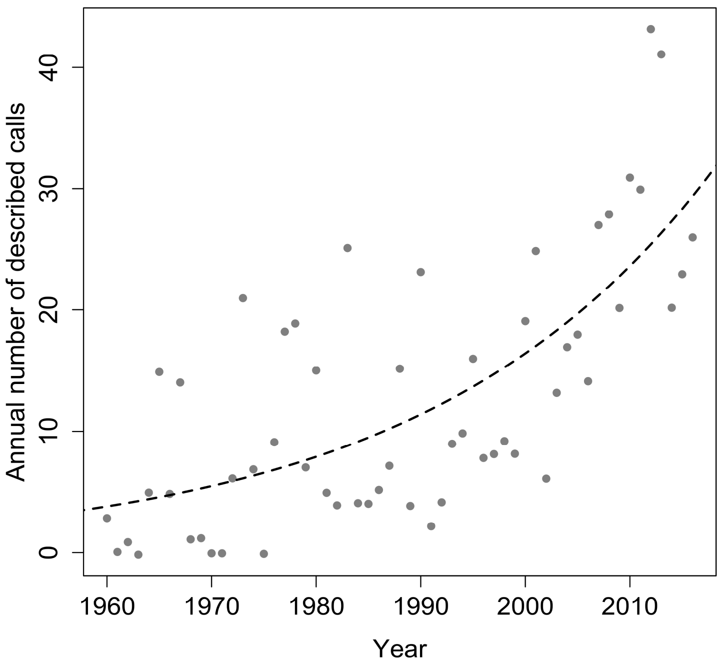

Fig. 1. Evolution in the descriptions of the advertisement calls of Brazilian anurans between 1960 and 2016. Regression function and line fitting the annual number of new described calls over the last six decades.

2.3.2 Described calls

During the last six decades, 719 Brazilian anuran species have had their advertisement call described (68.8% of the total, i.e., 1,045 species). More than half of them corresponded to species of the two largest anuran families in Brazil (Fig 2): 262 species to the family Hylidae (75.3% of its species) and 138 species to the family Leptodactylidae (88.0% of its species). Some families, with considerably less number of species, also show more than 75% of their species with described calls, namely, Aromobatidae (79.3%), Dendrobatidae (76.0%), Ceratophrynidae (83.3%), Ranidae (100%), and Allophrynidae (100%). On the contrary, the families Brachycephalidae (34.4%), Bufonidae (47.1%), Cycloramphidae (44.4%) and Pipidae (25.0%) have less than half of their species with described calls (Fig 2). The species *Limnomedusa macroglossa* (single representative of the Alsodidae family) does not have the call described. A large proportion of the species (302 species, 42.0% of all species with descriptions) have two or more published descriptions of their calls. As a result, a total of

1,403 descriptions have been published between 1960 and 2016. The species with greatest number of call descriptions are *Dendropsophus minutus* (15 descriptions), *Leptodactylus fuscus* (14 descriptions), and *L. pentadactylus* (13 descriptions).

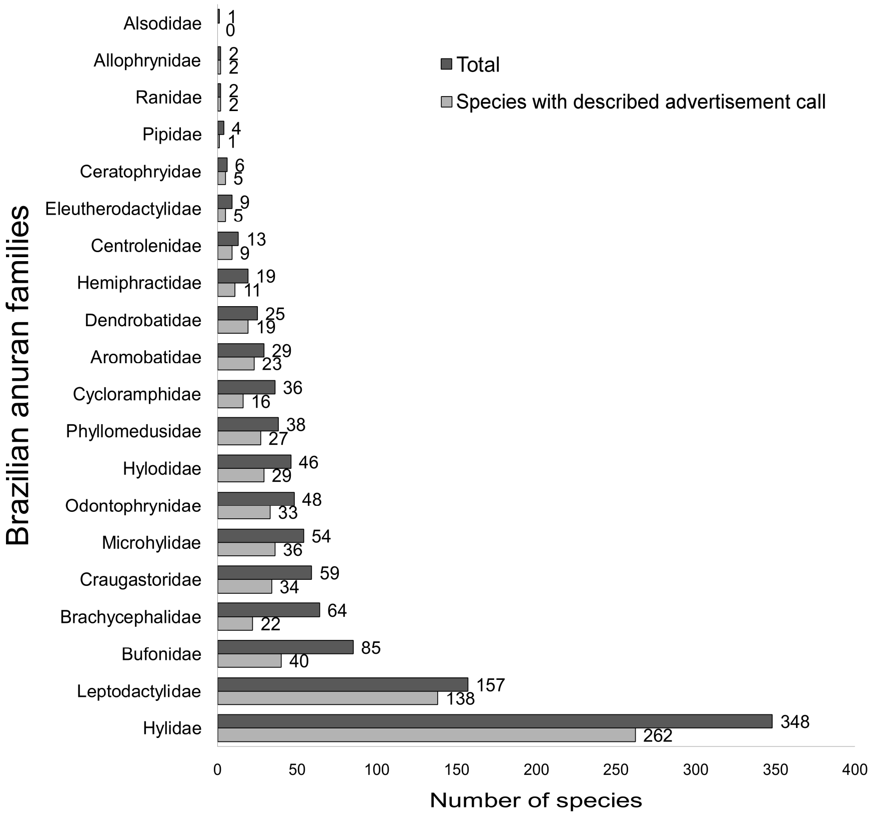

Fig 2. Total number of species with described advertisement call from each of the Brazilian anuran families.

2.3.3 Metadata

The presence of metadata associated with the call description is highly variable across studies. However, the number of metadata variables included in the description tended to increase over the time (estimate±SE = 0.011±0.001, z = 17.8, p<0.001, df = 1401; Fig 3). None of the reviewed studies presented all variables considered in the analysis. The most common information included in the descriptions were: call frequency (97.7%), call sonogram (90.0%), study locality (88.5%), call duration (81.9%), analysis software (81.0%), and note duration (75.7%; Table 1). Information of perch height, number of recorded males, voucher recording, power spectrum, call rate and presence of harmonics were showed in between 10 and 50% of the studies. Information of water temperature, relative humidity, distance to the nearest calling male, microphone distance, and sound pressure level were showed in less than 10% of the studies. Only a total of 547 Brazilian anuran species (52.3% of all species; 76.1% of species with described calls) have some of its calls deposited as voucher recordings in the scientific animal sound collections analysed. The Fonoteca Neotropical Jacques Vielliard (FNJV) included the greatest number of species with voucher recordings (445 species; 61.9% of the species with described calls). A small portion of species (59 species; 5.6% of all Brazilian anurans) has recordings deposited in the collections although their calls have not been described yet. Moreover, only 214 original descriptions of Brazilian anurans (20.5% of all species; 29.8% of species with described calls) included information about call parameters of the described species.

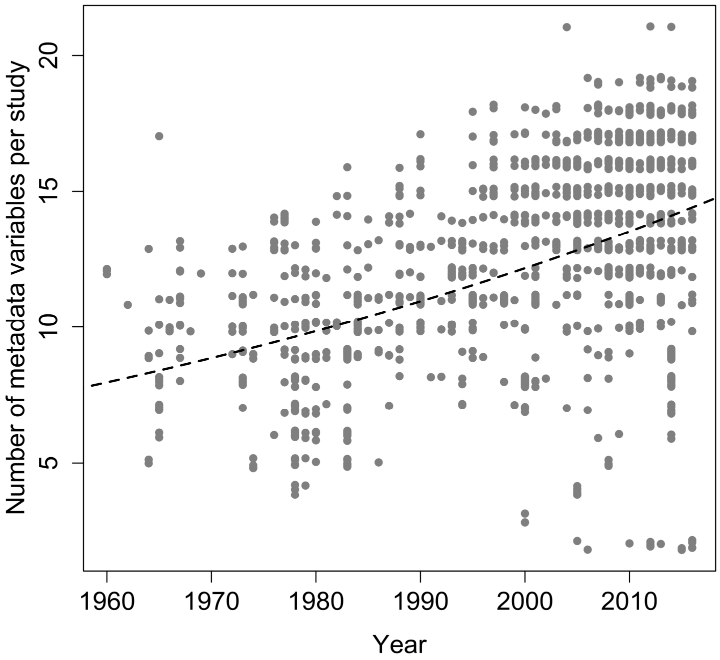

Fig 3. Evolution in the presence of metadata in the studies with descriptions of advertisement calls of Brazilian anurans between 1960 and 2016. Regression function and line fitting the number of metadata variables provided in each study and the year in which was published.

Table 1. Metadata variables shown in the 1,403 descriptions of advertisement calls of Brazilian anuran species.

Information	**Number of descriptions**	**%**
Locality	1242	88.52
Date	846	60.30
Water temperature	123	8.77
Air temperature	755	53.60
Relative humidity	64	4.56
Activity period	752	53.60
Habitat	895	63.79
Perch height	488	34.78
Distance to the nearest calling male	49	3.49
Number of recorded males	669	47.68
Recorder	995	70.92
Software	1136	80.97
Microphone distance	133	9.48
Voucher specimen	894	63.72
Voucher recording	335	23.88
Sonogram	1262	89.95
Oscillogram	941	67.07
Power spectrum	182	12.97
Call duration	1136	80.97
Note duration	1062	75.69
Pulse number	850	60.58
Call rate	621	44.26
Call frequency	1371	97.72
Sound Pressure Level	28	2.00
Harmonics presence	443	31.58

2.3.4 Call descriptions per biome

Among the six biomes presented in Brazil, the Atlantic Forest has the highest number of anuran species, followed by the Amazon and the Cerrado (Fig 4, Appendix B), whereas the level of endemism is maximum in the Amazon (90.1%), followed by the Atlantic Forest (67.2%) and the Cerrado (44.5%). Overall, biomes with higher diversity of amphibian anurans have lower percentage of species with their adverstisement calls described, namely, Atlantic Forest (65.1%), Amazon (71.5%), Cerrado (83.5%), Caatinga (87.2%), Pampa (83.9%), and Pantanal (90.6%). Among the three most diverse biomes, the highest percentage of endemic species with described calls corresponded to the Cerrado (77.1 %), followed by the Amazon (68.7%) and the Atlantic Forest (54.9%), being more than 50% for all biomes. On the other hand, the proportion of species with calls described more than once ranges from more than 50% in the Pantanal to 13.4% in the Atlantic Forest (Fig 4).

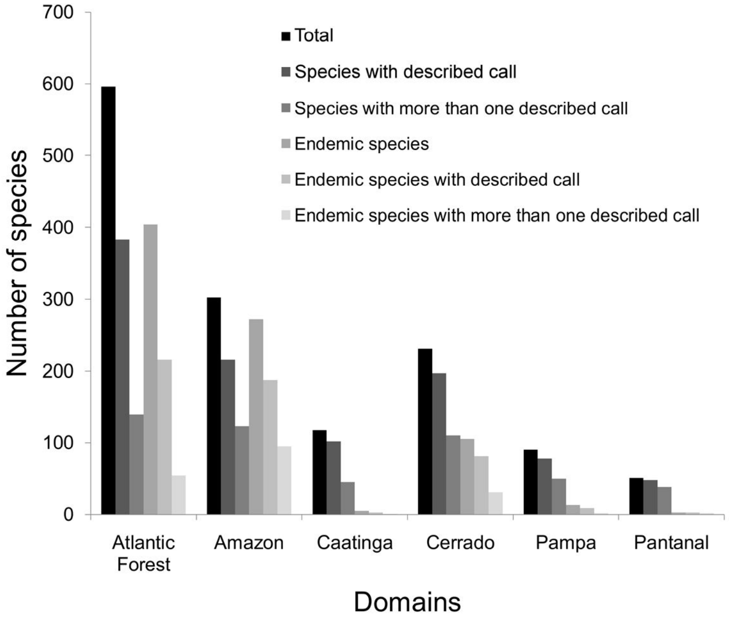

Fig 4. Number of anuran species (overall and endemic) in each Brazilian biome with one or more published descriptions of their advertisement call.

2.3.5 Call descriptions per categories of threat

Within the IUCN red list, 56.8% of the species included in threatened categories (critically endangered — CR, endangered — EN, and vulnerable — VU) have their advertisement call described, whereas 82.1% of the species in less-than-threatened categories (least concern — LC and near threatened — NT). The category CR (38.5%) has the lowest percentage of species with their calls described, while the remaining IUCN categories (including data deficient species — DD and not evaluated — NE) reach more than 54% (Fig 5). The call of the only extinct species (*Phyllomedusa fimbriata*) remains unknown. Concerning the Brazil Red Book of Threatened Species of Fauna, among the 46 species that are included in some threatened category (CR, EN and VU), 41.3% of them (19 species) do not yet have their advertisement call described. Only five threatened species included in the BRB (*Allobates goianus*, *Boana semiguttatus*, *Scinax duartei*, *Physalaemus maximus*, and *Proceratophrys moratoi*) have their calls described in more than one study.

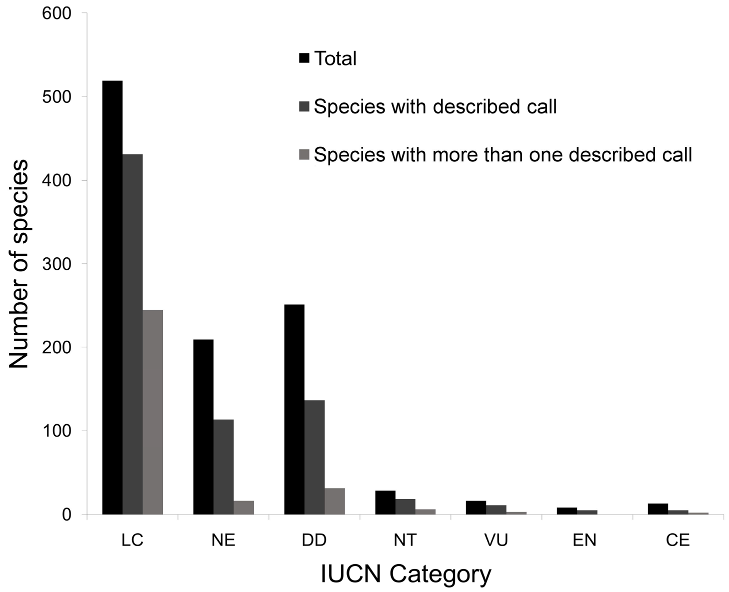

Fig 5. Number of Brazilian anuran species in each of the categories of threat of the IUCN red list with one or more published descriptions of their advertisement call.

2.3.6 Publication of call descriptions

The advertisement calls of Brazilian anurans have been reported in a series of 99 journals and books. The journals with the largest number of publications are Zootaxa, followed by Herpetologica, Brazilian Journal of Biology (previously "Revista Brasileira de Biologia"), South American Journal of Herpetology, Journal of Herpetology, and Copeia (Fig 6). From the 607 studies including descriptions of advertisement calls published so far, 88 were single-authored (14.5%), 199 published by two authors (32.8%), 168 by three authors (27.7%), 73 by four authors (12.0%), and the remainder by five or more authors (13.0%). Over time, the number of authors per study has also increased significantly (estimate±SE= 0.029±0.002, z=

16.3, p<0.001, df= 717; Fig 7). As expected, first authors of most of the articles were affiliated with Brazilian institutions (403 studies, 66.4% of the total; Fig 8). The Universidade Estadual Paulista (9.1%), Universidade Federal do Rio de Janeiro (8.6%), Universidade de São Paulo (8.1%), Universidade Federal de Goiás (3.6%), and Universidade Federal de Uberlândia (3.6%) contributed with the greatest proportion of studies. On the other hand, large number of studies described the calls of more than one species, highlighting the works of Lescure & Marty [54], with 72 call descriptions, Duellman [56], with 50, De Sá et al. [63], with 44, and Heyer et al. [53], with 36.

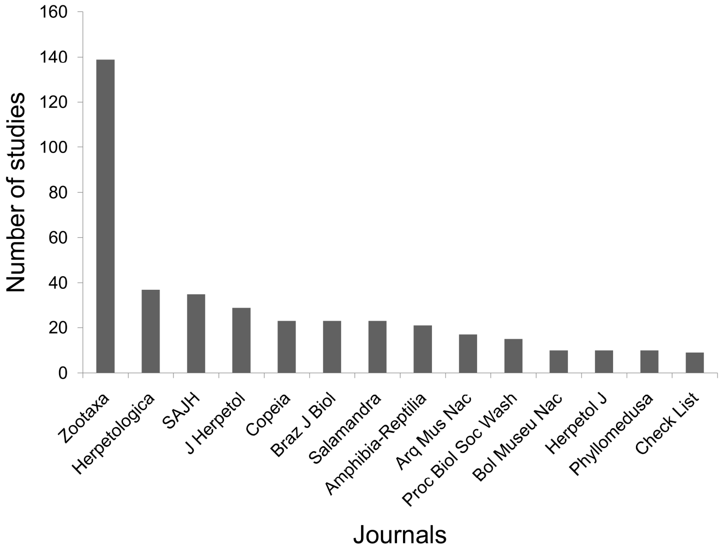

Fig 6. Scientific journals with publications about descriptions of advertisement calls of Brazilian anurans published between 1960 and 2016.

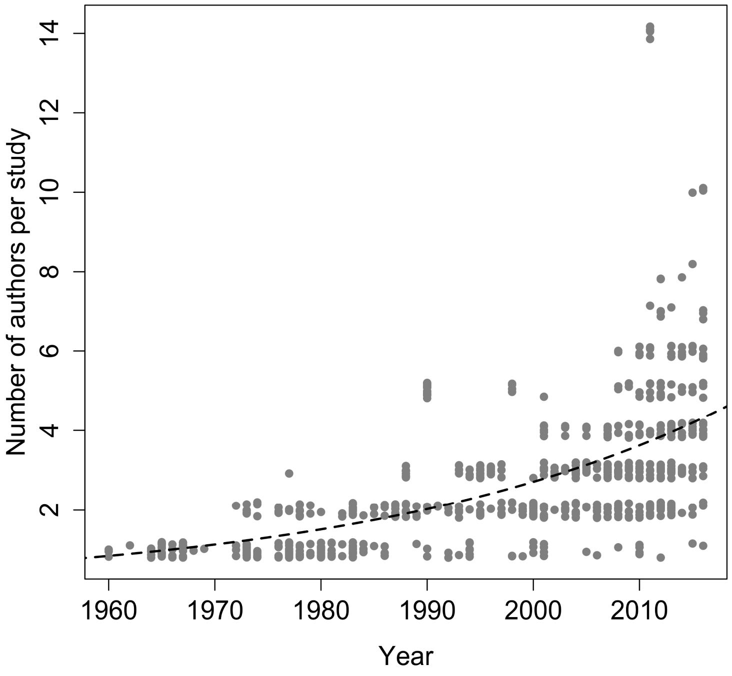

Fig 7. Evolution in the number of authors of studies with descriptions of advertisement calls of Brazilian anurans between 1960 and 2016. Regression function and line fitting the number of authors of each study and the year of publication.

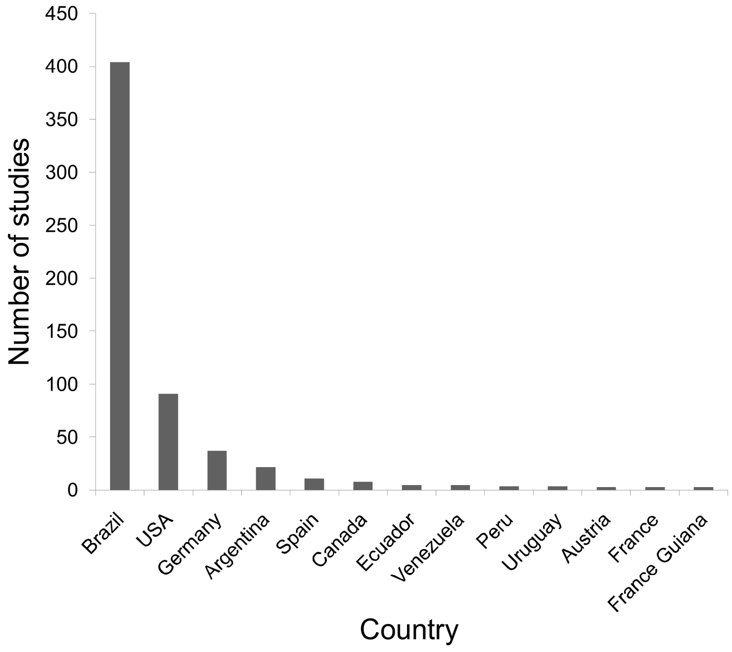

Fig 8. Number of studies with descriptions of advertisement calls of Brazilian anurans published per country (affiliated institution of the first author).

2.4 DISCUSSION

2.4.1 General perspective

Despite of intense efforts to characterize advertisement calls of Brazilian anurans during the last six decades, which have resulted in the publication of 607 studies and 1,403 call descriptions, the bibliographical review indicated a remarkable gap in the knowledge of this key functional trait (only 68.8% of the described species have their advertisement call known). Moreover, significant biases in the current understanding of the advertisement calls were identified across families, biomes, and categories of threat. This lack of information about the natural history of the species may compromise the amount, quality, and types of research accomplish in this group, such as studies on behaviour [17,20], ecology [21], conservation [23], thermal biology [24] or the evolutionary relationships among taxa [26–28], since calls may contain a phylogenetic signal [22].

Natural history information is important not only to known the biology of species and communities, but also to identify cryptic species or to prioritize conservation efforts over taxa that could be more vulnerable than others [1,8]. Since sophisticated technological tools allow us to better examine the patterns of nature and to make more accurate predictions of the responses of organisms to potential futures, it seems that fewer researchers have devoted to studying natural history [1]. Fieldwork is laborious, costly, and time-consuming; besides, journals that published works on natural history show generally low scientific impact in official journal rankings, been restricted to specific areas of biology. The risk that new generations of biologists disregard the importance of the natural history as an relevant component of their professional training or their scientific careers [3], may complicate the reduction of knowledge gaps on basic functional traits of the species and also deteriorate research in general, since natural history can contribute to all phases of research, from the development of hypotheses and concepts to the interpretation and discussion of results.

Nevertheless, an exponential growing in the annual number of published call descriptions was observed in the period 1960-2016. On average, more than 10 descriptions of advertisement calls of Brazilian anurans were reported every year, with a rate above 30-40 descriptions per year in the last decade. The emergent interest in bioacoustics [23] and the opportunities provided by the expansion of audio digital recording and other technologies [64] are likely responsible for this particular pattern, opposite to that observed in other areas of natural history. Therefore, the growing attention paied to the anuran calls enables to forecast a progressive mitigation of this knowledge gap about Brazilian anurans in future decades.

New technologies, including portable recorders, automated recording systems and novel analyses software, have played an important role in the advance of studies on animal acoustic communication [65,66]. A constant improvement and miniaturization of the sound devices, together with a decrease in price, have contributed to make studies on acoustic signals more affordable, allowing a greater number of researches to be completed [66]. However, at the same time that the interest in bioacoustics has recently increased, standardarization in sound recording, analyses or terminology are still scarce [14,64]

2.4.2 Knowledge gaps: taxa

Our review pointed out that the advertisement calls of anuran families with restrict distribution and including many recently described species (e.g., Brachycephalidae - genera *Brachycephalus* and *Ischnocnema*) were less studied. The genera *Amazophrynella*, *Melanophryniscus*, *Dendrophryniscus* and *Oreophrynella* (Bufonidae), *Cycloramphus* (Cycloramphidae), and *Megaelosia* (Hylodidae) have scarce or no studies on their calls. Other genera, such as *Phasmahyla* and *Phrynomedusa* (Phyllomedusidae), show less than a half of their species with their advertisement calls described. Many of the genera mentioned above are endemic to Brazil, being most of their species ranked in some threatened category of the IUCN or classified as data deficient (DD) due to noticeable lack of basic knowledge about their biology, distribution, population size and trends. Overall, species with their calls undescribed are found in places of difficult access, are poorly known in terms of geographic distribution or general biology, have cryptic habit, inhabiting forest floor and those that live near streams (rheophilic habit), or have a high morphological similarity with other taxa, becoming more challenging to study (e.g., *Pristimantis* spp. - familiy Craugastoridae).

Although no specific statistical analyses were performed, it is noticed that anurans with large distribution areas (e.g., *Leptodactylus fuscus* and *Dendropsophus minutus*) show a larger number of call descriptions. Some of these species are currently considered a complex of species (e.g., *Leptodactylus fuscus* in [67] and *Dendropsophus minutus* in [68]), and information on their vocalizations may help to elucidate such taxonomic problems. Besides, widely distributed species are generally more generalist in relation to their habitat and more abundant, occurring in a broad variety of environments [13]. Since these species are easily found, they are widely studied, serving as suitable models for testing ecological hypotheses (e.g., [69]) and evaluating how anthropic activities can affect this group of organisms (e.g., [70,71]).

2.4.3. Knowledge gaps: metadata

The most common and serious problems with respect to call descriptions are associated with the lack of standardized methodological procedures of analysis [14,34], which difficult comparisons of call characteristics among studies and species. Specifically, studies are highly variable in the metadata recorded and call descriptions are often poorly documented. In general, the studies analyzed often lack a description of the general properties of the calls (e.g., type of call, number of notes per call, number of calls per call series, structure of notes or calls, arrangement in groups or in series, presence or absence of harmonics). Our review identied only six among the twenty-five analyzed metadata variables being presented in more than 75% of the studies. These variables are considered a quality standard in call descriptions and also to offer some important information on the natural history of the species. Detailed information on all recording conditions such as precise locality, date, time of recording, abiotic (e.g., air and/or water temperature, and relative humidity) and biotic conditions (calling behaviour, social context), and also the description of the recording gear (e.g., recorder, microphone), recording settings (e.g., sampling rate, bit depth, format), and all procedures conducted during analysis (e.g., software, filtering, spectral settings) are part of a call description [14,64] and are crucial information for comparisons among studies and species. Activity period, calling habitat and perch height can also be important features to guide future research [66].

In addition to provide an exhaustive description of methods, context, circunstances, terminology and numerical parameters used in the studies, at least the following acoustic parameters should be analyzed and described in detail: (1) duration of calls, notes and/or pulses; (2) duration of intervals between calls, notes and/or pulses; (3) repetition rate of calls and/or notes per time unit; (4) pulse number (when applicable); (5) dominant frequency of calls and/or notes; (6) fundamental frequency of calls and/or notes; (7) bandwidth of calls and/or notes (or approximate prevalent bandwidth), i.e., difference between the uppest and lowest frequencies; and (8) harmonics. Besides, when possible, efforts to include information about the absolute or relative amplitude (sound pressure level ─ SPL) of the calls and of the background noise in which the calling males are inserted, would be highly appreciated. The knowledge of sound levels allows an estimation of the maximum distance in which intraspecific communication can occur (due to degradation and attenuation of sound), of the energy spent during communication, and of the active space in programs of passive acoustic monitoring [72]. Furthermore, this variable enables to guide the choice of sound amplitude levels in playback tests and behavioral studies [73,74]. The sound level information was previously reported for some American anurans (e.g., [73,75,76]). However, for most species of tropical anurans, as demonstrated in this study, this call parameter is unknown.

2.4.4 Knowledge gaps: biome

Some studies suggest that biomes with seasonally dry climatic features are historically neglected by taxonomists and geographers, as is the case of Cerrado and Caatinga [77,78]. However, these biomes, together with Pantanal and Pampa, showed the highest percentage of species with their advertisement calls described. This is likely because of the relative lower diversity and endemism rate of these biomes in comparison with humid forest biomes, such as the Amazon and the Atlantic Forest, where more studies on anuran species have traditionally been conducted [54,56,79]. Cerrado was lately considered as a hotspot of biodiversity [80], which has promoted a recent interest and a greater number of studies aimed at this biome. This trend has also been reinforced by the increase in the number of universities and researchers located in the region. On the other hand, the fact that Pantanal was the biome in which more species calls were studied may be explained by an inherent positive bias from taxonomists, higher number of common species and easier to sample [81,82]. In general, biomes with a greater density of access routes are those with higher biological knowledge [83], while regions having difficult access typically show better conservation status and distinct species, and should therefore be considered as priority areas for biodiversity inventories [83]. Efforts must also be allocated according to endemism rate of each region or biome. Atlantic Forest, Caatinga, and Pampa were the less studied biomes in terms of the advertisement calls of their endemic species. The low number of studies describing advertisement calls of endemic species can indicate that these species are in general still poorly studied and likely many of them have no data on reproductive biology or population size. Nevertheless, it should be highlight that studies across biomes in widely distributed species may help to identify the existence of cryptic species, when pronounced differences in their advertisement calls are found along the species range [84].

2.4.5 Knowledge gaps: categories of threat

A considerable gap of knowledge on the advertisement calls of species classified within the IUCN threatened categories were found, and specifically of species in categories VU and CR, which less than 40% have their advertisement calls described. Contrary to expectations, the species that do not have their threatened status evaluated (20% of the total) and those with data deficient (24% of total), showed a higher rate of their calls described, i.e. 54%, than species in the threatened categories. The IUCN's Red List is an important conservation tool, not only to estimate the threat status of species, but also to guide conservation strategies [85]. Species classified as threatened (CR, EN, or VU) are usually prioritized in conservation action plans [85] and, according to our review, they should also be prioritized in efforts for describing anuran calls and conducting bioacoustics studies. Data deficient species are those which data available during the assessment process is not adequate to determine threat category [86], and amphibians are the terrestrial vertebrate group with the highest proportion of DD species [86,87]. It is therefore necessary to give special attention to DD species which were described more than 50 years ago and those geographically restricte[86,87].

2.4.6 Publication of call descriptions

The description of advertisement calls of Brazilian anurans has been published in a wide variety of scientific journals and books, although most of the publications were gathered in a few journals, particularly Zootaxa and herpetological journals. In recent years, the number of authors collaborating on publications featuring call descriptions has been increasing noticeably. Likely, several benefits have promoted these scientific collaborations [88,89], such as addressing increasingly complex biological questions, sharing costs in laboratory studies, or exchanging information and building networks in multi-taxa inventories, with researchers specialized in various taxonomic groups [90], which also stimulate collaborations in different research areas [89,91]. Also, articles with more number of authors tend to be publicated in higher-ranked journals and have usually more citations [90,91]. Considering the

Brazilian scientific productivity, it is possible to observe an increase in number of authors per article that is similar to that of developed countries, however these publications remain less cited [90]. As Brazil is a country of large territorial size, with different biomes and restricted access to many areas, there are still abundant localities to be deeply explored, and it is possible to notice that regions close to large universities or protected areas are often more intensely studied [83,92].

2.4.7 Further directions

It is important to emphasize that anurans may present a broad vocal repertoire, with calls other than the advertisement call, and most of the species do not yet have all call types described. The acoustic social interactions during reproductive activity are complex and the species may present different strategies to communicate, depending of the social and ecological contexts. In a recently study, Toledo et al. [16] reviewed the calling repertoire terminology of anurans and proposed a classification based in three categories: reproductive, aggressive, and defensive calls. The calls classified in the reproductive category (e.g., advertisement, courtship, amplectant, and release calls) are the most studied and commonly described vocalizations. Due to the importance of acoustic signals in the communication of anurans and the existence of a complex vocal repertoire for many species, it is necessary not only to describe the advertisement calls and other vocaizations, but also to use experimental approaches to elucidate the function of each type of call. Thus, a considerable sampling effort under different social, ecological and climatic condictions is required to study the vocal repertoire of anurans.

The development of acoustic methodologies for biodiversity appraisal has been substantial in the last years [25,93]. The use of automatic sound recorders for acoustic monitoring terrestrial and aquatic environments is a relatively recent technique and has been applied on a large scale in many recent studies [94]. Besides being an efficient and non-invasive technique, passive acoustic monitoring enables simultaneous sampling at several scales in time and space, with reduced observer biases and animal disturbances [64]. Although there are methods to circumvent the need of knowledge about all species occurring in habitats with high diversity [79,80], information about the species-specific calls provides a more powerful and cost effective tool for the study of communities [25], and for monitoring of populations over the years in the light of global change [24,95] or other major evolutionary trends such as effects of introduced species [96]. Due to the relevance of bioacoustics in anuran species delimitation, the deposit of recorded calls in sound libraries may also facilitate the taxonomic studies [16,28]. Just like specimens and genetic samples curated in museums and other collections, sound archives are important repositories of worldwide biodiversity, storing significant information on the species [14,97]. Thus, the deposit of sound archives in audiovisual collections accessible to researchers must become a common practice for assisting further investigations.

The Raunkiaeran shortfalls are tipically present in poorly known regions of developing countries, characterized by recent and wide human occupation and often by high rate of biodiversity [8,98]. Knowledge about diversity and natural history of the species is relevant to identify current trends and to support future studies. Anuran species arised millions of years ago and have expanded into a wide range of environments [13], but many species are currently experiencing dramatic declines and even local and global extinctions mainly due to the anthropic activities [99,100]. Since the advertisement call and other acoustic signals are often species-specific and contain phylogenetic signal, they can be used for delimiting and identifying anuran taxa. A complete taxonomy is at the core of threat status assessments, and hence the knowledge about advertisement calls may assist for the effective planning of conservation and future management of amphibian diversity [14]. The exponential growth in the number of species and call descriptions suggests promising scientific advance for the coming years. However, in many developing countries such as Brazil, the adoption of laws that promote deforestation and other impacts on ecosystems as well as the reduction of investments in education and research may severely jeopardize this advance and conservation strategies. The long-term success of the efforts to know and protect biodiversity depends on a large series of factors, such as research investment, the establishment of research networks, preservation of natural habitats or public engagement in science and conservation, among many others [101,102]. Natural history may significantly contribute to this aim as shown in the present study, and we emphasize the need that key actors encourage its role in the scientific training and academic career of future generations of biologists.

2.5 CONCLUSION

The bibliographical review of the description of advertisement calls of anuran species of Brazil suggested the existence of Raunkiaeran shortfall on this key functional trait. This shortfall was highly variable across families and mainly focused on species classified as threatened taxa by the IUCN and the BRB and species occurring in biomes with the highest anuran diversity and endemism rate. In summary, our scientometric analysis provides a general insight about Brazilian anuran species, supplying a detailed guide for orienting future efforts on the description and study of their acoustic signals, behavior, taxonomy and natural history.

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APPENDIX A - Collected information and metadata variables from each study (only presence or absence)

(i) Locality: Specific site (with coordinates), state or county where the species call was recorded. Works with only country information were not considered.

(ii) Date: Days, months or years when the species call was recorded.

(iii) Water temperature; (iv) Air temperature; and (v) Relative Humidity: data obtained directly from the environment in which the species was recorded. Climatological data or data unrelated to the recorded calls were not considered.

(vi) Activity period: the period of calling activity described in the methodology, results and/or discussion. The period could be the hour or the period of day (day, twilight or night).

(vii) Habitat: the type of substract that the species typically uses to vocalize (soil, litter, leaf, bush, bromeliad, creek, pond, etc.).

(viii) Perch height: the height at which the species was perched while calling, described in the methodology, results and/or discussion. When there was information of the species calling on the ground, the perch height was considered as present.

(ix) Distance to the nearest calling male: the nearest distance between two conspecific calling individuals.

(x) Number of recorded individuals: the number of individuals included in the description of the call.

(xi) Recorder: the specific device used to record the calls described in the study.

(xii) Software: the software used to analyze the calls described in the study.

(xiii) Microphone distance: the distance at which the microphone was positioned in relation to the recorded individual.

(xiv) Voucher specimen: the number of the collected specimen associated to the species or call description and deposited in a collection. It could be different from the individual that emitted the recorded calls used for the description.

(xv) Voucher recording: the number of the recorded calls that were described in the study and deposited in a sound collection.

(xvi) Sonogram (or spectrogram): a visual representation of spectrum of frequencies of the recorded call across the time.

(xvii) Oscilogram: a visual representation of the sound wave of the recorded call across the time.

(xviii) Power spectrum: a visual representation of a time series of a recorded call that describes the distribution of power into frequency components which composing that signal.

(xix) Call duration: the time between the onset and the end of a call. The call was considered as a set of sounds constituted by either a single note (simple call) or a series of identical or group of different notes (composite call) emitted in a defined period of time.

(xx) Note duration: the time between the onset and the end of a note. The note was considered as a temporally uninterrupted sound element composing the call and could be made up of a pulses series.

(xxi) Pulse number: the number of pulsed in a note. The pulse was considered as sounds of short duration produced by a single energy impulses released in the temporal spectrum of a note.

(xxii) Call rate: the number of calls emitted in a defined period of time.

(xxiii) Call frequency: the frequency band with more energy in the call.

(xxiv) Sound pressure level (SPL): the effective pressure caused by a sound wave relative to a reference pressure.

(xxv) Harmonics presence: the presence or absence of harmonics in the described call.

APPENDIX B - Number and percentage of Brazilian anuran species with described calls per each biome

Species = Total number of species; Calls = Species with described calls; Calls >1 = Species with more than one description of their calls; Endemic Spp = Endemic species; ECalls = Endemic species with described calls; ECalls >1 = Endemic species with more than one description of their calls. Percentages in the first three columns refer to Species (the total number of species), while percentages in the two last columns refer to Endemic Spp (number of endemic species).

Biomes	**Species**	**Calls**	**Calls >1**	**Endemic Spp**	**ECalls**	**ECall >1**
Atlantic Forest	601	391	139	404	222	54
		65.1%	23.1%	67.2%	54.9%	13.4%
Amazon	302	216	123	272	187	95
		71.5%	40.7%	90.1%	68.7%	34.9%
Caatinga	117	102	45	5	3	1
		87.2%	38.5%	4.3%	60.0%	20.0%
Cerrado	236	197	110	105	81	31
		83.5%	46.6%	44.5%	77.1%	29.5%
Pampa	93	78	50	13	9	2
		83.9%	53.8%	14.0%	69.2%	15.4%
Pantanal	53	48	38	3	3	2
		90.6%	71.7%	5.7%	100%	66.7%

3 THE ADVERTISEMENT AND AGGRESSIVE CALLS OF *Rhinella abei* (BALDISSERA, CARAMASCHI, AND HADDAD, 2004) (ANURA: BUFONIDAE) FROM CAMPO LARGO, PARANÁ, BRAZIL

RESUMO

Rhinella abei é uma espécie do grupo *Rhinella crucifer*, e possui distribuição na Mata Atlântica brasileira, entre os estados do Paraná e o norte do Rio Grande do Sul. Neste estudo descrevemos os cantos de anúncio e agressivo de machos de *R. abei* que foram gravados no município de Campo Largo, estado do Paraná, Brasil. O canto de anúncio de seis indivíduos consistiu em uma série de notas pulsionadas não harmônicas, e a média da duração do canto e da frequência dominante foi de 6,839 s e 1418,8 Hz, respectivamente. O canto agressivo consistiu de uma a três notas pulsionadas, e a média da duração do canto e da frequência dominante foi de 0,676 s e 1312,6 Hz, respectivamente. O canto de anúncio de *R. abei* se diferencia do canto das outras espécies do grupo *R. crucifer* devido a sua maior duração e maior número de notas.

Palavras-chave: grupo *Rhinella crucifer*, Mata Atlântica, parâmetros do canto, taxonomia.

3.1 FULL TEXT

Rhinella abei is a medium-sized species (snout-to-vent length 57.0–76.4 mm in males; 60.4–83.9 mm in females – Baldissera *et al.* 2004) of the *Rhinella crucifer* species group, distributed in the Brazilian Atlantic Rainforest, from the State of Paraná to northern Rio Grande do Sul (Frost 2015). It is recorded in forested areas (Conte & Rossa-Feres 2007), or while breeding along forest edges and open habitat. Information on advertisement calls of species in the *R. crucifer* group is available for *R. casconi*, *R. crucifer*, *R. inopina* and *R. ornata* (Andrade *et al.* 2015; Heyer *et al.* 1990; Oliveira *et al.* 2014; Roberto *et al.* 2014). Here we describe the advertisement and aggressive calls of *R. abei* recorded in the municipality of Campo Largo (25.507472° S, 49.376632° W, datum "WGS84"), central State of Paraná, Brazil.

We recorded six *R. abei* males on September 23, 2015, calling from the edge of Passaúna Lake, near an Araucaria Forest remnant in Atlantic Forest biome. Recordings were obtained using a Marantz PMD 660 digital recorder and a Sennheiser ME66/K6 directional microphone. Calls were recorded at 44.1 kHz with 16-bit resolution. Data were analyzed using Raven Pro 64 1.5 software from the Cornell Lab of Ornithology (Bioacoustics Research Program 2014). Spectrograms were produced applying a window size of 256 samples, 75% overlap, a hop size of 64 samples, DFT of 1024 samples, and Hamming window type. We obtained oscillogram and spectrogram figures using TuneR 1.0 (Ligges *et al.* 2013) and Seewave 1.7.3 (Sueur *et al.* 2008) packages for R 3.0.3, 32-bit version (R Development Core Team, 2014). Voucher specimens are housed at the Zoological Collection of Federal University of Goiás, in Goiânia, Brazil (ZUFG 9884; ZUFG 9885). Samples of the recordings are curated by Fonoteca of the Federal University of Goiás (FUFG 1508; FUFG 1509). The following acoustic parameters were taken: call duration, silent interval between calls, call rate (calls/minute), note duration, note repetition rate (notes/s), silent interval between notes, pulse number, pulse duration, dominant frequency of the call, upper frequency of the call, lower frequency of the call and call´s bandwidth (see Batista *et al.* 2015, Forti *et al.* 2015). Parameters were measured from 30 advertisement calls and 13 aggressive calls, recorded from six and four males, respectively. Measurements are presented as the mean (or mode) ± one standard deviation (minimum–maximum). Call description and terminology follow Gerhardt (1998).

Average snout-to-vent length of recorded males was 58.97 ± 6.04 mm (52.40–69.87 mm; N = 6). Mean air and water temperatures were 16.7 ± 0.82 °C (15–17.6 °C, N = 6) and 19.01 ± 0.12 °C (18.8–19.1 °C; N = 6), respectively. The advertisement call of *R. abei* (Fig. 1a) consists of a series of pulsed notes. Mean call duration is 6.839 ± 2.109 s (2.621–12.710 s; N = 30); average duration of silent interval between calls is 28.712 ± 30.669 s (2.569–134.633 s; N = 30). The average number of notes per call is 85 ± 26 notes (32–152; N = 30). Mean note duration is 0.038 ± 0.003 s (0.007–0.055 s; N = 300). Average silent interval between notes is of 0.038 ± 0.004 s (0.008–0.073 s; N = 300). Average rate of note emission is 12.46 ± 0.82 notes/s (10.00–14.00 notes/s; N = 30). Notes are formed by 1–6 pulses (mode = 4 pulses; N = 300 notes). Average call rate is 1.959 ± 0.513 calls/minute (1.400–3.000 calls/minute; N = 4 males). Average dominant frequency of the call is 1418.8 ± 94.5 Hz (1312.5–1500.0 Hz; N = 30 calls). Average upper frequency of the call is 1606 ± 94.5 Hz (1500–1688 Hz; N = 30 calls). Average lower frequency of the call is 1131 ± 34.2 Hz (1125–1313 Hz; N = 30 calls). Average call´s bandwidth is 475 ± 95.14 Hz (375–562 Hz; N = 30 calls).

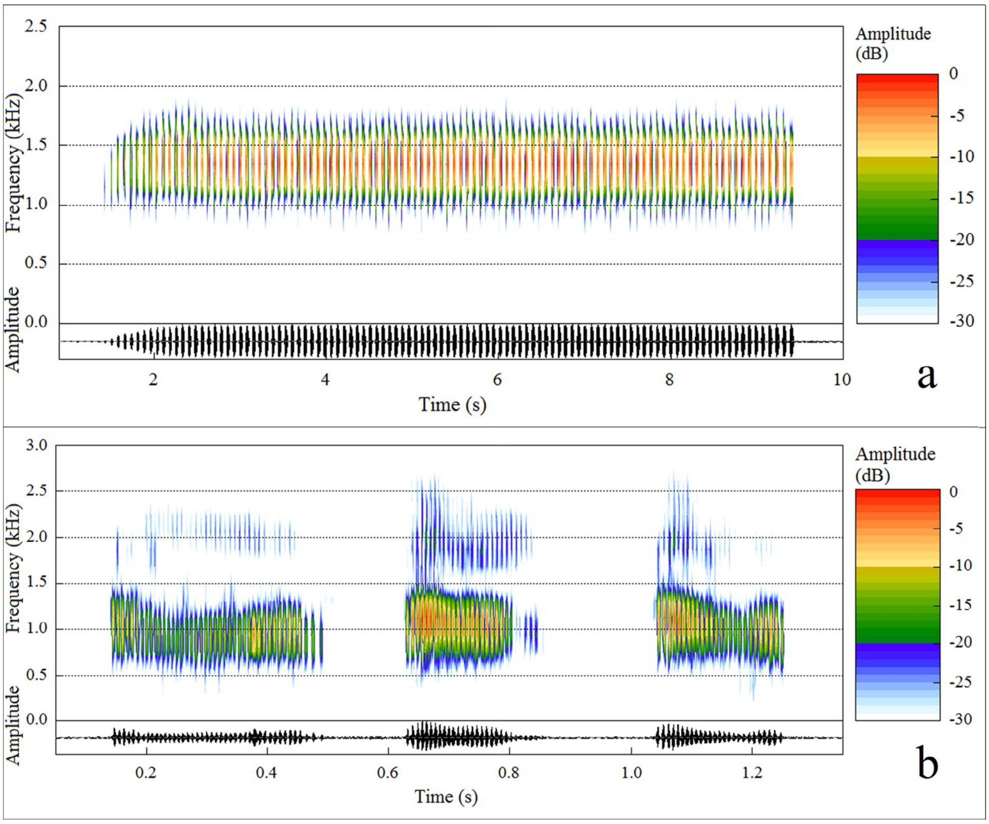

FIGURE 1. Advertisement (A) and aggressive (B) calls of a male *Rhinella abei* from Campo Largo, State of Paraná, Brazil. Upper graphs: Audiospectrograms. Lower

graphs: oscillograms. Air temperature at time of recording = 17.6°C. Water temperature = 18.9°C. Male´s SVL = 69.87 mm. Voucher specimen: ZUFG 9884. Voucher recording: FUFG 1508.

O Only four males emitted aggressive calls (Fig. 1b), consist of one to three pulsed notes (mode = 1 note; N = 13 calls). Aggressive calls were emitted only during aggressive interactions between conspecific males vocalizing close to each other (<1.5 m). Call duration averaged 0.676 ± 0.410 s (0.168–1.368 s; N = 13 calls). Note duration averaged 0.332 ± 0.177 s (0.168–0.565 s; N = 21 calls) and the number of pulses per call ranged from 11–110 pulses/call (mode = 44 pulses/call; N = 13 calls). The interval between notes averaged 0.209 ± 0.067 s (0.126–0.15 s; N = 8 notes), and the pulse duration averaged 0.007 ± 0.001 s (0.003 – 0.011 s; N = 130 pulses). Dominant frequency of the call averaged 1312.577 ± 76.547 Hz (1125–1500 Hz; N = 13 calls).

The advertisement call of *R. abei* is the longest among those emitted by species in the *R. crucifer* group, being characterized by a greater number of notes. Note duration, number of notes per call, rate of note emission and dominant frequency overlap with those of the calls of *R. ornata* (Heyer *et al.* 1990), differing mainly in call duration. The taxonomy of *R. ornata* and *R. abei* is problematic due to their similar external morphology and by accounts of hybridization between them (Thomé *et al.* 2012). Hence, detailed descriptions of advertisement calls may aid integrative approaches (*e.g.* Padial & De La Riva, 2009), targeted at clarifying their taxonomic status.

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4 VOCALIZATIONS OF *Limnomedusa macroglossa* (AMPHIBIA: ANURA: ALSODIDAE)

RESUMO

Limnomedusa macroglossa é uma espécie monotípica que ocorre na Argentina, Paraguai, Uruguai e sul do Brasil. Embora a biologia reprodutiva desta espécie seja conhecida o seu canto ainda não foi formalmente descrito. Neste trabalho, nós fazemos a descrição de dois tipos de cantos de *L. macroglossa*. O canto de anúncio foi composto de uma nota pulsionada com estrutura harmônica, sendo emitido sozinho ou em conjuntos de dois a sete cantos. A duração e a frequência média do canto de anúncio é 0,07 s e 1002,01 Hz, respectivamente. O canto agressivo também é composto por uma nota pulsionada e estrutura harmônica, e as médias da duração e frequência dominante são 0,40 s e 884,84 Hz, respectivamente. Os dados de canto de *L. macroglossa* podem auxiliar a compreender posição taxonômica desta espécie.

Palavras-chave: canto de anúncio, canto agressivo, Alsodidae, taxonomia.

4.1 FULL TEXT

The anuran family Alsodidae is currently composed of 30 species in three genera: *Alsodes*, *Eupsophus* and *Limnomedusa* (FROST 2017). The single species of the monotypic genus *Limnomedusa* is distributed throughout northeastern Argentina, adjacent Paraguay, Uruguay and southern Brazil (states of Paraná, Santa Catarina and Rio Grande do Sul) (KWET et al. 2010). *Limnomedusa macroglossa* (DUMÉRIL & BIBRON, 1841) is a medium-sized frog (average length in males 46.7 mm and in females 52.9 mm; KAEFER et al. 2009), with dorsal skin covered by large warts and elongated tubercles on the back. The dorsum is brown or gray, with a light-colored triangular spot on the snout and a dark spot between and behind the eyes (STRANECK et al. 1990, KWET et al. 2010). *Limnomedusa macroglossa* has been considered a threatened species in Paraguay (MOTTE et al. 2009), whereas in Brazil it is listed as critically endangered in the state of Paraná and endangered in the state of Santa Catarina (SEGALLA & LANGONE 2004, CONSEMA 2011). However, *L. macroglossa* is considered least concern according to global and Brazilian lists of threatened species (IUCN 2016, BRB 2016).

Limnomedusa macroglossa is terrestrial and occurs widely among open habitats and forests where it lives on the ground near streams on basalt or rocky outcrops (GUDYNAS & GEHRAU 1981, KWET et al. 2010). The most complete study on the reproductive biology of this species was that of Kaefer et al. (2009), who reported detailed information on reproductive season, reproductive mode, number and diameter of eggs, calling period, calling site and breeding habitat. The eggs (396 ± 65.8 eggs per clutch; KAEFER et al. 2009) are laid during the months of September, October and November in flooded pools and temporary ponds along rivers and small rocky streams while the tadpoles grow up in both ponds and streams (KAEFER et al. 2009). Despite this information on its reproductive biology and even published audio recordings of calls in guides from Argentina and Brazil (STRANECK et al. 1990, KWET & MÁRQUEZ 2010), quantitative parameters of the advertisement call of *L. macroglossa* have yet to be described. Herein, we provide the first description of the quantitative parameters of two different calls of *L. macroglossa*.

A calling male *L. macroglossa* (Fig. 1) was recorded on 10 October 2013 in the municipality of Três Barras do Paraná, state of Paraná, Brazil (-25.4061°; -53.1173°). The calls were recorded at a sampling rate of 22 kHz and 16-bit resolution (wav file format) using a Tascam recorder coupled to a Sennheiser ME66 microphone positioned at about 50 cm from the calling male. Air temperature was measured with a digital thermo-hygrometer (precision to ± 1ºC). The voucher specimen was collected and is deposited at the Amphibian Collection at Universidade Tecnológica Federal do Paraná, Francisco Beltrão (RLUTF 918). Recordings were deposited at Fonoteca Zoológica (FonoZoo – the animal sound library of the Museo Nacional de Ciencias Naturales, Madrid, Spain; 10038). Additional call recordings were also obtained from FonoZoo. Some of these vocalizations were found published in an acoustic guide containing calls of amphibian species from southern Brazil and Uruguay (KWET & MÁRQUEZ 2010). Analyses of all recordings of *L. macroglossa* were performed with sampling frequencies of 22 kHz and 16-bit resolution. For each recording, data on site, date, hour and air temperature at time of recording were compiled. Two specimens were deposited in the collection of the Museu de Ciência e Tecnologia (MCT) at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) (MCT 2566, 5607).

Figure 1. Adult male *Limnomedusa macroglossa* from Três Barras do Paraná, Paraná State, Brazil. Photo by R. Lingnau.

Calls were analyzed on a personal computer using Raven Pro software, version 1.5, 64-bit version (Bioacoustic Research Program, 2013) with the following settings: window size = 256 samples; window type = Hann; overlap = 50%; DFT size = 256 samples, grid spacing = 188 Hz. Sound figures were produced using the Seewave package version 1.6.4 (Seur et al. 2008) of the R platform, version 2.15.1, 64-bit version (R Development Core Team 2012). Seewave settings employed were: window name (Fourier transform window) = Hanning; window length = 256 samples; overlap = 80%.

Terminology and definitions in call descriptions follow Köhler et al. (2017). We analyzed 20 advertisement calls from each individual (*n* = 120 calls analyzed, *n* = six individuals), and 22 aggressive calls from four males. Five call parameters were measured from the oscillogram: call duration (s), pulse number, pulse duration (s), inter-call interval (s), and call rate (calls/minute). Spectral parameters were obtained by selecting three variables in Raven 1.5: (1) Frequency 5% (Hz) and (2) Frequency 95% (Hz), where these two parameters included the lowest and highest frequency, ignoring the upper and lower 5% of the frequency band formed by the energy distribution; and (3) Peak Frequency (Hz). The number of harmonics and the frequency of each harmonic of the analyzed calls were measured from spectrogram. Measurements were presented as the mean ± standard deviation (minimum–maximum) or mode.

Recorded males of *L. macroglossa* called from the ground, on flooded soil or rocks, or partially submerged at the edge of rivers or ponds (Fig. 1). Air temperature of analyzed recordings averaged 20.8 ± 2.4 °C (17–24 °C). Most recorded males emitted two different calls: an advertisement call and an aggressive call. Numerical parameters of these calls are presented in Table 1.

Table 1. Call parameters of vocalizations of adult males *Limnomedusa macroglossa*. Measurements were presented as the mean ± standard deviation (minimum–maximum) or mode (for pulse number and call group).

Parameter	**Advertisement call (*n* = 120 calls analyzed, *n* = six males)**	**Aggressive call (*n* = 22 calls analyzed, *n* = four males)**
Call duration (s)	0.07±0.01 (0.05—0.09)	0.40±0.03 (0.35—0.45)
Pulse number	6 (4—9)	19 (15—29)
Pulse duration (s)	0.01±0.00 (0.01—0.03)	0.01±0.00 (0.01—0.03)
Silent interval between calls (s)	0.55±0.74 (0.04—6.56)	1.20±0.46 (0.57—2.15)
Call rate (s)	91.17±42.12 (46—152)	3.32±1.04 (1—4)
Call group	1 (1—7)	—
Dominant frequency (Hz)	1002.01±92.04 (861.30—1205.90)	884.84±201.00 (689.10—1205.90)
Upper frequency (Hz)	1633.67±387.45 (1033.60—2325.60)	1887.11±350.55 (1033.60—2239.50)

	Advertisement call	**Aggressive call**
Parameter	**(*n* = 120 calls analyzed, *n* = six males)**	**(*n* = 22 calls analyzed, *n* = four males)**
Lower frequency (Hz)	735.01±103.49 (516.80—947.50)	555.95±91.03 (516.80—861.30)
Harmonic number	3 (2—6)	3 (2—3)
Harmonic 2 (Hz)	1949.49±221.35 (1550.40—2239.50)	1660.03±146.03 (1378.10—1894.90)
Harmonic 3 (Hz)	2910.57±403.58 (2067.20—3445.30)	2385.91±245.35 (2239.50—2928.50)
Harmonic 4 (Hz)	4193.03±290.21 (3617.60—4651.20)	—
Harmonic 5 (Hz)	5135.69±69.46 (4995.70—5168.00)	—
Harmonic 6 (Hz)	6263.09±249.25 (5857.00—6546.10)	—

Advertisement call (Fig. 2) was composed of a single pulsed note with harmonic structure, was emitted alone or in groups of two to seven calls (Table 2). The number of harmonics ranged from two to six, but calls with three harmonics were most common; the dominant frequency was in the first harmonic (Fig. 2, Table 1). The advertisement call was emitted by all males of *L. macroglossa* recorded, and was the most frequently heard call. Advertisement calls emitted alone were the most common vocalizations (34.2 % of all calls), followed by calls emitted in groups of two (30 % of all calls); groups of seven calls were recorded only once (0.8 % of all calls).

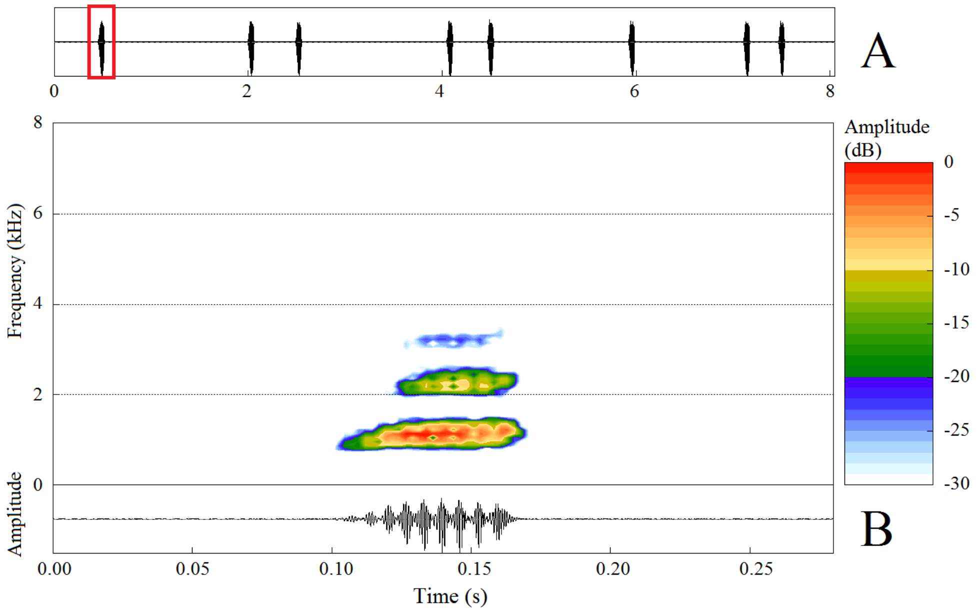

Figure 2. Advertisement call of *Limnomedusa macroglossa* from Rio Guarani, Três Barras do Paraná, Brazil. (A) Oscillogram of a series of advertisement calls; (B) spectrogram (above) and oscillogram (below) of the advertisement call highlighted with

a red outline (air temperature = 21.6°C). Voucher specimen RLUTF 918. Voucher recording: FonoZoo 10038.

Aggressive calls comprised a pulsed note with harmonic structure (Fig. 3). This call exhibited a gradually increasing amplitude (call intensity) and an ascendant frequency modulation towards the end of the call. Males emitted this call only sporadically. Aggressive calls possessed two or three well-defined harmonics and higher values of call duration, pulse number, pulse duration, and upper frequency than the advertisement call (see Table 1 for complete numerical values).

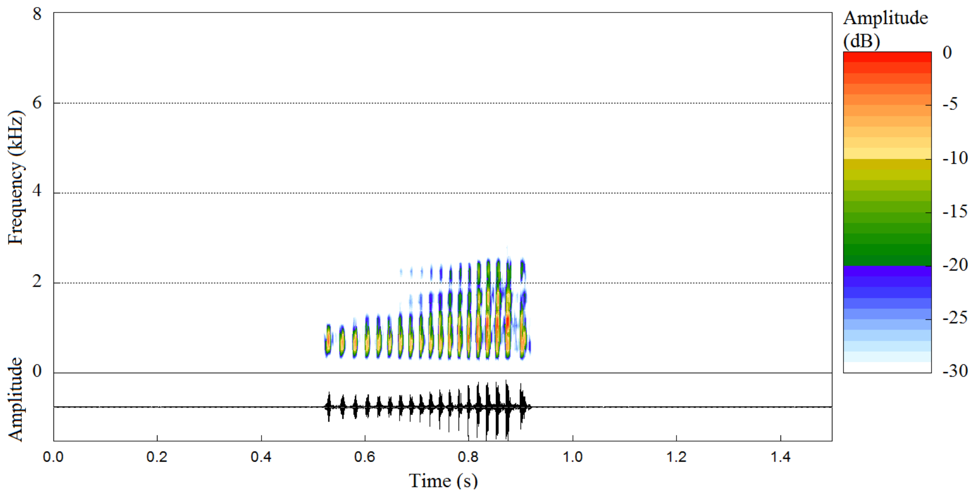

Figure 3. Aggressive call of *Limnomedusa macroglossa* from near Tacuarembo, Uruguay. Spectrogram (above) and oscillogram (below) (air temperature = 17°C). Unvouchered specimen. Voucher recording: FonoZoo 17300.

Limnomedusa macroglossa possesses two different call types that we refer to as an advertisement call and an aggressive call (sensu Toledo et al. 2015; Köhler et al. 2017). Aggressive calls were emitted by males in response to advertisement calls from other males. Since there are no previous reports on territoriality or physical combat in *L. macroglossa* (KAEFER et al. 2009, LANGONE & PRIGIONI 1985) further studies should be performed to confirm its aggressive function. The vocal behavior of males of *L. macroglossa* seems to comprise the frequent emission of both calls to attract females and in a context of aggressiveness.

Vocalization by *L. macroglossa* was first reported in a sound book accompanying a cassette tape with anuran recordings from Argentina (STRANECK et al. 1990), but parameters of these calls were not provided. The first sonogram provided on page 47 in STRANECK et al. (1990) seems to represent the vocalization that we identified as the advertisement call, whereas the sonogram below refers to what we consider the aggressive call.

Vocalizations of males play a key role during the reproductive period for most anuran species (WELLS 2007), and accurate quantitative descriptions of anuran calls can provide important information for taxonomic and phylogenetic studies by uncovering relationships between taxa (KÖHLER et al. 2017). *Limnomedusa* is a monotypic genus and comparisons with calls of related species are limited. As with other species of the family Alsodidae, the advertisement call of *L. macroglossa* is composed of a single harmonic note, although call duration and dominant frequency is lower than in calls of most species of *Eupsophus* (FORMAS 1989, FORMAS & BRIEVA 1994, NUÑEZ et al. 2012). The spectral structure (without a series of sidebands) and temporal structure (pulsed call) of the *L. macroglossa* vocalizations also differs from the call structure in *Eupsophus*, thus apparently not corroborating a close relationship between *Limnomedusa* and the *Eupsophus–Alsodes* clade. The advertisement call of *L. macroglossa* seems to be more similar to the calls of some species of the family Cycloramphidae. On the other hand, closely related species may have completely different call structure, as in the case of *Boana riojana* and *Boana marianitae* (KÖHLER et al. 2017), thus different call structure does not necessarily reject close relationships. Our bioacoustic data support BLOTTO et al. (2013), who reported on the molecular phylogenetics of Alsodidae and suggested that the inclusion of *Limnomedusa* within Alsodidae should be considered provisional (see FROST 2017).

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5 VARIATION OF THE ADVERTISEMENT CALL OF *Physalaemus centralis* BOKERMANN, 1962 (ANURA: LEPTODACTYLIDAE) IN THE CERRADO OF CENTRAL BRAZIL

RESUMO

Os sinais acústicos são utilizados para auxiliar a designação correta das espécies e permite a discriminação entre indivíduos. Neste estudo analisamos a variabilidade intra e interindividual do canto de anúncio de *Physalaemus centralis* com base em indivíduos registrados em oito localidades do Cerrado, no centro do Brasil. Para a variação intraindividual, a maioria dos parâmetros do canto demonstraram ser propriedades estáticas. Os parâmetros espectrais e temporais dos cantos foram dinâmicos entre os indivíduos. Todos os parâmetros do canto apresentaram maior variação inter do que intraindividual. Este estudo acrescenta dados importantes para futuros estudos sobre a taxonomia e filogenia do clado *P. cuvieri*.

Palavras-chave: grupo *Physalaemus cuvieri*; variação intraindividual; variação interindividual; seleção sexual; reconhecimento de espécies.

ABSTRACT

Acoustic signals are employed to support the correct designation of species and enables the discrimination between individuals. We analyzed both intra and interindividual variability of the advertisement call of *Physalaemus centralis* on the basis of specimens recorded in eight localities of the Cerrado in central Brazil. Within-individual variation, most of the call parameters were static properties. Both spectral and temporal call properties were dynamic between individuals. All call proprieties varied more between individuals than within an individual. Our study adds important data for future studies on the taxonomy and phylogeny of the *P. cuvieri* clade.

Keywords Physalaemus cuvieri group; intraindividual variation; interindividual variation; sexual selection; species recognition.

5.1 INTRODUCTION

Acoustic signals are the most conspicuous mechanism of communication in anuran amphibians (Wells 2007). There are several kinds of vocalizations (Toledo et al. 2014) capable of playing different roles in the social context of the species, such as in attracting conspecific females (Poole & Murphy 2007) and defending territories (Bee et al. 1999). The most frequently documented type of call for anurans is the advertisement call, which has reproductive function (Wells 2007; Morais et al. 2012; Gambale & Bastos 2014; Batista et al. 2015). Bioacoustic studies are useful to establish boundaries between species, since call properties represent a source of species recognition (Ryan & Rand 1993).

Since anurans are ectothermic animals, acoustic signals may vary with external factors such as temperature (Lemes et al. 2012; Morais et al. 2012). Signal variation also depends on social context, such as chorus size and distance between conspecific neighbors (Lemes et al. 2012; Morais et al. 2012; Gambale & Bastos 2014). Calls also have properties that are able to provide information on morphological features such as snout–vent length (SVL) and body size (Morais et al. 2012; Gingras et al. 2013; Gambale & Bastos 2014). Therefore, many factors can influence the emission of calls by anurans.

The natural variation between species and individuals helps to recognize conspecifics and may enable discrimination between individuals (Wells 2007). Variation in call properties in anurans is well documented (Bee & Gerhardt 2002; Rodríguez et al. 2010; Gambale & Bastos 2014). At the species level, variation in acoustic signals may be investigated from an evolutionary perspective regarding choruses (e.g. niche partition), whereas variation between individuals may provide cues for female choice (sexual selection) (Searcy & Nowicki 2005). Spectral parameters (e.g. dominant frequency) of the call that are under control of morphological features are classified as static, while temporal parameters (e.g. call duration), which are influenced by social or environmental conditions, are classified as dynamic (Gerhardt 1991; Gerhardt & Huber 2002). Variation in call trails is a continuum between static and dynamic properties (Gerhardt 1991) due to different selection pressures on vocalizations (Rodríguez et al. 2010). Since calling individuals are exposed to environmental and genetic changes (Brooke et al. 2000; Wells 2007), call signals may change over time

(Smith & Hunter 2005; Gambale et al. 2014). Thus, understanding the range of variation between species and individuals is important for providing information about conspecific identity and sexual selection.

The genus *Physalaemus* currently comprises 47 described species, distributed in South America, east of the Andes, with a range including the Guianas, lowlands of southern Venezuela, llanos of southeastern Colombia, western Ecuador, Bolivia, Paraguay, Uruguay, northern and central Argentina, and Brazil (Nascimento et al. 2005; Lourenço et al. 2015; Frost 2016). Five species groups are recognized in the *P. cuvieri* clade (Lourenço et al. 2015). The *P. cuvieri* group is composed of nine small- to medium-sized species (Lourenço et al. 2015). It is distributed from the north to the south of South America and east of the Andes (Nascimento et al. 2005; Lourenço et al. 2015; Frost 2016). Information on advertisement calls of some species of the group is available in Gambale and Bastos (2014).

Physalaemus centralis (central dwarf frog) is distributed in the eastern and northeastern borderlands of Bolivia (Department Beni), east-central Paraguay, and east and northeast Brazil throughout Mato Grosso, Goiás, São Paulo, Minas Gerais, Bahia and Tocantins up to the border of Maranhão and into southern Piauí (Frost 2016). This species occurs in open areas associated with semi-deciduous Atlantic Forest, flooded regions and Cerrado domain (Brasileiro & Martins 2006; Zina et al. 2007). Brasileiro and Martins (2006) provided information on the breeding ecology of *P. centralis*, and the call was first described by Padial and Köhler (2001). Available call information for *P. centralis* is based on only few individuals (Padial & Köhler 2001; Vasconcelos et al. 2014). Therefore, knowledge about intraspecific variation based on a more robust call sample is needed to improve comparisons between species of the *P. cuvieri* clade and to better understand species delimitation. Herein, we analyzed both intra and interindividual variability of the advertisement call of *P. centralis* from specimens recorded from the Cerrado of central Brazil.

5.2 MATERIAL AND METHODS

5.2.1 Fieldwork and acoustic recordings

We recorded the advertisement calls of 15 males of *P. centralis* in eight localities of Goiás state, Brazil (Table 1), between 19:00 h and 00:00 h. We used Marantz PMD660 (Kanagawa, Japan) and Tascam DR-40 (Montebello, California, USA) digital recorders coupled to a Sennheiser K6/ME66 (Wedemark, Alemanha) microphone, with 44,100 Hz and 16-bit sampling. After each recording, we measured SVL of the males with a digital caliper (0.05 mm precision) and weighed them with a digital scale (0.01 g precision). The air temperature at each calling site was recorded with a digital thermometer (0.1°C precision). Voucher specimens are housed at Coleção Zoológica da Universidade Federal de Goiás (ZUFG 9572; ZUFG 9602), and the Fonoteca da Universidade Federal de Goiás holds the recordings (FUFG 1489, FUFG 1490).

Table 1. Localities, dates and temperature of 15 males of *Physalaemus centralis* recorded in the state of Goiás, Brazil.

Municipality	**Latitude**	**Longitude**	**Date**	**SVL (mm)**	**Weight (g)**	**Temperature (°C)**
Rio Verde	-17.800028	-51.084722	8-Dec-2014	33	4.1	19.2
			8-Dec-2014	42.88	4.6	19.4
			8-Dec-2014	33.16	3.2	19.4
Caldas Novas	-17.867116	-48.707992	18-Nov-2014	37.48	5.3	21.7
			18-Nov-2014	37.56	5.4	21.7
Pontalina	-17.558265	-49.568742	5-Dec-1999	37.1	5.2	24.1
			16-Nov-2000	35.3	3.4	26.4
			16-Nov-2000	33.7	3.9	26.4
			17-Nov-2000	36.2	4.8	19.6
Mambaí	-14.491201	-46.118865	15-Oct-2001	-	-	23.8
Aragarças	-15.897208	-52.252014	7-Mar-2000	36.1	4.6	26.3
Jataí	-17.736667	-51.631667	5-Jan-2001	36.3	4.7	23.4
Palmeiras de Goiás	-16.789528	-49.932639	13-Oct-2001	35.3	3.3	22.5
				35.6	3.5	22.5
Serranópolis	-18.30963	-51.955564	Nov-2003	-	-	-

5.2.2 Data analysis

The calls were analyzed using the software Raven Pro 64 1.4, Cornell Lab of Ornithology (Bioacoustics Research Program Workstation, Ithaca, NY, USA). Six temporal parameters were measured in the oscillogram: note duration (ms) (ND), pulse number (PN), pulse duration (ms) (PD), pulse rate (pulses s–1) (PR), silent time interval between calls (ms) (SC), and call rate (calls min–1) (CR). The spectral parameters dominant frequency (Hz) (DF), maximum frequency (Hz) (MaxF), minimum frequency (Hz) (MinF), and frequency amplitude (Hz) (FA) were analyzed in the spectrogram (see Forti et al. 2015). A total of 147 calls from 15 males were analyzed (10 calls for 14 males and seven calls for one male). The values are presented as mean ± standard deviation or mode (for pulse number, which consisted of only integers) followed by range (minimum−maximum). There was no call edition before analysis. The call and pulse rates were obtained by cross-multiplication. For call rate, we multiplied the number of calls in the record by 60 (s), and the result was then divided by the total time of the recording in seconds. For pulse rate, we divided the pulse number of a call by call duration in seconds. Call description and terminology followed Duellman and Trueb (1994) and Gerhardt (1998).

The spectrograms were made with a window size of 256 samples, 75% overlap, hop size of 64 samples, DFT of 1024 samples, and Hanning window type. Default settings were employed for resolution, contrast, and brightness. We obtained both the oscillogram and spectrogram figures using the TuneR version 1.0 (Ligges et al. 2013) and Seewave version 1.7.3 (Sueur et al. 2008) packages of R platform version 3.2.3, 32/64 bit version (R Development Core Team 2015).

The variation of each call parameter was expressed as coefficient of variation [(CV = SD/mean) x 100]. The calculation of the average coefficient of variation per parameter was made using CV values per male (within-individual CV:CV_W) and for all analyzed males (between-individual CV:CV_B). Acoustic parameters with low coefficient of variation (normally below 5%) were classified as static, and parameters with higher coefficients of variation (normally above 12%) were considered dynamic, while those in between were considered intermediate (5%<CV<12%) (Gerhardt 1991). We determined the point of occurrence of the highest variation of the acoustic parameters, both for intra- or interindividual, by calculating the ratio between CV_B and

CV_W (CV_B/CV_W). If $CV_B/CV_W>1$, then the variation of a particular acoustic parameter is higher between individuals than within the individual.

We performed a discriminant function analysis (DFA) to determine whether acoustic parameters of *P. centralis* could be used to distinguish individuals. Before DFA, we adjusted the acoustic parameters to a common air temperature (mean = 22.5°C) in accordance with Kaefer et al. (2012). To reduce the dimensionality of the data, we performed a principal component analysis (PCA), and the PCA scores were used as input variables for the DFA. All statistical analyses were performed using R platform version 3.2.3, 32/64 bit version (R Development Core Team 2015), with significance level set at $p = 0.05$.

5.3 RESULTS

Physalaemus centralis advertisement calls were composed of one multipulsed note with no harmonic structure (Figure 1). There was a modulation in the call frequency, in which the dominant frequency increased from 740.7 to 1246.1 Hz. There were two recognizable parts, the initial part (1/3 of the call) had a call duration of 150 ± 61 ms (81−740 ms; N = 147), pulse number of 13 pulses (6−22 pulses; N = 147), and dominant frequency of 740.7 ± 175.5 (430.7 −947.5 Hz); the final part had a call duration of 294 ± 70 ms (175−448 ms; N = 147), pulse number of 26 pulses (15−41 pulses; N = 147), and dominant frequency of 1246.1 ± 294.1 (775.2−1636.5 Hz).

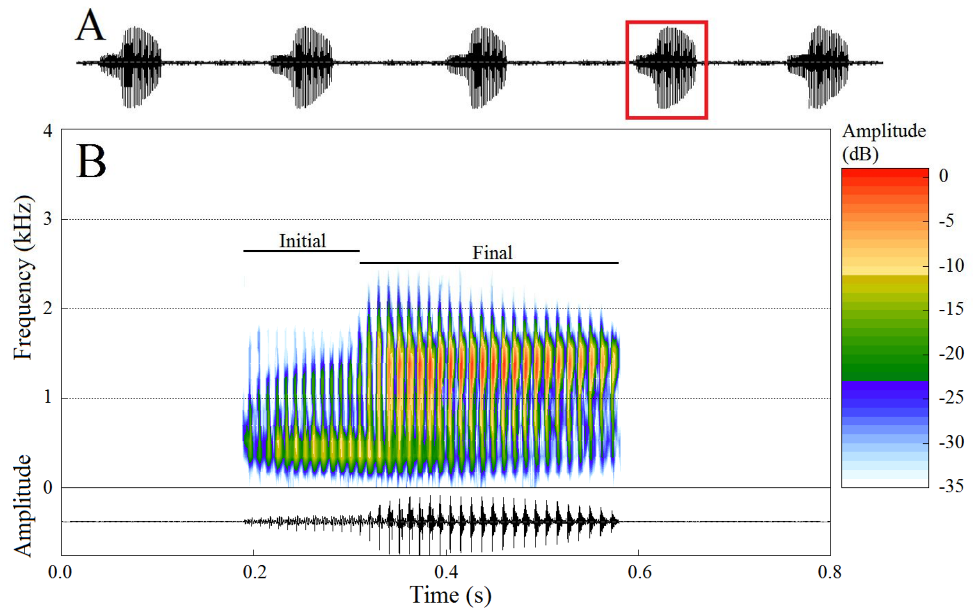

Figure 1. Advertisement call of *P. centralis* from municipality of Caldas Novas, Goiás state. (a) A waveform section (5.3 s) with five calls; (b) Audiospectrogram (above) and respective oscillogram (below) of the fourth call (a note) highlighted by a red outline (air temperature = 21.7°C; air humidity = 94%; SVL = 37.56 mm). The horizontal lines represent the two recognizable parts of the call. Voucher specimen: ZUFG 9572. Voucher recording: FUFG 1489

The advertisement call had a call duration of 440 ± 99 ms (266−686 ms, N = 147 calls), pulse number of 35 (21−58; N = 147 calls), pulse duration of 11 ± 1 ms (7−15 ms; N = 735 pulses), and pulse rate of 90.29 pulses s–1. The silent interval between calls was 871.5 ± 659.7 ms (203−4439 ms; N = 147), and repetition rate was 39.8 ± 16.7 calls per minute (13.8−71.1 calls per minute; N = 15 males). Dominant frequency was 1216.1 ± 254.3 Hz (775.2−1636.5 Hz; N = 147 calls), maximum frequency was 1578.9 ± 115.5 Hz (1378.1 −1894.9 Hz, N = 147 calls), minimum frequency was 613.2 ± 115.1 Hz (334.5−775.2 Hz, N = 147 calls), and frequency amplitude was 965.6 ± 122.2 Hz (602.9 −1205.9 Hz, N = 147 calls) (see Table 2).

Table 2. Advertisement call traits for species of the *Physalaemus centralis* clade reported in different studies. Mean±SD or range. Abbreviations: CD = call duration; PN = pulse number; PR = pulse rate (pulses s–1); SC = silent time interval between calls; DF = dominant frequency; S = presence or absence of harmonics; FM = frequency modulation; H = harmonic structure; N = no harmonic structure; ms = milliseconds.

Species	**CD (ms)**	**PN**	**PR**	**SC (ms)**	**DF (Hz)**	**S**	**FM**	**Reference**
P. biligonigerus group								
P. biligonigerus (Cope, 1861)	700−1000	−	−	−	1800−2800	H	D	Barrio 1965
P. biligonigerus (Cope, 1861)	1049±117.1	−	−	−	568.3±21.6	H	D	Márquez et al. 1995
P. biligonigerus (Cope, 1861)	773.29±92.1	−	−	3523.92±1225.65	1750	H	D	Salas et al. 1998
P. marmoratus (Reinhardt & Lütken, 1862)	−	−	−	−	2640	H	D	Giaretta & Menin 2004
P. riograndensis Milstead, 1960	800−900	−	−	−	1200−6000	H	−	Barrio 1965
P. santafecinus Barrio, 1965	230	−	−	−	1800−3200	H	D	Barrio 1965
P. cuvieri group								
P. albifrons (Spix, 1824)	450±20	−	−	550±210	2820±80	H	D	Pederassi et al. 2015
P. albonotatus (Steindachner, 1864)	1400−1500	−	−	−	2100−4000	H	D	Barrio 1965
P. albonotatus (Steindachner, 1864)	1778.9±114.7	−	−	−	2398.8±33.2	H	−	Márquez et al. 1995
P. albonotatus (Steindachner, 1864)	1704.4±82.1	−	−	−	2230	H	D	Köhler 2000
P. centralis Bokermann, 1962	273.5±16.6	20.8±1.1	77	−	1000−1530	−	−	Padial & Köhler 2001
P. centralis Bokermann, 1962	167.0±34.9	9−17	−	861.48±329.87	965.7±138.4	−	−	Vasconcelos et al. 2014
P. centralis Bokermann, 1962	440±99	21−58	90.3	871.5±659.7	1216.1 ± 254.3	N	A	This work
P. cuqui Lobo, 1993	1580.1 ± 224.8	17−37.4	22.9±2.2	−	2284.3±75.6	H	−	Ferrari & Vaira 2001
P. cuqui Lobo, 1993	1525±260	27−33	−	−	2293−2777	H	−	Iglesias & Natale 2013
P. cuvieri Fitzinger, 1826	200	−	−	−	700	H	D	Barrio 1965
P. cuvieri Fitzinger, 1826	250−350	−	−	−	580−800	H	−	Heyer et al. 1990
P. cuvieri Fitzinger, 1826	240±40	−	−	−	680±70	H	−	Silva et al. 2008

P. cuvieri Fitzinger, 1826	315.2±22.4	−	−	−	400−1100	H	D	Pombal 2010
P. cuvieri Fitzinger, 1826	250±30	−	−	−	730.94±135.41	H	D	Gambale & Bastos 2014
P. ephippifer (Steindachner, 1864)	410±80	−	−	4650±7650	839.8±77.56	H	D	Kaefer et al. 2011
P. erikae Cruz & Pimenta, 2004	560	−	−	13420	3220	H	D	Cruz & Pimenta 2004
P. fischeri (Boulenger, 1890)	722±92	−	−	3810±1100	898±90	H	A/D	Tárano 2001
P. kroyeri (Reinhardt & Lütken, 1862)	700−900	−	−	−	−	H	−	Bokermann 1966a
P. henselii group								
P. fernandezae (Müller, 1926)	500−700	−	−	−	−	H	A	Barrio 1965
P. henselii (Peters, 1872)	200	−	90	−	4800−6200	N	−	Barrio 1965
P. henselii (Peters, 1872)	177±21	13−28	113±18	462±90	3706±132	H	−	Maneyro et al. 2008
P. gracilis group								
P. barrioi Bokermann, 1967	1200−1400	−	−	−	−	H	D	Bokermann 1967
P. barrioi Bokermann, 1967	1240±20	−	−	5.41±0.39	2265.39±15.92	H	D	Provete et al. 2012
P. evangelistai Bokermann, 1967	1000−1200	−	−	−	2000−4000	H	D	Bokermann 1967
P. gracilis (Boulenger, 1883)	900−1000	−	−	−	4000−5000	H	D	Barrio 1965
P. jordanensis Bokermann, 1967	1400−1600	17−19	−	−	−	N	−	Bokermann 1967
P. jordanensis Bokermann, 1967	1068±186	12–24	19.0±1	4940±1698	2742±196	H	−	Giaretta et al. 2009
P. lisei Braun & Braun, 1977	1300±380	−	−	18830±27520	1272±354	H	A	Morais et al. 2012
P. olfersii group								
P. feioi Cassini, Cruz & Caramaschi, 2010	4300±660	30−70	8−16	3720±2740	2560±75	N	A/D	Cassini et al. 2010
P. maximus Feio, Pombal & Caramaschi, 1999	2010±260	−	−	2390±1040	732−775	H	−	Baêta et al. 2007
P. maximus Feio, Pombal & Caramaschi, 1999	2100±190	−	−	2400±550	1230	−	−	Peres & Simon 2012
P. olfersii (Lichtenstein & Martens, 1856)	2000	−	−	−	1000−2000	H	−	Bokermann 1966b
P. olfersii (Lichtenstein & Martens, 1856)	3500±4000	−	200−225	−	−	−	−	Heyer et al. 1990
P. olfersii (Lichtenstein & Martens, 1856)	4035±199	886–1012	234±11	3847±4379	2375±99	N	N	Giaretta et al. 2009
P. olfersii (Lichtenstein & Martens, 1856)	4040±400	−	149±5	2070±800	1920±330	N	N	Cassini et al. 2010
P. olfersii (Lichtenstein & Martens, 1856)	3040±510	−	−	1540±300	1400−2500	−	−	Pombal 2010

P. soaresi Izecksohn, 1965	1830	−	−	−	3300	N	A	Weber et al. 2005
P. lateristriga (Steindachner, 1864)	3600±700	404−990	183±10	1800±800	1680±230	N	N	Cassini et al. 2010
Without defined group								
P. aguirrei Bokermann, 1966	230−250	−	−	500−700	2000−3000	H	D	Bokermann 1966b
P. aguirrei Bokermann, 1966	230	−	−	1.22	3000	H	D	Pimenta & Cruz 2004

Table 3. Within-individual (CVW) and between-individual (CVB) coefficients of variation, CVB<i>/CVW ratios of 15 individuals of *Physalaemus centralis* recorded in eight localities of the Cerrado in central Brazil.

	CV_W	**CV_B**	**CV_B/CV_W**
CD	6.41 (I)	22.15 (D)	3.46
PN	6.20 (I)	18.46 (D)	2.98
PD	4.84 (S)	9.09 (I)	1.84
PR	2.35 (S)	7.24 (I)	3.08
SC	38.66 (D)	75.70 (D)	1.95
DF	1.66 (S)	20.83 (D)	12.55
MaxF	1.59 (S)	7.31 (I)	2.81
MinF	7.72 (I)	18.77 (D)	2.43
FA	5.82 (I)	12.66 (D)	2.18

Call properties are in parentheses: D = dynamic; S = static; I = intermediate. Abbreviations: CD: call duration; PN: pulse number; PD: pulse duration; PR: pulse rate; SC: silent time interval between calls; DF: dominant frequency; MaxF: maximum frequency; MinF: minimum frequency; FA: frequency amplitude.

When considering the within-individual variation (CV_W), the temporal parameters PD and PR were static, while SC was dynamic (Table 3). Regarding spectral parameters, MaxF and DF were static properties (Table 3). When considering the between-individual variation (CV_B), with exception of PD, PR, and MaxF (which were intermediate properties), all other parameters were dynamic (Table 3). The CV_B/CV_W ratio was above 1.0 for all acoustic parameters analyzed, with between-individual variation being 1.82 to 12.55 times higher than within-individual variation.

In the PCA, the first three principal components accounted for 73% of the variation. PCA1 axis explained 42% of the total variation and was mostly influenced by CD (factor score = 0.825), MaxF (factor score = −0.808) and PR (factor score = −0.764); PCA2 axis was influenced by DF (factor score = 0.805), which explained 18% of the variance; and PCA3 axis was influenced by FA (factor score = −0.740) and MinF (factor score = 0.727), which explained 13% of the variance. The first three PCA scores were used as input for DFA. Three discriminant functions (DFun) were generated and all had eigenvalues above 1.0, explaining 100% of the total variation. The first DFun was represented by PCA1 (eigenvalue of 14.85), and explained 68.28% of the variation. The second DFun was represented by PCA2 (eigenvalue of 9.48), and explained 27.82% of the variation, and the third DFun was represented by PCA3 (eigenvalue of 3.55) and explained 3.90% of the variation. The Wilks Lambda was close to 0 (<0.01), indicating that the variables of PCA1 (CD, MaxF and PR), PCA2 (DF) and PCA3 (FA and MinF) were appropriate to discriminate males. The percentage of cases correctly classified was 90.55%.

Males of *P. centralis* were found calling at the edge of temporary or permanent water bodies, always associated with low vegetation, where they remained floating in the water. This species displays explosive breeding, calling during the rainy season (November– February). Daily chorus activity occurs from 19:00 h to 01:00 h. Snout–vent length, weight and air temperature are shown in Table 1.

5.4 DISCUSSION

The advertisement call is the most common vocalization emitted by *P. centralis* males during the breeding season, a pattern commonly observed in anuran amphibians (Lemes et al. 2012; Morais et al. 2012; Gambale & Bastos 2014). The advertisement calls recorded in the present study were similar to those in earlier descriptions by Padial and Köhler (2001) and Vasconcelos et al. (2014), except for call duration, pulse number and pulse rate, which were higher in this study. Although many studies have reported that temperature (Morais et al. 2012), morphological features (Bee & Gerhardt 2001) and social context (Wells 2007) may influence the temporal parameters, they may be stable over time in the same population (Gambale et al. 2014). These parameters (e.g. call duration and pulse rate) can indicate differences between groups of closely related anuran species (Padial et al. 2008; Padial & De La Riva 2009) and can also be used as characters for distinguishing clades (Köhler et al. 2010). Temporal parameters play a significant role in species recognition among leptodactylids, and particularly in the genus *Physalaemus* (Márquez et al. 1995; De la Riva et al. 1997).

The advertisement call of *P. centralis* differs from that of most species of the *P. cuvieri* group, since it lacks harmonic structure. Furthermore, the pulsed feature occurs in *P. cuqui* (*P. cuvieri* group; Iglesias & Natale 2013), *P. jordanensis* (*P. gracilis* group; Bokermann 1967; Giaretta et al. 2009), *P. feioi* (Cassini et al. 2010), *P. olfersii* (Giaretta et al. 2009), and *P. lateristriga* (Cassini et al. 2010), where the last three are members of the *P. olfersii* group. Call duration of *P. centralis* is similar to that of *P. albifrons* (Pederassi et al. 2015), *P. ephippifer* (Kaefer et al. 2011), *P. erikae* (Cruz & Pimenta 2004), and *P. fernandezae* (Barrio 1965); pulse number is similar as in *P. feioi* (Cassini et al. 2010), and pulse rate is similar as in *P. henselii* (Barrio 1965). Dominant frequency of *P. centralis* is similar to *P. lisei* (Morais & Kwet 2012) and *P. maximus* (Baêta et al. 2007; Peres & Simon 2012). Although we can make comparisons between calls, it is important to remember that variation in call parameters may be affected by differences in recording equipment used and methodological procedures used during the analysis. Also, many calls were described in the 1960s (Barrio 1965; Bokermann 1967), where call analysis was limited due the technology available.

Advertisement calls for only 39 of the 47 *Physalaemus* species occurring in Brazil have been described (Ferrari & Vaira 2001; Padial & Köhler 2001; Gambale & Bastos 2014), and other types of calls have been reported for some species (e.g. Gally & Zina 2013; Gambale & Bastos 2014). Since calls can provide insight into the taxonomy validity of former species, differences in acoustic parameters could be very useful to discover cryptic species (e.g. Carvalho & Giaretta 2013). Thus, knowledge of the call parameters of anuran species may be useful in studies that use an integrative taxonomy approach.

Regarding within-individual variation, temporal parameters of anuran calls generally have dynamic properties (Tárano 2001). These dynamic parameters may be associated with environmental and/or social context in which males are inserted (e.g. density of conspecific calling males or presence of females), as found for other anuran species (see Gerhardt 1991; Gerhardt & Huber 2002). The high variability of temporal parameters, as found for the silent time interval between calls in *P. centralis*, allows males to rapidly change their vocal behavior during acoustic interactions (e.g. Castellano et al. 2002). Similarly to other anuran species (Tárano 2001; Briggs 2010;

Rodríguez et al. 2010; Morais et al. 2012), most spectral parameters were static. Spectral parameters (e.g. dominant frequency) are under morphological control, and thus they show low variability (Bee & Gerhardt 2001). Within-individual variation may indicate which acoustic parameters are more variable, while data on between-individual variation suggest the call signals that may be involved in interindividual recognition, sexual selection, and species recognition (Márquez & Eekhout 2006). *Physalaemus centralis* males show higher acoustic variation between individuals than within individuals, which can indicate genetic variance, environmentally induced phenotypic morpho-physiological variance, different adopted tactics, and/or directional sexual selection (Giacoma & Castellano 2001; Morais et al. 2012). This pattern has been found in other anuran species (*Oophaga pumilio* – Pröhl 2003; *Allobates femoralis* – Gasser et al. 2009; *Dendropsophus minutus* – Morais et al. 2012).

Acoustic parameters (CD, PR, DF, MaxF, MinF, and AF) of *P. centralis* are potentially useful in the discrimination of males. The four call parameters selected by DFun1 and DFun2 that showed higher between-individual variation, were also those that had the highest CVB/CVW. Temporal parameters have been reported as important traits for the recognition of individuals in many anuran species (Gasser et al. 2009; Gambale et al. 2014). Spectral parameters such as dominant frequency carry information about body condition of the male, so it is an important parameter for discriminating individuals (e.g. Morais et al. 2012; Gambale et al. 2014). *Physalaemus centralis* is an anuran species that shows explosive reproduction and males are found calling in chorus with many individuals (Brasileiro & Martins 2006). Choruses may increase attraction to females and reduce predation risk due the number of males (Wells 2007), so the higher cost of calling in choruses is worthwhile to offset the difficulty of achieving individual reproductive success; hence, it is important that call signals be able to discriminate individuals.

Previous studies on the advertisement call of *P. centralis* were based on few recordings (one individual for both – Padial & Köhler 2001 and Vasconcelos et al. 2014), which could affect the assessment of a greater bioacoustic variability in future studies. Our data indicated a higher variation in advertisement calls compared with previous reports, enabling the measurement of intra and interindividual differences. In addition, the coefficient of variation for *Physalaemus* species is known only for *P. fischeri* (Tárano 2001) and *P. cuvieri* (Gambale & Bastos 2014). Bioacoustic data have been employed to support the correct designation of the species, as it is a trace of recognition and specific characterization (Carvalho & Giaretta 2013), and also to suggest the most important parameters for mate selection during breeding season, due the features of the call properties (static or dynamic) (Tárano 2001, Gambale & Bastos 2014). It is important to emphasize the importance of further bioacoustics studies on the variability of acoustic trails to improve research in taxonomy and phylogeny, which may provide better identification and description of cryptic species in the *P. cuvieri* clade.

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6 VOCALIZATIONS AND BIOACOUSTIC ANALYSIS OF *Boana jaguariaivensis* (CARAMASCHI, CRUZ, AND SEGALLA, 2010) (ANURA: HYLIDAE)

RESUMO

Os sinais acústicos constituem o principal mecanismo de comunicação em para a maioria dos anfíbios anuros. Neste estudo descrevemos o repertório vocal, a variação do canto de anúncio e o sítio de canto de *Boana jaguariaivensis* de uma população do município de Tibagi, Paraná, sul do Brasil. Dezessete machos foram gravados em dezembro de 2012 e fevereiro de 2013. Os machos de *B. jaguariaivensis* usaram arbustos, ervas e gramíneas como local de vocalização, e os ovos foram depositados em galhos dentro do corpo d'água. O canto de anúncio foi composto de 1–4 notas com estrutura harmônica. O canto B foi composto por uma única nota pulsada com estrutura harmônica. O canto de 'aflição' foi composto por uma única nota harmônica pulsionada. Todos os parâmetros espectrais apresentaram propriedades estáticas, enquanto que todos os parâmetros temporais apresentaram propriedades dinâmicas. Além disso, todas as propriedades acústicas variaram mais entre do que dentro dos indivíduos. A variação observada nos parâmetros acústicos de *B. jaguariaivensis* não foi influenciada pelo tamanho do corpo ou pela temperatura do ar. Dados sobre repertório vocal e fatores que podem alterar os parâmetros acústicos são importantes para o avanço nas pesquisas sobre taxonomia, filogenia e evolução dos anfíbios anuros.

Palavras-chave: Discriminação acústica; Canto de anúncio; Clado *Boana polytaenia*; Cerrado; Coeficiente de variação; *Hypsiboas*; Sul do Brasil.

ABSTRACT

Acoustic signals are the main communication mechanisms in anuran amphibians. Herein is described the vocal repertoire, call variation, and calling site of *Boana jaguariaivensis* from a population of the Tibagi, Paraná State, southern Brazil. Seventeen males were recorded in December 2012 and February 2013. Males of *B. jaguariaivensis* used shrubs, herbs and grass as calling sites, and the eggs were deposited on branches in the water body. The advertisement call was composed of 1–4 notes with harmonic structure. Call B was composed of a single, pulsed note with harmonic structure. The distress call was composed of a single, pulsed harmonic note. All spectral parameters had static properties, whereas all temporal parameters had dynamic properties. Also, all acoustic properties varied more between than within males. The variation observed in the acoustic parameters of *B. jaguariaivensis* advertisement calls was not influenced by body size or air temperature. Data on vocal repertoires and factors that can change acoustic parameters are important for progress in research on the taxonomy, phylogeny and evolution of anuran amphibians.

Key-Words. Acoustic discrimination; Advertisement call; *Boana polytaenia* clade; Cerrado; Coefficient of variation; *Hypsiboas*; Southern Brazil.

6.1 INTRODUCTION

The main communication mechanism in anuran amphibians is vocalization at both intra- and interspecific levels (Morais et al., 2012; Forti et al., 2015; Heard et al., 2015). Acoustic signals play an essential role during the reproductive period (Wells, 2007). Advertisement calls are the main type of vocalization, being species specific and, therefore, considered a pre-zygotic mechanism of reproductive isolation (Duellman and Trueb, 1994; Morais et al., 2012). Aggressive calls are also common in anurans, used by conspecific males to maintain territories and spacing between them (Reichert, 2010; Caldart et al., 2011).

Acoustic signals are remarkable and an important tool for species identification (Bastos et al., 2011; Gambale and Bastos, 2014; Gambale et al., 2014) and, therefore, it is likely that accurate, quantitative descriptions of anuran calls will help in the correct designation of species that are often misidentified due to their similar morphologies (Padial and De La Riva, 2009; Carvalho and Giaretta, 2013). Previous bioacoustics studies have provided important information for taxonomic and phylogenetic studies, uncovering the relationships between taxa and description of cryptic species (Padial and De La Riva, 2009; Carvalho and Giaretta, 2013). Although advertisement call descriptions have been published for many anuran species, the number of recorded males and/ or the number of analyzed calls used in these descriptions is often small, not representing the intraspecific variation (Batista et al., 2015; Forti et al., 2015). Moreover, information about other types of calls besides advertisement calls is needed to improve comparisons between species (Morais et al., 2012; Gambale and Bastos, 2014; Batista et al., 2015).

The *Boana* (= *Hypsiboas*) *polytaenia* clade is composed of 11 species that are distributed throughout central, southeastern and southern Brazil (Caramaschi and Cruz, 2013). These species are recognized as a distinct clade in the *B. pulchella* group (Faivovich et al., 2004; 2005; Caramaschi et al., 2010; Pyron and Wiens, 2011; Caramaschi and Cruz, 2013). Species of this clade have been defined by their small size (snout–vent length [SVL] of 25.6–40.6 mm in males; 29.0–51.6 mm in females), slender body, narrow head, prepollex with a single curved spine, absence of outer metatarsal tubercle, color pattern consisting of longitudinal lines or stripes, and absence of transverse bars or spots on the anterior and posterior surfaces of the thigh and inguinal region (Caramaschi and Cruz, 2013).

Despite the importance of vocalization in taxonomic studies of *Boana* species (Garcia et al., 2007; Köhler et al., 2010; Batista et al., 2015), in the *B. polytaenia* clade only the vocalizations of *B. beckeri* (Caramaschi and Cruz, 2004), *B. botumirim* (Caramaschi et al., 2009), *B. cipoensis* (Lutz, 1968), B. goiana (Lutz, 1968), *B. leptolineata* (Braun and Braun, 1977), *B. phaeopleura* (Caramaschi and Cruz, 2000), and *B. polytaenia* (Cope, 1870) have been described (Batista et al., 2015). *Boana jaguariaivensis* (Caramaschi et al., 2010) (Fig. 1) is characterized by medium size (SVL of 23.9–28.8 mm in males), striped dorsal pattern, and overall similarity to the members of the *B. polytaenia* clade, which supports its inclusion in this group (Caramaschi et al., 2010). Apart from its distinct morphology, *B. jaguariaivensis* is allopatric to all other members of the *B. polytaenia* clade and is currently known only from the type locality (Parque Estadual do Cerrado; Caramaschi et al., 2010).

Data on the natural history of *Boana jaguariaivensis* are scarce and acoustic parameters have not been described (Caramaschi et al., 2010). As mentioned above, knowledge about the acoustic repertoire of anuran amphibians is important for taxonomic and phylogenetic studies. Herein, we describe the vocal repertoire, vocal variability, and calling site of *B. jaguariaivensis*.

Figure 1. Calling male of *Boana jaguariaivensis* (ZUFG 7327) recorded from a population in Tibagi, Paraná state, southern Brazil.

6.2 MATERIAL AND METHODS

6.2.1 Study site

The study was performed at a site in Tibagi, Paraná State, South Brazil, next to Parque Estadual do Guartelá (24°41′14.92″S, 50°12′37.14″W, 1,213 m above sea level). The regional climate corresponds to the Cfa and Cfb temperate climate types of Köppen (Peel et al., 2007), with mean temperatures of 21.1–24.3°C in the warm months (December–February) and 13.8–16.0°C in the cold months (June and July; Peel et al., 2007). Annual rainfall is highly variable, with greater rains December–March (approximate means of 150 mm in March and 230 mm in January), decreasing in subsequent months to July and August when lowest rainfall occurs (approximate means of 75 and 66 mm, respectively; Instituto Ambiental do Paraná, 2002).

6.2.2 Data analysis

Seventeen males of *Boana jaguariaivensis* were recorded in December 2012 and February 2013. Vocalizations were obtained from 19:00–00:00 h (disregarding daylight saving time) and recorded at a sampling rate of 44.1 kHz and 16 bit resolution (WAVE file format) using a Marantz PMD 222 recorder coupled to a Sennheiser ME66 microphone positioned ca. 50 cm from the calling male. After each recording session, SVL and mass of the recorded male were measured with digital calipers (accurate to 0.05 mm) and a digital scale (accurate to 0.01 g), respectively. Air temperature and relative humidity were measured with a digital thermo-hygrometer (precision to ± 1°C and ± 3% RH). Measurement of calling site height, the distance between the recorded calling male and the closest conspecific calling male, and the distance from the calling male to the edge of the water body were taken using a tape measure (accurate to 1 mm). Sound intensity (sound pressure level, SPL) of 10 calls (from 10 males) was measured with a Minipa digital decibel meter (fast setting) at a distance of 50 cm from the calling male. All sound level measurements are expressed as dB SPL, with reference to 2 × 10^{-5} Pa.

Calls were analyzed on a personal computer using the Raven Pro software, version 1.5, 64-bit version (Bioacoustic Research Program, 2012). The Raven Pro settings were as follows: window size = 256 samples; window type = Hanning; overlap = 50%; DFT size = 256 samples, grid spacing = 188 Hz. Sound figures were obtained using the Seewave version 1.6.4 package (Sueur et al., 2008) of the R platform, version 3.3.2, 64-bit version (R Development Core Team, 2016). The Seewave settings were as follows: window name (Fourier transform window) = Hanning; window length = 256 samples; overlap = 80%. Individual specimens were killed with 5% lidocaine, fixed with 10% formalin and preserved in 70% alcohol. Five voucher specimens were deposited in the Zoological Collection of the Federal University of Goiás (ZUFG 7327–7331). Recordings are stored in the acoustic collection of the Federal University of Goiás (FUFG 1514–1515; FUFG 1521–1535).

Acoustic terminology follows Duellman and Trueb (1994), Gerhardt (1998), and Toledo et al. (2014). Were analyzed six advertisement calls from each individual (n = 102 calls analyzed), eight Call B calls from five males, and four distress calls from one male of *Boana jaguariaivensis*. Nine temporal parameters were measured from the oscillogram: call duration (s), number of notes, note duration (s), number of pulses per call, pulse duration (s), silent interval between calls (s), interval between notes (s), interval between pulses (s), and call rate (calls/min). Spectral parameters were obtained by selecting three variables in Raven 1.5: (1) Frequency 5% (Hz) and (2) Frequency 95% (Hz), where these two parameters included the lowest and highest frequencies, ignoring the upper and lower 5% of the frequency band formed by the energy distribution, and (3) Peak Frequency (Hz). Measurements are presented as the mean ± SD (minimum–maximum) or mode (for number of pulses).

For each bioacoustic parameter, variation was expressed as a coefficient of variation (CV), where CV = (SD/ mean) x 100. For each male, the CV of each bioacoustic parameter was calculated considering all of the individual call samples. The mean CV per parameter was determined from CV values per male (CV within males, CV_W) and pooled for all analyzed males (CV between males, CV_B). Acoustic parameters with low CV (normally below 5%) were classified as static; parameters with higher CV (normally above 12%) were considered dynamic, and other values were considered intermediate (5% < CV < 12%; Gerhardt, 1991).

The point of occurrence of the highest variation of the acoustic parameters, both intra- and interindividual, was determined by calculating the ratio between CV_B and CV_W (i.e., CV_B/CV_W), where values > 1 mean that the variation of a particular acoustic parameter is higher between males than within males. A high ratio indicates acoustical parameters that might function in individual recognition, as demonstrated in previous studies (Jouventin et al., 1999; Bee et al., 2001). Multiple regression analyses were performed to test the influence of air temperature (°C) and body size (obtained by multiplying the SVL by mass) on the acoustic parameters of the advertisement call. Before performing the analyses, all variables were tested for homoscedasticity and normal distribution, and log-transformed when necessary (Zar, 1999). Each acoustic parameter was used as response variable and air temperature and body size were the predictor variables. All statistical analyses were performed in R 3.3.2, 64-bit version (R Development Core Team, 2016), using the significance level of $p \leq 0.05$.

6.3 RESULTS

Males of *Boana jaguariaivensis* used shrubs, herbs, and grass as calling sites, at a perch height of 46.82 ± 13.65 cm (25–70 cm; n = 17 males). The distance between calling males was 391.59 ± 106.17 cm (140– 550 cm; n = 17 males) and the distance from males to the edge of the water body was 375.35 ± 143.21 cm (95– 635 cm; n = 17 males). SVL and body mass of the recorded males were 27.77 ± 1.41 mm (25.17–29.99 mm; n = 17 males) and 0.89 ± 0.18 g (0.7–1.4 g; n = 17 males), respectively. During field work, air temperature and humidity were 22.97 ± 2.60°C (17.7–25.5°C) and 85.59 ± 6.55% (73–95%), respectively.

Individuals of *Boana jaguariaivensis* were found at a human-made pond with clear water and scrub as marginal vegetation. Around this pond other species were also observed, including *Dendropsophus minutus* (Peters, 1872), *Phyllomedusa tetraploidea* Pombal and Haddad, 1992, *Scinax* cf. *fuscovarius* (Lutz, 1925), *Scinax perereca*

Pombal et al., 1995, *Scinax* sp. (gr. ruber), *Leptodactylus* aff. *latrans* (Steffen, 1815), and *Leptodactylus gracilis* (Duméril and Bibron, 1840). During fieldwork, two pairs of *B. jaguariaivensis* were observed in amplexus perched in the marginal vegetation and their eggs were deposited on a branch in the water. The egg clutch is a gelatinous mass of eggs with pigmented animal pole (Fig. 2). This was the second record of a population of *B. jaguariaivensis* and the first for Tibagi, Paraná State, Brazil, extending the species distribution approximately 78 km southwest from the type locality (Caramaschi et al., 2010).

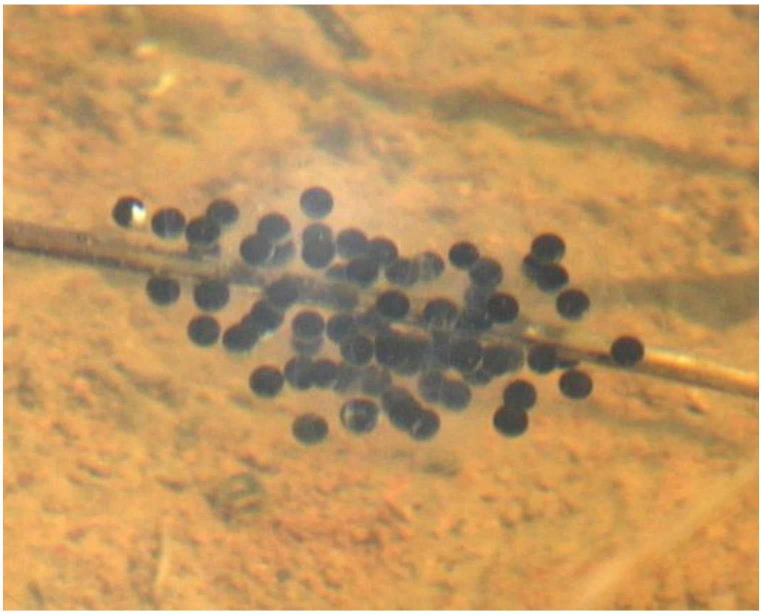

Figure 2. Clutch of *Boana jaguariaivensis* deposited on a branch in the water (photographed on the first day after clutch deposition) in Tibagi, Paraná state, southern Brazil.

Males of *Boana jaguariaivensis* emitted three call types. The advertisement call (Fig. 3) was composed of 1–4 pulsed notes with harmonic structure (Table 1). The duration of the first note was 0.037 ± 0.010 s (0.008– 0.060 s; n = 102 notes) with the number of pulses ranging from 1–3 (3 pulses; n = 102 notes). The duration of the second and third notes was 0.043 ± 0.017 s (0.009– 0.082 s; n = 97 notes) and 0.043 ± 0.012 s (0.010–0.079 s; n = 51 notes), respectively, with the number of pulses per note being 1–5 (2 pulses; n = 97 notes) and 1–5 (3 pulses; n = 51 notes), respectively. The duration of the fourth note was 0.034 ± 0.013 s (0.005–0.047 s; n = 14 notes) with the number of pulses per note ranging from 1–3 (2 pulses; n = 14 notes). The advertisement call presented 3–5 well-defined harmonics, but calls with three harmonics were most common (the first is the dominant frequency; Fig. 1, Table 1). The advertisement call was the most common call type and was emitted as a single call or in groups of 2–15 calls. Advertisement calls with one note were emitted by 12 males (4.63% of all calls), and all males emitted calls with two and three notes (25.69 and 66.44% of all calls, respectively); only seven males emitted advertisement calls with four notes (0.93% of all calls).

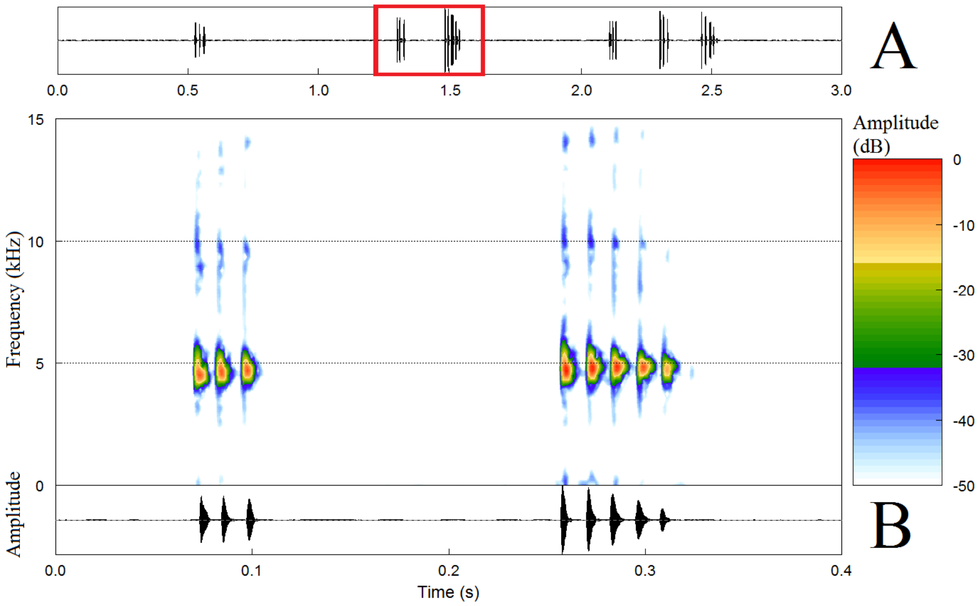

Figure 3. Advertisement call of *Boana jaguariaivensis* from Tibagi, Paraná state, southern Brazil. (A) Waveform of a series of three calls with different note numbers (one, two and three). (B) Spectrogram (above) and corresponding oscillogram (below) of the second call (two notes; red box; air temperature = 25.5°C; air humidity = 73%; SVL = 26.45 mm). Specimen not collected; recording FUFG1515.

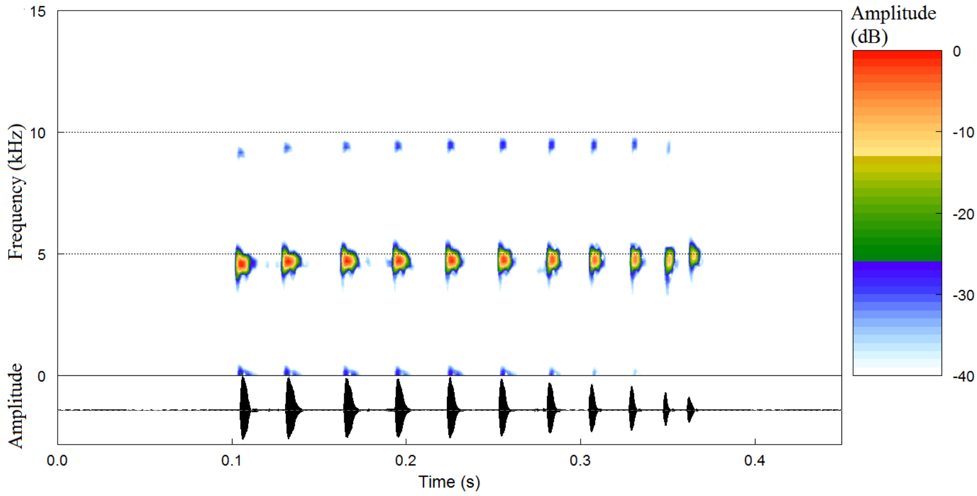

Figure 4. Call B of *Boana jaguariaivensis* from Tibagi, Paraná state, southern Brazil. Spectrogram (above) and corresponding oscillogram (below; air temperature = 19.5°C; air humidity = 95%; SVL = 28.96 mm). Specimen not collected; recording FUFG1535.

Table 1. Call parameters, within-male (CV$_{\mathrm{W}}$) and between-male (CV$_{\mathrm{B}}$) coefficients of variation, and CV$_{\mathrm{B}}$/CV$_{\mathrm{W}}$ ratios of *Boana jaguariaivensis* recorded at Tibagi, Paraná State, Brazil. Abbreviations of the call properties: CD = call duration; ND = note duration; NN = note number per call; PD = pulse duration; PN = pulse number; Ical = silent interval between calls; Inot = interval between notes; Ipul = interval between pulses; CR = call rate; DF = dominant frequency (1st harmonic); MaxF = maximum frequency; MinF = minimum frequency; Harm2 = 2nd harmonic; Harm3 = 3rd harmonic; SPL = sound pressure level.

Call	Acoustic parameters	mean ± SD (range, males/calls)	CV$_{\mathrm{W}}$	CV$_{\mathrm{A}}$	CV$_{\mathrm{B}}$/CV$_{\mathrm{W}}$
Advertisement call	CD (s)	0.271±0.106 (0.021–0.508, N=17/102)	35.358	39.011	1.103
	ND (s)	0.040±0.014 (0.005–0.082, N=17/253)	18.091	25.785	1.425
	NN	2.471±0.625 (1–4, N=17/102)	22.336	25.284	1.132
	PD (s)	0.010±0.003 (0.003–0.020, N=17/670)	14.637	20.104	1.373
	PN	7.167±0.983 (13.719–20.951, N=17/102)	20.951	25.619	1.223
	Ical (s)	2.417±3.385 (0.206–33.980, N=17/102)	60.896	140.034	2.3
	Inot (s)	0.110±0.047 (0.003–0.204; N=17/173)	18.378	26.531	1.444
	Ipul (s)	0.010±0.008 (0.001–0.104, N=17/393)	26.336	52.35	1.988

Call	Acoustic parameters	mean ± SD (range, males/calls)	CV_W	CV_A	CV_B/CV_W
	CR (calls/min)	23.265±7.0 (14.50–34.930, N=17/17)	–	–	–
	DF (Hz)	4704.044±129.536 (4500–4875, N=17/102)	1.596	2.754	1.726
	MaxF (Hz)	5000±112.97 (4687.50–5250, N=17/102)	0.981	2.259	2.303
	MinF (Hz)	4457.72±117.72 (4125–4687.50, N=17/102)	1.39	2.641	1.9
	Harm2 (Hz)	9500.00±311.45 (8812.50–10125, N=17/102)	–	–	–
	Harm3 (Hz)	13661.76±774.22 (11250–14812.50, N=17/102)	–	–	–
	SPL (dB)	81.12±3.58 (59.20–91.20, N=10/100)	–	–	–
Call B	CD (s)	0.278 ±0.072 (0.170–0.426, N=5/8)	–	–	–
	PN	8.750±1.928 (7–12, N=5/8)	–	–	–
	PD (s)	0.008±0.001 (0.003–0.012, N=5/71)	–	–	–
	Ipul (s)	0.023±0.016 (0.003–0.0677, N=5/61)	–	–	–
	DF (Hz)	4687.5±100.223 (4500–4875, N=5/8)	–	–	–
	MaxF (Hz)	4968.750±100.223 (4875–5062.5, N=5/8)	–	–	–
	MinF (Hz)	4382.813±97.040 (4312.5–4500, N=5/8)	–	–	–
	Harm2 (Hz)	4382.813±97.040 (4312.5–4500, N=5/8)	–	–	–
	Harm3 (Hz)	4382.813±97.040 (4312.5–4500, N=5/8)	–	–	–
Distress call	CD (s)	0.235±0.058 (0.174–0.302, N = 1/4)	–	–	–
	PN	350.50±87.73 (225–429, N = 1/4)			
	DF (Hz)	11812.50±2286.17 (10125–15187.50, N=1/4)	–	–	–
	MaxF (Hz)	14859.38±1622.90 (12750–16312.50, N=1/4)	–	–	–
	MinF (Hz)	6843.75±1087.93 (5437.50–8062.50, N=1/4)	–	–	–

Call B was composed of a pulsed note with harmonic structure (Fig. 4) and was emitted during aggressive interactions between conspecific males vocalizing close to each other (< 3 m). It's structure was similar to that of the advertisement call but seemed to be a fusion of the notes of a composite advertisement call, showing a longer call duration (Table 1). Call B presented 2–4 well-defined harmonics.

Distress calls were emitted with an open mouth by one male when it was captured by a researcher in the field. This call was composed of a single, pulsed harmonic note (Table 1). In the oscillogram, the call is bell-shaped, with ascending frequency modulation at the start and slowly descending frequencies until the end of the call (Fig. 5). In the four recorded distress calls, the dominant frequency was found in the fourth, fifth, and sixth harmonics and there were at least seven clearly defined harmonics.

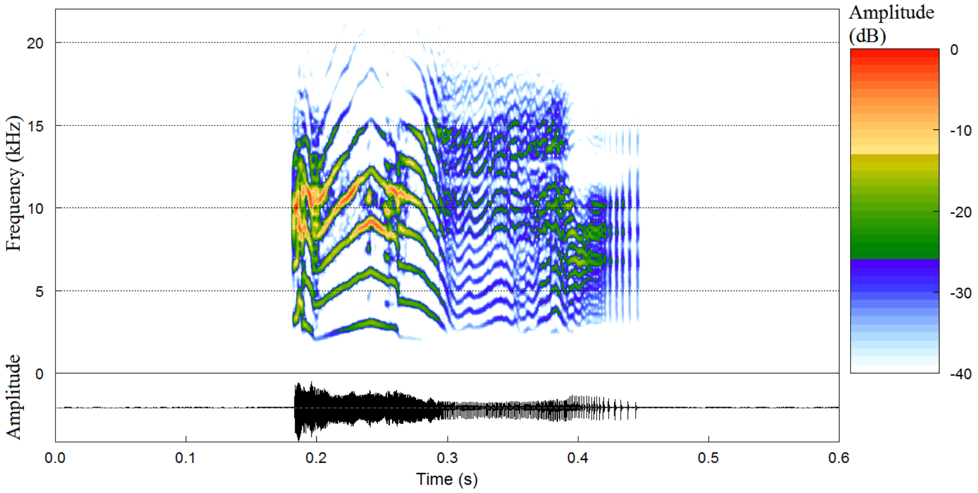

Figure 5. Distress call of *Boana jaguariaivensis* from Tibagi, Paraná State, South Brazil. Spectrogram (above) and respective oscillogram (below) (air temperature = 24°C; air humidity = 89%; SVL = 27.69 mm). Voucher specimen ZUFG 7518; recording FUFG1514.

Considering the variation of the acoustic parameters within individuals (CV_W) and between individuals (CV_B), all spectral parameters had static properties, while all temporal parameters had dynamic properties (Table 1). Additionally, all acoustic properties varied more between than within males, with CV_B/CV_W ratios > 1 (Table 1). The maximum frequency and the interval between calls were the main acoustic parameters that discriminated individuals, due to the high CV_B/CV_W ratio. Air temperature and body size did not have a statistically significant influence on call parameters ($p > 0.05$).

6.4 DISCUSSION

The advertisement call of *Boana jaguariaivensis* was similar to the calls of *B. caingua* (Carrizo, 1991), *B. caipora* (Antunes et al., 2008), *B. goiana*, B. phaeopleura, and *B. riojana* (Koslowsky, 1895) in terms of call duration and note number (Guimarães et al., 2001; Antunes et al., 2008; Köhler et al., 2010; Pinheiro et al., 2012; Batista et al., 2015), although it showed a shorter note duration than *B. caingua* (0.097–0.143 s; Batista et al., 2015), a longer note duration than *B. phaeopleura* (0.012–0.058 s; Pinheiro et al., 2012) and an increased note number compared with *B. caipora* (1–2; Antunes et al., 2008). Note duration in *B. jaguariaivensis* is similar to that of *B. leptolineata* (0.040– 0.080, Kwet, 2001) and call duration is longer than in *B. botumirim* (0.037–0.064; Caramaschi et al., 2009) and *B. leptolineata* (0.180–0.220, Kwet, 2001). As only note number and pulse rate are known for *B. cipoensis* (see Batista et al., 2015), it was not possible to make a complete comparison with the calls of this species. The dominant frequency was similar to that of *B. palaestes* (Duellman et al., 1997) (4,430–4,450 Hz; Duellman et al., 1997), being higher than in most species of the *B. pulchella* group (Batista et al., 2015), possibly as a result of the smaller body size of *B. jaguariaivensis*. Only *B. beckeri* (6,890–7,320 Hz; Acioli and Toledo, 2008) and *B. polytaenia* (5,075–8,057 Hz; Pinheiro et al., 2012) have higher dominant frequencies than *B. jaguariaivensis* in the *B. pulchella* group (Batista et al., 2015). The vocalizations of *B. jaguariaivensis* had a similar call rate as those emitted by *B. riojana* (21–24; Duellman et al., 1997). As in most species in the *B. pulchella* group, the calls showed a pulsed structure (Batista et al., 2015).

Call B of *Boana jaguariaivensis* was similar to the aggressive calls of *B. ericae* (Caramaschi and Cruz, 2000) in call duration (0.103–0.560 s; Garcia and Haddad, 2008), note number (1), and pulse number (2–11), but it had a higher dominant frequency (2,120–3,540 in B. ericae). Like the Call B of *B. ericae* (Garcia and Haddad, 2008) and Call C of *B. polytaenia* (Pinheiro et al., 2012), the call B of *B. jaguariaivensis* had a structure similar to the advertisement call, but only males close to each other or in aggressive interactions with other calling males emitted this call type. The vocal repertoire of anuran amphibians is composed of different types of calls emitted in different social contexts (Toledo et al., 2014). For species of the *B. pulchella* group the aggressive calls can be shorter or longer than the advertisement calls (Batista et al., 2015).

The distress call is a loud scream emitted during subjugation by a potential predator (Toledo et al., 2014). This type of call has been described for other *Boana* *pulchell*a species (*B. bischoffi*, Toledo and Haddad, 2009; *B. caingua*, Toledo and Haddad, 2009; and *B. caipora*, Antunes et al., 2008) and can be emitted to warn conspecifics of predator presence, to surprise (frighten) auditively oriented predators or to attract other animals (Toledo et al., 2014). Call duration is similar to that of the distress call of *B. bischoffi* (0.290 ± 0.060; Toledo and Haddad, 2009) and the dominant frequency is the highest among all known distress calls of the genus *Boana* Gray, 1825.

The most useful diagnostic call parameter in *Boana jaguariaivensis* that enables this species to be distinguished from others of the *B. pulchella* group is the dominant frequency. Spectral parameters are known to be the most reliable feature for differentiating closely related frog species because they are associated with body size, which is generally a static parameter (Morais et al., 2012; Gambale et al., 2014).

None of the studied measured acoustic parameters were influenced by temperature and body size. In Neotropical anurans, the influence of temperature on acoustic parameters is more subtle and difficult to detect (Pröhl, 2003; Morais et al., 2012; Gambale et al., 2014). No influence of morphological traits or temperature on the temporal parameters of the calls has been found for other anurans (Pröhl, 2003; Giasson and Haddad, 2006; Bastos et al., 2011; Morais et al., 2012; Gambale and Bastos, 2014). The absence of body size influence on the spectral parameters may be because there is not a lot of body size variation among males (Bee et al., 2001), because males fake the acoustic signals in an attempt to disguise their size and physical strength (Wagner, 1989), or because of other complex social interactions.

On the basis of CV values within individuals, all temporal parameters of the calls were classified as dynamic, whereas all spectral parameters were static. Temporal parameters varied more than spectral parameters, because they are dependent on social interactions between individuals within the chorus and the density of calling males (Bastos et al., 2011; Gambale et al., 2014). Spectral parameters are associated with species recognition (Morais et al., 2012) and are directly related to body size (Bastos et al., 2011; Gambale et al., 2014). Within-individual variation can provide information about more variable acoustic parameters, whereas between-individual variation suggests call parameters involved in inter-individual recognition, sexual selection and species recognition (Márquez and Eekhout, 2006; Gasser et al., 2009). Greater call variation among than within individuals could be a result of genetic variation, environmentally induced phenotypic morpho-physiological variation, different tactics adopted, and/or directional sexual selection (Giacoma and Castellano, 2001). However, if social interactions favor changes in temporal parameters, variation suggests the existence of individual discrimination between calling males. The CV_B/CV_W ratio for all call parameters was similar to those found within *Allobates femoralis* (Boulenger, 1884) (0.68–1.85; Gasser et al., 2009) and *Oophaga pumilio* (Schmidt, 1857) (1.2–1.7; Bee et al., 2001). Thus, the CV_B/CV_W ratio suggests a potential for neighborstranger discrimination, although behavioral experiments are necessary to confirm this notion. As individual discrimination in a chorus is based on a subset of signal properties that varies between males (Bee et al., 2001; Gasser et al., 2009), both temporal and spectral call parameters are important for discriminating individuals in *B. jaguariaivensis*.

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7 NIGHTLY CALLING PATTERN, ACOUSTIC AND SPATIAL SEGREGATION OF THREE SYMPATRIC *Dendropsophus* SPECIES (ANURA: HYLIDAE)

RESUMO

Durante a época de reprodução, os machos da maioria das espécies de anuros cantam para atrair as fêmeas. Diferentes estratégias comportamentais foram selecionadas para minimizar os custos energéticos associados à atividade de vocalização e evitar a sobreposição do nicho acústico e espacial. Neste estudo nós investigamos as estratégias de vocalização de três espécies de *Dendropsophus* simpátricas (*D. minutus*, *D. rubicundulus* e *D. cruzi*) durante a noite. Foram testadas quatro hipóteses: (i) os machos emitem cantos mais agressivos no início da noite, quando estão estabelecendo seus territórios; (ii) a atividade acústica possui um período máximo, quando as fêmeas estão chegando no sítio reprodutivo; (iii) os parâmetros do canto mais dependentes do meio social atingem valores máximos durante o pico de atividade, enquanto os dependentes da morfologia permanecem estáveis; e (iv) as espécies estudadas apresentam partição acústica e segregação do sitio de canto para evitar interferências sonoras. Em seus habitats naturais, os machos das três espécies de *Dendropsophus* foram gravados individualmente durante cinco sessões consecutivas ao longo da noite (a cada 30 min) e os parâmetros dos cantos foram analisados. Como esperado, as espécies estudadas apresentaram partição do espaço acústico, bem como dos sítios de canto. Nossos resultados também confirmaram que *D. minutus* apresentou variação no repertório vocal, com mais cantos agressivos no início da noite, quando os machos estão estabelecendo seus territórios, seguido de uma diminuição da atividade que pode estar relacionada a um efeito de habituação. Além disso, todas as espécies estudadas modificaram seus parâmetros acústicos durante a noite, o que pode estar associado a preferências das fêmeas ou a estratégias de prevenção de sobreposição acústica. As variações temporais observadas em *Dendropsophus* sugerem uma dinâmica social complexa, com ampla plasticidade no comportamento de vocalização das espécies durante o período reprodutivo.

Palavras-chave: sobreposição de nicho, sítio de canto, espaço acústico, segregação espacial, estratégia reprodutiva.

ABSTRACT

During breeding season, males of most anuran species call to attract females. Different behavioral strategies have been selected to minimize energetic costs associated with calling activity and to avoid acoustical and spatial niche overlap. We investigated the calling strategies of three sympatric *Dendropsophus* species (*D. minutus*, *D. rubicundulus*, and *D. cruzi*) throughout the night. Four hypothesis were tested: (i) males emit more aggressive calls in the beginning of the night, when they are establishing calling sites; (ii) calling activity is particularly concentrated at a peak period, when females are arriving in the calling site; (iii) social-dependent call parameters reach maximum values during the activity peak, whereas morphological-dependent ones remain stable; and (iv) the studied species show acoustic partitioning and segregation of calling site to avoid sound masking interference. In natural settings, males of the three sympatric *Dendropsophus* species were individually recorded for five consecutive sessions throughout night (every 30 min) and call parameters were analyzed. As expected, studied anurans used species-specific portions of the acoustic space as well as calling sites, with no acoustic and spatial niche overlap between them. Our results also confirmed that *D. minutus* presented variation in the calling repertoire, with more aggressive calls in the beginning of the night, when males are establishment their calling sites, followed by a decrease in activity that may be related to a habituation effect. Moreover, all study species modified their call parameters throughout night, which could be associated with female preferences or strategies of avoidance of acoustic overlap. We demonstrated that temporal variations observed in *Dendropsophus* suggest a complex social dynamic, with broad plasticity in the vocal behavior of the species during the breeding period.

Keywords: niche overlap, calling site, acoustic space, spatial segregation, reproductive strategy.

7.1 INTRODUCTION

Acoustic communication is one of the main ways of sending and receiving information among animals in different social contexts (Toledo et al., 2015; Köhler et al., 2017). The emission of acoustic signals involves multiple factors that are associated with energetic costs (Gerhardt and Huber, 2002; Wells, 2007). A number of motor systems contribute to song production (buccal floor and respiratory musculature) and modification (vocal sac and body movements; Duellman and Trueb, 1994; Wells, 2007). Calling activity must therefore increase the metabolic requirements of the caller, once muscular activity requires energy (Gerhardt and Huber, 2002). During the breeding season, males of most anurans call at high rates (Gerhardt and Huber, 2002; Wells, 2007). It is expected that males choose the best opportunities to produce vocalizations while minimizing energetic costs (Gerhardt and Huber, 2002; Ophir, Schrader, and Gillooly, 2010; Brepson, Voituron, and Lengagne, 2013).

Calls play an important role in establishing and defending territories, in conspecific and individual recognition and in attracting mates (Lemes et al., 2012; Morais et al., 2012; Köhler et al., 2017). Thus, some acoustic parameters may indicate the current physical condition of the calling male, reflecting developmental conditions (Gerhardt and Huber, 2002; Wells, 2007; Köhler et al., 2017). Consequently, calling behavior should be subject to strong sexual selective forces (Wilkins, Seddon, and Safran, 2013; Köhler et al., 2017). Furthermore, acoustic parameters may be influenced by morphological traits and many social and environmental variables (e.g. Lemes et al., 2012; Morais et al., 2012; Gambale and Bastos, 2014). The spectral parameters (e.g. dominant frequency) of the calls are usually correlated with body size, while temporal parameters (e.g. call duration) are more influenced by environmental factors, such as air temperature (Lemes et al., 2012; Morais et al., 2012; Gambale and Bastos, 2014). Vocal behavior, morphological traits of the individuals and the nearest distance between males are important to determine calling sites and to increase the reproductive success (e.g. Bertram, Berrill, and Nol, 1996; Wogel, Abrunhosa, and Pombal Jr., 2002; Hoffman et al., 2010). Males should establish themselves in the calling sites at the beginning of the night, defending their territories before females arrive in the reproductive site (Bastos et al., 2011; Dias, Prado, and Bastos, 2017). The selection of calling sites influences sound propagation, which may affect the capacity of female attraction (Kime, Turner, and Ryan, 2000; Llusia et al., 2013). Besides, females often choose breeding partners based specially on calling traits (Márquez and Bosch, 1997; Tárano and Fuenmayor, 2013).

As calls emitted by males may be highly variable within- and between individuals (Morais et al., 2012; Gambale and Bastos, 2014), the variation in call activity throughout the night may be important to determine reproductive success during breeding season (Brepson, Voituron, and Lengagne, 2013; Dias, Prado, and Bastos, 2017). The analysis of entropy derived from information theory (Shannon and Weaver, 1949), used to investigate the complexity of communication signals (Lengagne et al., 1999; da Silva et al., 2006; Morais et al., 2012), and the duty cycle, which is an estimate of call effort per hour (Wilson and Mennill, 2011), may also be good proxies to investigate the nightly calling pattern, since these parameters provide a more accurate quantitative approach of highly variable acoustic signals.

In large aggregations of anuran amphibian communities of the tropical region, males face a strong competition to find mates for reproduction (Wells, 2007). In syntopic sympatric anurans (that breed in the same site), heterospecific calls represent a noise that may interfere in the acoustical signal detection by conspecific males and females (Brumm and Slabbekoorn, 2005; Bee, 2008; Vieira, Batista, and Bastos, 2016). Thus, males may adjust both their calls and their temporal patterns of calling activity as well as their calling sites in order to minimize sound masking interference (Martins, Almeida, and Jim, 2006).

The habituation effect (Thompson and Spencer, 1966) and the energetic constrain (Bevier, 1997) have been proposed to explain the intra-individual variation in acoustic signals along the night (Reichert, 2010; Dias, Prado, and Bastos, 2017; Brepson, Voituron, and Lengagne, 2013). As anuran species vary in their calling sites, seasonal and daily activity, and thus in the energetic cost spent to vocalize, they may have different strategies during breeding season to reduce the costs of calling activity (Ophir, Schrader, and Gillooly, 2010; Brepson, Voituron, and Lengagne, 2013; Jansen, Masurowa, and O’Hara, 2016; Dias, Prado, and Bastos, 2017). Furthermore, there are few studies that investigated variation of the call parameters throughout the night in anurans (e.g. Runkle et al., 1994; Castellano and Gamba, 2011; Brepson, Voituron, and Lengagne, 2013; Jansen, Masurowa, and O'Hara, 2016; Dias, Prado, and Bastos, 2017).

To investigate the calling strategies of three sympatric *Dendropsophus* species throughout the night, we tested the following hypothesis: (i) males emit more aggressive calls (and also complex calls) in the beginning of the night, when they are establishing calling sites, than in subsequent periods; (ii) calling activity is particularly concentrated at a peak period, when females are arriving in the calling site; (iii) social-dependent call parameters (temporal domain) reach maximum values during the activity peak, whereas morphological-dependent ones (spectral domain) remain stable; and (iv) the studied species show acoustic partitioning and segregation of calling site to avoid sound masking interference.

7.2 MATERIAL AND METHODS

7.2.1 Study site

Calling behavior of the study species was examined in three permanent ponds, located at the municipality of Caldas Novas, Goiás State, Brazil (Appendix A), during January and February 2015, January and March 2016 and February 2017. This period was chosen because males of the three species were found in calling activity at the study site. The study area is dominated by grasslands and pastures with disperse shrubs and small trees. The local climate is tropical with two distinct seasons (wet summer and dry winter), classified as Aw in the Köppen classification system (Peel, Finlayson, and McMahon, 2007). The dry season occurs during the coldest months, from May to September, while the rainy season in the warmest ones, from October to April. The monthly mean temperaturewas 24.54ºC, the monthly mean relative humidity was 73.02% and the monthly mean rainfall was 231.76 mm (averages obtained for the sampled months; INMET, 2017).

7.2.2 Focal species

Three species of the genus *Dendropsophus* were selected as study species. This choice was due to their ecological similarities and to reproduce in the same environment and during the same period (Pombal and Bastos, 1998; Morais et al., 2012; Teixeira and Giaretta 2015). All species have prolonged reproduction and social behavior is mediated mainly by acoustic signals (Morais et al., 2012; Teixeira and Giaretta, 2015; Tessarolo et al., 2016). The three species usually select shrubs and herbaceous around ponds as calling sites, they are typically found in open areas and males may be found in large abundance (Cardoso and Haddad, 1984; Pombal Jr. and Bastos, 1998; Teixeira and Giaretta, 2015).

Dendropsophus minutus (Peters, 1872) is a small-sized hylid (3.14±1.36 mm in males; Morais et al., 2012; Figure 1A) widely distributed in South America (Frost, 2017). The first studies to describe their vocalizations were Bokermann (1967) and Barrio (1967). Cardoso and Haddad (1984) investigated the acoustic variability in different populations and Morais et al. (2012) studied the intra- and inter-individual coefficient of variation and factors that may affect the acoustic signals. In this species, vocal repertoire is composed by three different notes (typically termed as A, B and C) that can be emitted separately (single calls) or combined (complex calls). Each note has a distinct function related to the social context in which the individuals are inserted (Morais et al., 2012; Toledo et al., 2015). The note A is mainly used to attract females, while the notes B and C are used in aggressive interactions, especially in breeding sites with high density of males (Haddad, 1987; Toledo et al., 2015).

Dendropsophus rubicundulus is a small-sized species (18.0─23.4 mm in males and 21.6─25.1 mm in females; Napoli and Caramaschi, 1999; Figure 1B) widely distributed in Brazil (Frost, 2017). The call was first described by Cardoso and Vielliard (1985) and later by Napoli and Caramaschi (1999). Teixeira and Giaretta (2015) re-described the call, comparing it with other species of the *D. microcephalus* group. *Dendropsophus cruzi* is a small-sized hylid (18±0.9 mm for males and 22.9±1.1 mm for females) endemic of the Cerrado (Pombal and Bastos, 1998; Frost, 2017; Figure 1C). The advertisement call was firstly described by Pombal Jr. and Bastos (1998). Tessarolo et al. (2016) studied the geographic variation in the advertisement call of ten populations.

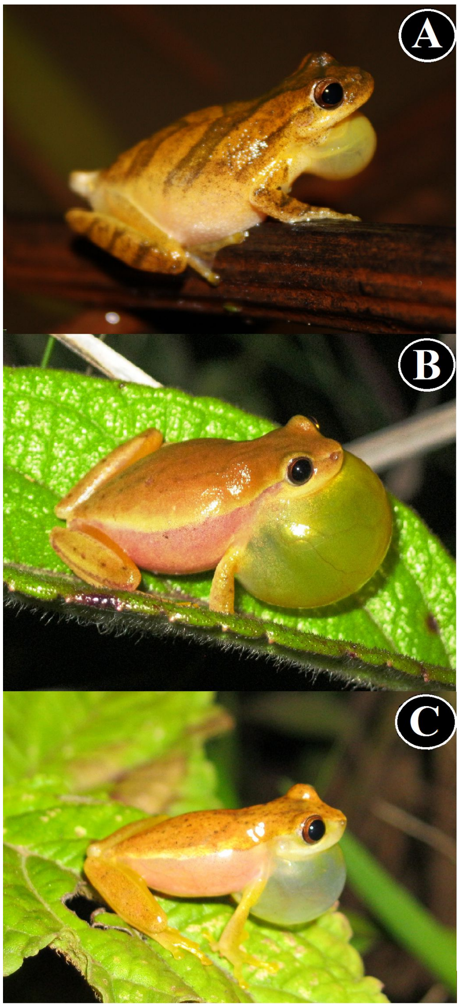

Figure 1. Calling males of *Dendropsophus minutus* (A), *D. rubicundulus* (B) and *D. cruzi* (C) in the municipality of Caldas Novas, Goiás, Brazil.

7.2.3 Data collection

To investigate the existence of variation in the acoustic parameters throughout night we first searched for males during one and two hours after the sunset (~18:50 h) around the study sites, when they were starting their calling activity. Once the focal individuals were found, their calling behaviour was recorded five times for two minutes each (sessions from 1 to 5), separated by 30-minute intervals, and totalling a 10-minutes recording per male. During the interval between recordings the focal males were monitored to make sure the individuals were still in the same site (they did not move away). Recording sessions began between 20:00 and 20:30 h for *D. minutus* and *D. rubicundulus*, and between 21:00 and 21:30 h for *Dendropsophus cruzi*, since calling activity of the last species often starts later in the night. The focal individuals that stopped to call before the conclusion of all five recording sessions were excluded from further statistical analysis. After each 2-min session, we registered from the calling males their perch height, distance to the nearest calling neighbour and distance from the edge of the water body, using a tape measure (accurate to 0.01 m). Air temperature to the nearest 0.1 ºC was measured with a digital thermo-hygrometer (Instrutemp, ITHT 2210 model). When we found amplexed pairs, we recorded the exact hour in which they were observed.

The anuran calls were recorded at a sampling rate of 44.1 kHz and 16-bit resolution (WAV Mono file format) with digital recorders Tascam DR-40 or Marantz PMD 660 coupled to a Rode NTG-2 and/or Sennheiser microphone positioned at about 50 cm from the focal males. The calls were analysed with Raven Pro 1.5 software (Bioacoustics Research Program, 2014), using the following settings: window size = 512 samples; window type = Hamming; overlap = 90%; DFT size = 512 samples.

At the end of the recording sessions, the body size of each individual was measured (Snout-Vent Length, SVL) with a digital calliper (0.1 mm). To avoid recording the same male more than once, each calling site was marked and most of the focal individuals were collected. These specimens were euthanized in a 5% lidocaine, fixed in 10% formalin, preserved in 70% ethanol and housed in the zoological collection of the Universidade Federal de Goiás (ZUFG, Appendix A). The recordings performed during the second session of each individual were deposited in the Fonoteca Zoológica (FonoZoo – the animal sound library of the Museo Nacional de Ciencias Naturales, Madrid, Spain; Appendix A).

We analysed ten advertisement calls from each individual for each of the five recorded sessions. Our data set consisted of a total of 750 calls for *D. minutus* (15 males), 650 calls for *D. rubicundulus* (13 males) and 450 calls for *D. cruzi* (nine males). For each call, five call parameters were measured in the oscillogram: call duration (s), number of pulses per call, pulse duration (s; mean of 10 pulses for *D. minutus*; mean of all pulses for *D. rubicundulus* and *D. cruzi*), silent interval between calls (s) and call rate (calls/minute). For *D. minutus*, call rate (CR) was calculated both for simple calls (CR.A: rate of the advertisement calls with one note A) and complex calls (CR.O: calls with more than one note) as well as the number of each type of notes (A, B and C) within the two-minute recordings. Furthermore, two additional parameters per call were measured in the spectrogram: amplitude frequency (Hz) and dominant frequency (Hz), which were obtained by using Bandwidth Frequency 95% and Peak Frequency, respectively, in Raven Pro 1.5.

Duty cycle of the advertisement calls of the three *Dendropsophus* species (*sensu* Klump and Gerhardt, 1992, as the ratio of call duration to call period) was also calculated. We considered call period as the interval between the beginning of the call to the beginning of subsequent call (e.g. Jansen, Masurowa, and O'Hara, 2016). Bioacoustics terminology was adapted from Köhler et al. (2017). Although aggressive calls of *D. rubicundulus* and *D. cruzi* were occasionally recorded during the recording sessions, these calls were emitted only when a conspecific male was very close of the focal individual or even during physical combats, so they were excluded for further analysis.

Finally, to synthesize in a single measurement, the complexity of vocal behaviour of *D. minutus* (which is the only study species with more than one type of note in its advertisement call), we employed information theory (Shannon and Weaver, 1949) and calculated the entropy (E) per recording (see da Silva et al., 2006; Morais et al., 2012), which corresponds to the summation of the entropy of each notes. For doing so, the number of notes for each type (A, B, and C) emitted by each male was counted and the relative frequency of each note (pi) relative to the total number of notes was calculated within the two minutes of recording. The individual information (Ii) of each note was calculated per each focal male and recording sessions following the equation $Ii = log2\ (1/pi)$, where pi is the total number of times that the note (type i) was issued in the two minutes recorded. Finally, we obtained the entropy values (E) of each type of note (Ei) and of the recording as the summation of the entropy of all note:

$$(E = \sum_{i=1}^{N} p_i . I_i)$$

7.2.4 Data analysis

All variables were tested for normal distribution and log-transformed when necessary (Zar, 1999). Given that certain call parameters are typically affected by environmental temperature (Gerhardt and Huber, 2002; Morais et al., 2012; Brepson, Voituron, and Lengagne, 2013; Tessarolo et al., 2016), we tested with linear regression analyses to what extent this factor influenced each of the call parameters analysed in the study species (Appendix B). For parameters that were affected by temperature, we used temperature-adjusted acoustic data to control for such effect, by applying the following equation: *Yadj = y−(b×Tcalling site)+(b×Tmean)*, where *Yadj* is the adjusted value of call measurement, *y* is the intercept term*, b* the regression coefficient, and *T* is the temperature (Kaefer, Tsuji-Nishikido, and Lima, 2012).

To evaluate if calling site, aggressive behaviour and call parameters changed among the recorded sessions we performed repeated measures ANOVAs (RM ANOVAS), considering individual averaged variables as response factors and the five recording sessions as predictor factors. The repeated measures ANOVA compares means across one or more variables that are based on repeated observations, as in our case, in which many recordings were made with the same male. Aggressive behaviour was measured considering the number of aggressive calls (notes B and C, and complex calls — more than one note; Morais et al., 2012) emitted by males of *Dendropsophus minutus*. Were performed six RM ANOVAS for calling site (considering the three measurements of calling site for each of the three species); three RM ANOVAS for aggressive behaviour (considering number of notes B and C, and the the total number of complex calls); and twenty-four RM ANOVAS for call parameters (eight call parameters measured for each of the three species). An additional RM ANOVA was done to evaluate the entropy change in *D. minutus*.

To verify the variation in acoustic parameters and calling site between the three *Dendropsophus* species we performed a Principal Component Analysis (PCA) on a correlation matrix. Seven acoustic parameters (note duration, pulse number, pulse duration, dominant frequency, bandwidth frequency, silent interval between calls and call rate) of the advertisement calls and three features of the calling sites (perch height, distance to the edge of the water body and distance to the nearest calling male) were used. For that, we averaged ten advertisement calls recorded for each individual in the second session (session with the highest activity) for each species. The selection of the principal components (PCs) was based on the results on the Broken-Stick method (Jackson, 1993), which indicated the greatest contribution of the first two PCs. Then we tested the significant axes with an ANOVA (post hoc test) to verify if the species presented differences in the acoustic parameters and calling sites. All statistical analyses were performed in R 2.14.2 (R Core Team, 2017), with the significance level set at a *p*-value ≤ 0.05.

7.3 RESULTS

As expected, the number of aggressive notes, B and C, emitted by *D. minutus* significantly varied throughout the night (Table 1, Figure 2A). The highest emission rates of these notes occurred during the second and third recording sessions. Similarly, RM ANOVA revealed a significant difference in the emission of complex calls across the five recording sessions, and was more frequently produced at the beginning of the night than at later periods (Table 1, Figure 2B), while no difference was found in the emission of simple calls, i.e. those without presence of aggressive notes. In agreement with these findings, the averaged entropy, which reached 1.315±0.717 (0.216 ─ 2.513; n=21 males), also decreased progressively throughout the night (Table 1, Figure 2C).

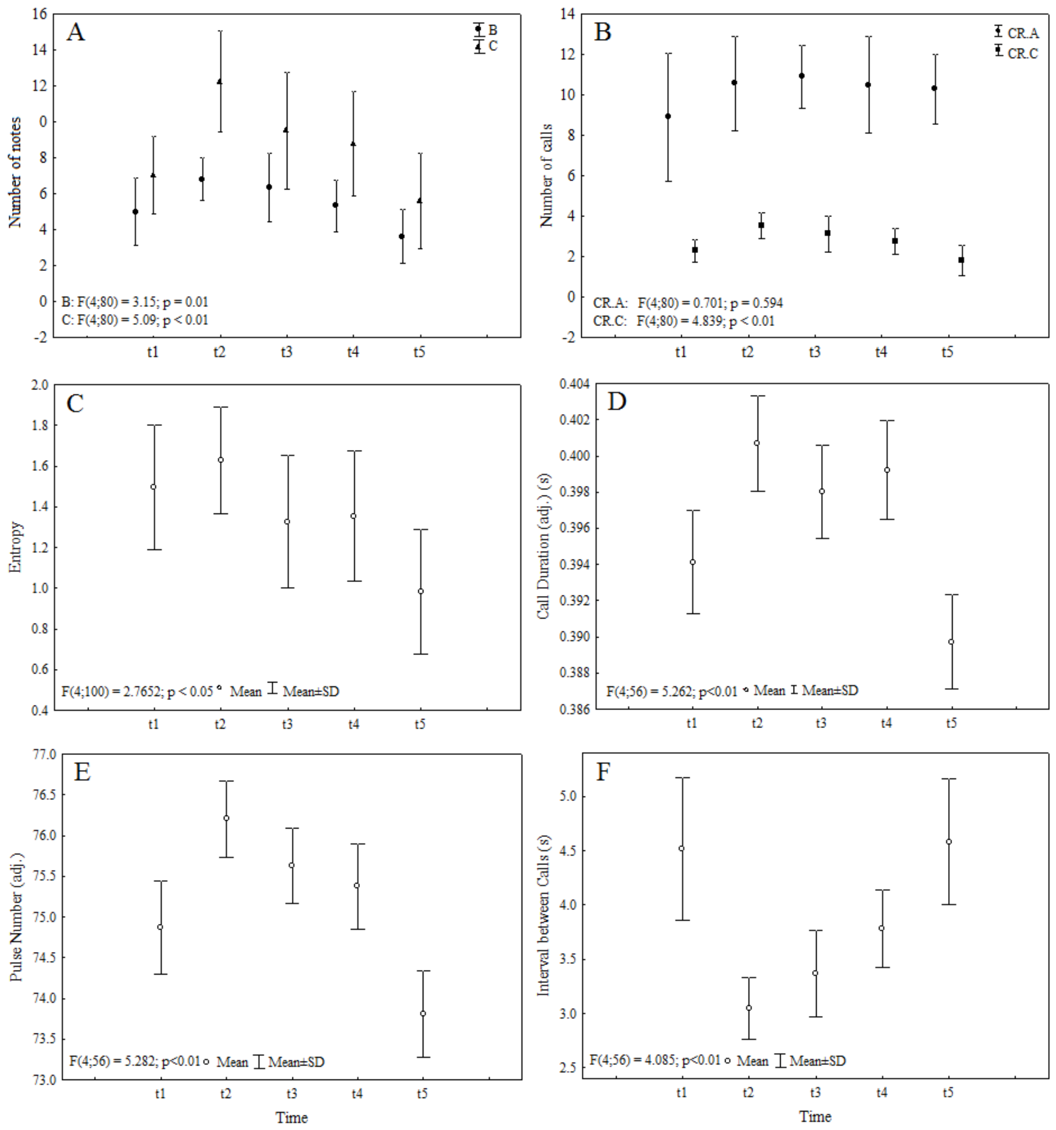

Figure 2. Variation in the acoustic parameters of the advertisement call of *Dendropsophus minutus* along the night in Central-Western Brazil. (A) number of notes B and C in two recording minutes; (B) Call rate of advertisement (CR.A) and complex calls (CR.C); (C) Entropy; (D) Call duration; (E) Pulse Number; (F) Silent Interval Between Calls. Call duration and pulse number were adjusted by temperature.

Table 1. Results of the repeated measured ANOVAs of the acoustic parameters of advertisement calls throughout the night (five periods) for *Dendropsophus minutus*, *D. rubicundulus* and *D. cruzi*. Statistically significant values (p <0.05) are shown in bold. * Acoustic parameters adjusted to air temperature.

Species	Call parameter	F	df	*p*-value	N
D. minutus	A note	2.07	4	0.092	21
	B note	3.15	4	**0.01**	21
	C note	5.09	4	**<0.01**	21
	Call rate simple	0.701	4	0.594	21
	Call rate complex	4.839	4	**0.001**	21
	Call rate total	2.204	4	0.076	21
	Entropy	3.275	4	**<0.05**	21
	Call Duration* (s)	5.262	4	**<0.01**	15
	Pulse Number*	5.282	4	**<0.01**	15
	Pulse Duration (s)	1.117	4	0.358	15
	Bandwidth Frequency * (Hz)	0.93	4	0.453	15
	Peak Frequency* (Hz)	1.601	4	0.187	15
	Interval between calls (s)	4.085	4	**<0.01**	15
	Call rate	0.044	4	0.996	15
	Duty cycle	4.638	4	**<0.01**	15
D. rubicundulus	Call Duration* (s)	1.385	4	0.253	13
	Pulse Number*	2.092	4	0.096	13
	Pulse Duration* (s)	0.671	4	0.616	13
	Bandwidth Frequency * (Hz)	0.981	4	0.427	13
	Peak Frequency * (Hz)	1.017	4	0.408	13
	Interval between calls * (s)	4.376	4	**<0.01**	13
	Call rate	13.31	4	**<0.01**	13
	Duty cycle	7.503	4	**<0.01**	13
D. cruzi	Call Duration* (s)	4.311	4	**<0.01**	9
	Pulse Number*	2.683	4	**0.04**	9
	Pulse Duration* (s)	0.948	4	0.449	9
	Bandwidth Frequency (Hz)	0.767	4	0.555	9
	Peak Frequency (Hz)	0.854	4	0.502	9
	Interval between calls (s)	2.683	4	**0.04**	9
	Call rate	1.508	4	0.223	9

Species	Call parameter	F	df	*p*-value	N
	Duty cycle	14.05	4	**<0.01**	9

In addition to the aggressive behavior and the vocal repertoire, other call parameters, such as call duration, pulse number and silent interval between calls, varied along the recording sessions, in the case of *D. minutus* and *D. cruzi* (Figure 2 and 3, Table 1). *Dendropsophus minutus* showed significantly longer call duration, larger pulse number and shorter silent intervals between calls in the second session than in the other periods (Figure 2). *Dendropsophus cruzi* showed an increase in call duration along the night, while interval between calls reached the lowest values in the third session (Figure 3). *Dendropsophus rubicundulus* showed significantly lower values of silent interval between calls and higher values of call rate in the third session (Figure 4). Finally, call duty cycle varied for the three *Dendropsophus* species studied, with a peak during the second (*D. minutus*) and third sessions (*D. rubicundulus* and *D. cruzi,* Table 1; Figure 5). The mean±SD (Mínimum–Máximum) of the call parameters of the three *Dendropsophus* species studied and the sonograms of the calls are listed in the Appendix C and D, respectively.

Male calling activity in *D. minutus* started just after sunset, while *D. rubicundulus* was the second species studied begin to call, followed by *D. cruzi*. Although 21 individuals of *D. minutus* completed all five recorded periods, six of them did not emit enough calls to perform statistical analyses, three other individuals stopped to vocalize in the third session and four ones stopped in the fourth session. Two and three individuals of *D. rubicundulus* stopped calling in the third and fourth recording session, respectively, and one and four individuals of *D. cruzi* stopped calling in the third and fourth sessions, respectively.

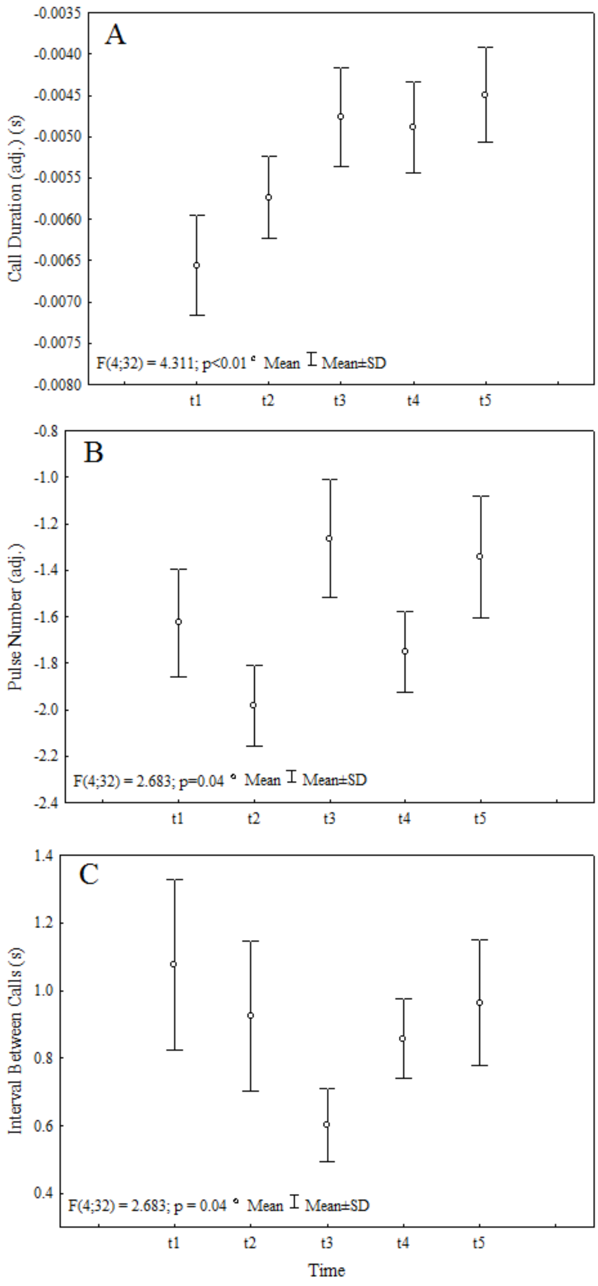

Figure 3. Variation of the acoustic parameters of the advertisement call of *Dendropsophus cruzi* along the night in Central-Western Brazil. (A) Call Duration; (B) Pulse Number; (C) Silent Interval Between Calls. Call duration was adjusted by temperature.

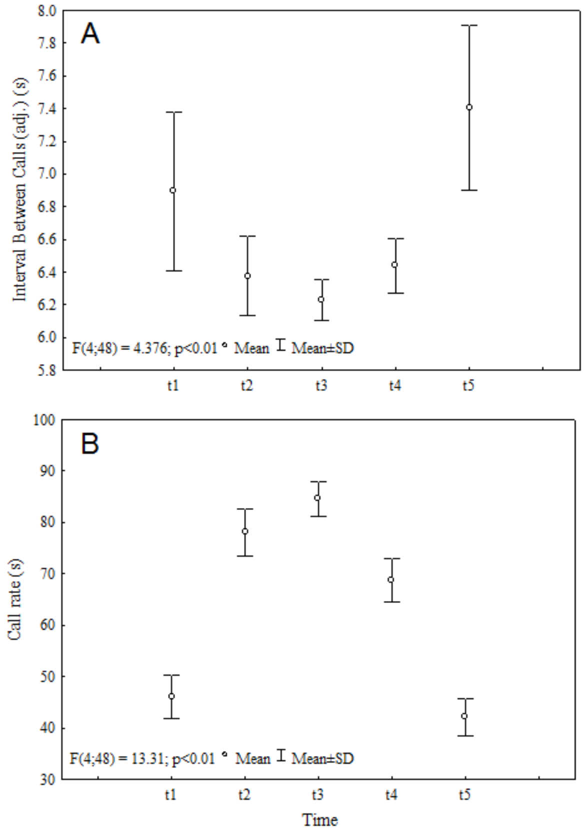

Figure 4. Variation of the acoustic parameters of the advertisement call of *Dendropsophus rubicundulus* along the night in Central-Western Brazil. (A) Silent Interval Between Calls; (B) Call Rate. Interval between calls was adjusted by temperature.

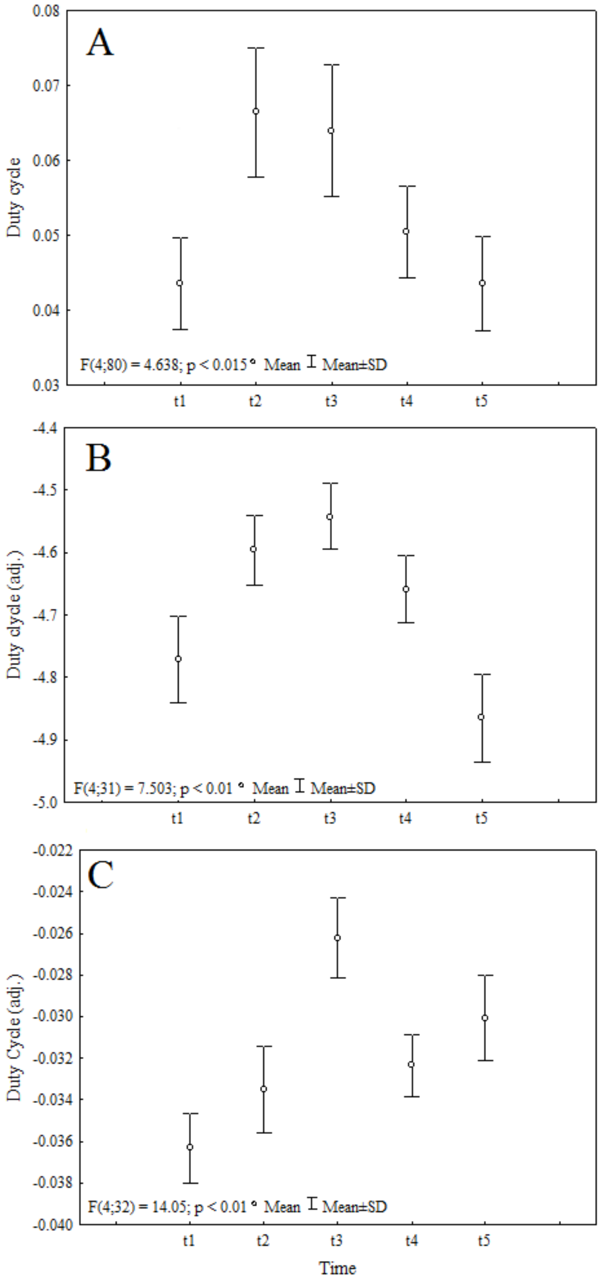

Figure 5. Variation of the duty cycle of the advertisement call of *Dendropsophus minutus* (A), *D. rubicundulus* (B) and *D. cruzi* (C) along the night in Central-Western Brazil. Duty cycle B and C were adjusted by temperature.

In relation to calling site, only *D. cruzi* presented a significant variation in height of the calling site throughout night (Table 2). The rest of spatial variables remained stable along the night. The average distance between nearest calling males of *D.*

minutus was 94.9±20 cm (20—430 cm, N=21), and the average distance to the edge of water body and perch height were 30.2±28.7 cm (0—90 cm, N=21) and 25.9±18.5 cm (0—63 cm, N=9), respectively. In *D. rubicundulus* the average distance between nearest calling males was 304.73±121.25 cm (40—450 cm, N=11), and the average distance to the edge of water body and perch height were 46±41.84 cm (0—120 cm, N=11) and 30.93±28.84 cm (0—79 cm, N=11), respectively. The average distance between nearest calling males of *D. cruzi* was 179.11±83.67 cm (45—330 cm, N=9), and the average distance to the edge of water body and perch height were 123.03±92.06 cm (0—230 cm, N=7) and 70.27±16.06 cm (53—123 cm, N=9), respectively. The females of *D. minutus* and *D. rubicundulus* in amplexus were found mainly between 9:30 p.m. and 10:00 p.m., while those of *D. cruzi* were found at approximately 10:30 p.m (Figure 6).

Table 2. Results of the repeated measured ANOVAs of the distance between nearest calling males (DNCM), height of calling site (HCS) and distance to the edge of the water body (DEWB) throughout the night (five periods) for *Dendropsophus minutus*, *D. rubicundulus* and *D. cruzi*. Statistically significant values (*p* <0.05) are shown in bold.

Species	Factor related to calling site	df	F	*p-value*	N
D. minutus	DNCM	4	1.127	0.35	20
	HCS	4	0.998	0.414	21
	DEWB	4	1.208	0.314	21
D. rubicundulus	DNCM	4	0.632	0.642	13
	HCS	4	0.993	0.423	13
	DEWB	4	0.331	0.855	13
D. cruzi	DNCM	4	0.271	0.894	9
	HCS	4	3.154	**0.027**	9
	DEWB	4	0.262	0.9	9

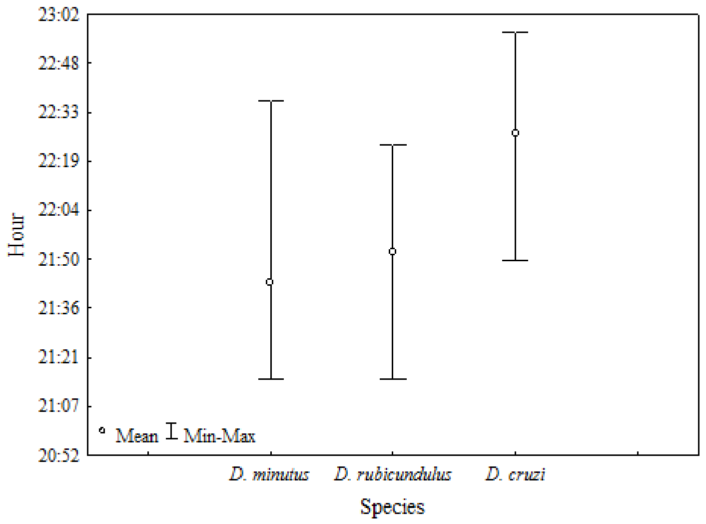

Figure 6. Moment of the night (in hours) showing the time in which couples of *Dendropsophus minutus* (N = 6), *D. rubicundulus* (N = 5) and *D. cruzi* (N = 4) were found in amplexus in the sampled sites, municipality of Caldas Novas, Goiás, Brazil.

The variation in the measured call parameters was summarized by two principal components, which accounted for 86.64% of the variation (Table 3; Figure 7A); PCA1 axis explained 71.66% of the total variation and was mostly influenced by call duration, pulse number, and bandwidth frequency; PCA2 axis was influenced by pulse duration and dominant frequency, which explained 14.96% of the variance. The PCA for calling site was also summarized in two principal components that accounted for 82.31% of the variation (Table 3; Figure 7B); PCA1 axis explained 44.33% of the total variation and was mostly influenced by perch height and distance to the edge of the water body; PCA2 axis was influenced by distance to the nearest calling male, which explained 33.88% of the variance. The ANOVA showed that the three *Dendropsophus* species used species-specific portions of the acoustic space ($PC1 = F_{(32,2)} = 420.5$, $p<0.001$; $PC2 = F_{(34,2)}=21.77$, $p<0.001$) as well as calling sites ($PC1 = F_{(28,2)} = 11.57$, $p<0.001$; $PC2 = F_{(28,2)}=5.34$, $p=0.01$), with no acoustic and spatial niche overlap between them.

Table 3. Results of a Principal Component Analysis (PCA) of the advertisement call parameters of *Dendropsophus minutus*, *D. rubicundulus* and *D. cruzi*. The factor loadings for all five acoustic parameters are provided and those higher than 0.7 are in

bold. CD: call duration; PN: pulse number; PD: pulse duration; BF: bandwidth frequency; PF: dominant frequency; IBC: intercal between calls; TX: call rate; HCS: height of the calling site; DEWB: distance to the edge of waterbody; DNCM: distance to the nearest calling male; EV: eigenvalue; V: variance (%); CV: cumulative variance (%).

Acoustic parameters	CD	PN	PD	BF	PF	IBC	TX	EV	V (%)	CV (%)
PC1	**-0.96**	**-0.96**	-0.49	**-0.94**	**-0.72**	**-0.88**	**0.86**	5.02	71.68	71.68
PC2	-0.12	-0.07	**-0.78**	-0.09	0.58	-0.02	-0.28	1.05	14.96	86.64
PC3	0.19	0.22	-0.39	0.19	-0.28	0.09	0.30	0.44	6.31	92.94
PC4	-0.10	-0.08	-0.04	-0.18	-0.14	0.45	-0.08	0.28	4.06	97.00
PC5	0.06	0.05	0.06	-0.14	0.21	0.09	0.27	0.16	2.22	99.22
PC6	-0.09	-0.10	0.01	0.16	0.05	0.05	0.07	0.05	0.75	99.98
PC7	0.03	-0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	100.00
Calling site	HCS	DEWB	DNCM							
PC1	**0.85**	**0.85**	-0.01					1.45	48.33	48.33
PC2	-0.14	0.13	**-0.99**					1.02	33.89	82.22
PC3	0.51	-0.51	-0.14					0.53	17.78	100

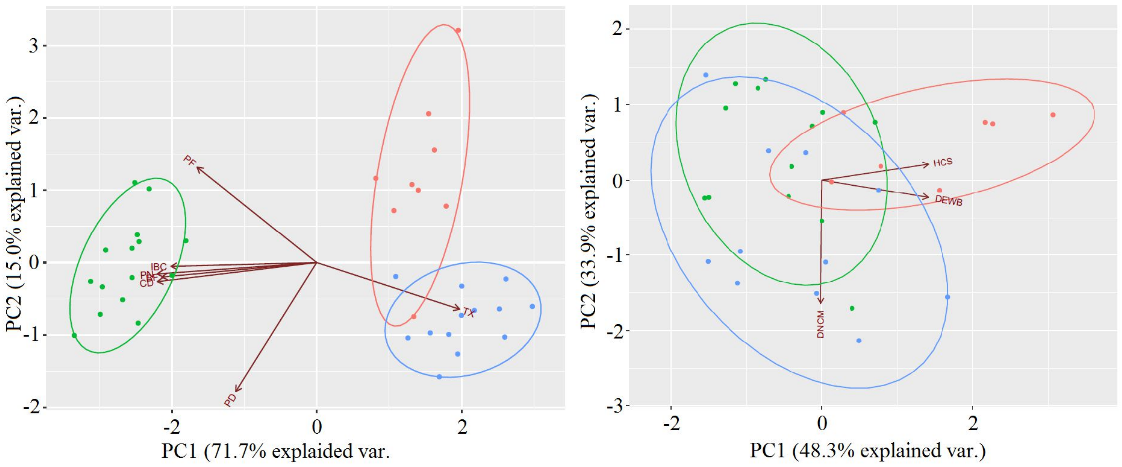

Figure 7. Principal Component Analysis (PCA) of the call parameters (A) and calling sites (B) from *Dendropsophus minutus* (green), *Dendropsophus rubicundulus* (blue) and *Dendropsophus cruzi* (red).

7.4 DISCUSSION

Males of *D. minutus* changed their calling behavior throughout the night, decreasing the aggressive behavior and altering some of their call parameters, but not their calling sites. Such a temporal variation in aggressive behavior was also found previously in other anuran species and may be related to the establishment of the calling sites by males (Reichert, 2010; Bastos et al., 2011; Dias, Prado, and Bastos, 2017). During field work we observed that at the moment of the second and thirty record sessions many males of *D. minitus* were involved in acoustical and/or physical fights, emitting more complex calls composed mainly by B notes while disputing for better calling sites (V.G. pers. observ.). The processes of habituation and sensitization have been used to explain the aggressive behavior in some anuran species (Bee, 2001; Marshall, Humfeld, and Bee, 2003; Reichert, 2010; Dias, Prado, and Bastos, 2017). We hypothesized that aggressive calls decreasing throughout the night in *D. minutus*, as observed in *Pseudacris crucifer* (Marshall et al. 2003) and *Hypsiboas goianus* (Dias et al. 2017), may be result of habituation effect (*sensu* Thompson and Spencer, 1966). The habituation indicates that a continuous stimulation by neighbors' advertisement and aggressive calls may elevate the aggressive threshold of males (Reichert, 2010), resulting in a decrease of some parameters as the night progresses (Reichert, 2010; Dias, Prado, and Bastos, 2017).

The variation of some acoustic parameters of the advertisement call throughout night for the three *Dendropsophus* species may be related especially to the arrival of females in the reproductive site in the middle of the night (Bevier, 1997; Murphy, 1999; Dias, Prado, and Bastos, 2017). The competition for females is more intense during the peak of calling activity, since the number of males in the chorus increases (Wells, 2007). Therefore, males may increase the quality of vocalizations by adjusting their call parameters and making them more attractive to potential mates (Gerhardt et al., 2000; Wells, 2007; Tárano and Fuenmayor, 2013). Females may have preference for calls with longer duration (Gerhardt et al., 2000; Poole and Murphy, 2007), higher pulse number (Gerhardt, Dyson, and Tanner, 1996), smaller interval between calls and higher call rate (Márquez and Bosch, 1997), which may explain the values of these call parameters recorded in the middle of the night, when females are arriving to the chorus. Pombal and Bastos (1998) observed females of *D. cruzi* in amplexus with males between one and two hours after the beginning of calling activity. In the field we found females of *D. minutus*, *D. rubicundulus* and *D. cruzi* amplected with males between the second and fourth recording sessions. As expected, spectral parameters such as dominant frequency and bandwidth frequency, which are directly related to the body size of the males (Morais et al., 2012; Tessarolo et al., 2016), did not vary throughout night.

We can hypothesize that by increasing the entropy during the beginning of the night *D. minutus* enhance the amount of information of its call repertoire, since during this period there is an increase of individuals in the chorus and noise is high. By repeating the same information many times (redundancy in the calls) king penguins increase the probability of communications in noisy environment (Lengagne et al., 1999). Information theory predicts that increasing redundancy in the signal improves the probability of receiving a message in a noisy channel (Shannon and Weaver, 1949; Lengagne et al., 1999). By increasing the duty cycle males of the three *Dendropsophus* species can also increase signal detectability during the period in which there is a greater number of males in the chorus (see Brumm and Slabbekoorn, 2005; Wiley, 2006). Variation in duty cycle communicates the signaler's motivational state, which would be relatively high in an aggressive situation (Wilson and Mennill, 2011). Signaling sequences with a high duty cycle attracted more conspecific and heterospecific receivers in environments with fluctuating ambient noise (see Wilson and Mennill, 2011).

Acoustic communication is an especially prominent activity for many anuran species due to its importance for reproduction, and appears to entail considerable energetic costs, involving high metabolic rates (review in Wells, 2001). Although we did not attempt to measure directly the energy cost of calling, the lowest observed value for the call parameters, lowest duty cycle and decrease in entropy (for *D. minutus*) in the last recording sessions of the night may be an indication of such a cost (i.e. 'decrease of metabolic energy' or a cost measured in terms of decreased opportunities for future reproduction; Wells, 2007). This pattern was also observed for *Dendropsophus microcephalus* (Wells and Taigen, 1989), *Hyla intermedia* (Castellano and Gamba, 2011), *Hyla arborea* (Brepson, Voituron, and Lengagne, 2013), *Leptodactylus* *mystacinus* (Jansen, Masurowa, and O'Hara, 2016) and *Hypsiboas goianus* (Dias, Prado, and Bastos, 2017). As the species mentioned before, the breeding season of the three studied *Dendropsophus* species lasts for some months and males may remain in the chorus for many nights, compromising their reserves and emphasizing the relevance of strategies to minimize energetic costs.

The most likely hypothesis to explain the decrease of the energetic reserves over the reproductive season (e.g. Tejedo, 1992) and even during a single night of calling (e.g. Bevier, 1997) is the energetic constraint hypothesis (Mac Nally, 1981; Tejedo, 1992). Although the energetic constraint seems to affect more both maintaining of calling activity and within-bout call rate of the males, instead of call properties (see Brepson, Voituron, and Lengagne, 2013), males of different species can modify their reproductive strategies to maintain vocal activity throughout the reproductive season and remain attractive to females. The amount of time spent in a chorus is a major determinant of a male's mating success (Wells, 2007), and thus the sexual selection may potentially balance the energetic demands of calling over a time period (for example, an alternation of dominance at a calling site along breeding season).

Nightly site fidelity was verified for the three *Dendropsophus* species studied. Through acoustic signals, male attendance at the mating site has been found to be a major determinant of male mating success in some species (Bertram, Berrill, and Nol, 1996; Hoffmann et al., 2010; Sinsch, 2014). Although *D. cruzi* presented a significant change in perch height along the night, calling males do not move horizontally from the vocalization site, defending their territory with the use of aggressive calls. Many anurans return consistently to vocalization sites, and males can be found for many nights in the same site (Sinsch, 2014), defending their territories with visual signals, vocalizations and physical fights (e.g. Wogel, Abrunhosa, and Pombal Jr., 2002; Lemes et al., 2012; Morais et al., 2012). The factors that act to avoid niche overlap between species and even communities may show different patterns, for example, while for some species the calling site was not important to avoid niche overlap (e.g. Vieira, Batista, and Bastos, 2016), for species studied here, this seems to be a relevant factor. In these species, males did not actively search for females but used calls to attract mates and defend their territory sites (Pombal and Bastos, 1998; Morais et al., 2012). Males should face a trade-off between increasing investment in display properties selected by females or increasing the amount of sexual display (Brepson, Voituron, and Lengagne, 2013), since sexual selection should act by selecting characteristics that increase the ability to obtain energy and/or spend it efficiently (Murphy, 1999).

As hypothesized, the congeneric *Dendropsophus* species in sympatry experience selection favoring stronger divergence in acoustic signals and calling site. Females must recognize the calls of conspecific males to avoid hybridization (Wells, 2007; Bee, 2008; Köhler et al., 2017). Then, sympatric species that communicate by sounds and share the same acoustic space should avoid masking interference affecting the signal recognition processes (Brumm and Slabbekoorn, 2005; Bee, 2008). The processes that shape species coexistence patterns can act in different ways between environments, communities or species, and while some studies have found that calls of closely related allopatric species were more similar than those of closely related sympatric species (competitive exclusion; Duellman and Pyles, 1983), others showed that calls were less similar when the pairs of species were sympatric (coexistence principle; Höbel and Gerhardt, 2003). Marquez, la Riva, and Bosch (1993) found that the differences in sympatry were expressed in female preferences rather than in male call characteristics. Differences in call structure, seasonal or nightly periods of calling activity prevent signal overlap and helps in species recognition (Marquez, la Riva, and Bosch, 1993; Wells, 2007; Silva, Martins, and Rossa-Feres, 2008). In the species studied here, it seems that the coexistence caused them to differ not only in relation to the parameters of the call, but also in the call site and in the activity period.

Dendropsophus minutus was the first species to vocalize at the reproductive sites, then began *D. rubicundulus* and finally *D. cruzi*. The phenology is an important characteristic of the natural history of the species, since the period of seasonal and daily activity influences the amount of energy necessary for the accomplishment of the reproductive activity, territory defense and maintenance of metabolic rate for survival (Wells, 2007). For example, *D. cruzi* is one of the last species to stop to vocalize, and individuals of these species may be found calling until four o clock, while individuals of *D. minutus* and *D. rubicundulus* call until mid-night (V.G. unpublished data). Therefore, not only the characteristics of the vocalization and the calling site may be important for the segregation of niche between species, the beginning and end of the activity period are also important to determine the calling patterns, which may also be related to the preferences of females of each species.

Call parameters of *D. minutus*, *D. rubicundulus* and *D. cruzi* are similar to those described by Morais et al. (2012), Teixeira and Giaretta (2015) and Tessarolo et al. 2016), respectively. Our results showed that *D. minutus* presented a calling pattern throughout night, emitting more aggressive calls in the beginning of the night, which could be related to the establishment of males at their calling sites, and a subsequent decrease that may be related to the habituation effect (Thompson and Spencer, 1966). Besides that, we hypothesized that females of *D. minutus*, as well as females of other anuran species, may have preference for more complex calls (Gerhardt, Humfeld, and Marshall, 2007; Henderson and Gerhardt, 2013), and by increasing the number of notes of the calls the males of *D. minutus* may be increasing their attractiveness. Future studies should focus on the female preferences by simple or complex calls and also for call properties with higher values. We also suggest investigate the use of complex calls in particular social contexts in *D. minutus*, for example, using call ordering to extract a complex meaning from call sequences, evoking specific responses in receivers (e.g. Suzuki, Wheatcroft, and Griesser, 2017).

We concluded that the three *Dendropsophus* species studied differed in both call parameters, avoiding acoustic space overlap, and calling site, avoiding spatial overlap. We demonstrated that the acoustic behavior throughout the night (difference in the number of calls, notes, acoustic parameters and phenology) seems to be the multiple mechanisms allowing reproductive isolation during the breeding season in the *Dendropsophus* species.

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APPENDIX A - Metadata on the *Dendropsophus minutus*, *D. rubicundulus* and *D. cruzi* recorded in the Municipality of Caldas Novas, Goiás state, Brazil

Taxa	Individual	Latitude	Longitude	Date	Temperature	Voucher	FonoZoo
D. minutus	1	-17.818536	-48.646447	23/01/2016	25.1	NA	10320
*D. minutus**	2	-17.875487	-48.684175	27/01/2016	24.7	10449	10330
*D. minutus**	3	-17.875487	-48.684175	28/01/2016	25.4	10451	10337
D. minutus	4	-17.818536	-48.646447	23/01/2016	24.3	NA	10321
*D. minutus**	5	-17.818536	-48.646447	24/01/2016	24	NA	10326
*D. minutus**	6	-17.875487	-48.684175	27/01/2016	24.5	10459	10331
*D. minutus**	7	-17.875487	-48.684175	28/01/2016	25.4	10461	10338
*D. minutus**	8	-17.875487	-48.684175	23/01/2016	25.6	NA	10322
*D. minutus**	9	-17.875487	-48.684175	27/01/2016	24.5	10460	10332
*D. minutus**	10	-17.875487	-48.684175	28/01/2016	25.4	10462	10339
D. minutus	11	-17.875487	-48.684175	23/01/2016	24.4	NA	10323
D. minutus	12	-17.875487	-48.684175	27/01/2016	25.1	10446	10333
*D. minutus**	13	-17.875487	-48.684175	28/01/2016	25.4	10447	10340
*D. minutus**	14	-17.875487	-48.684175	23/01/2016	24.2	NA	10324
*D. minutus**	15	-17.818536	-48.646447	24/01/2016	24.5	NA	10325
*D. minutus**	16	-17.875487	-48.684175	25/01/2016	24.8	10452	10327
*D. minutus**	17	-17.875487	-48.684175	27/01/2016	25	10455	10334
*D. minutus**	18	-17.875487	-48.684175	26/01/2016	24.5	10453	10328
*D. minutus**	19	-17.875487	-48.684175	27/01/2016	25	10456	10335

Taxa	Individual	Latitude	Longitude	Date	Temperature	Voucher	FonoZoo
D. minutus	20	-17.875487	-48.684175	26/01/2016	24.2	10463	10329
D. minutus	21	-17.875487	-48.684175	27/01/2016	24.5	10466	10336
D. rubicundulus	1	-17.875487	-48.684175	05/03/2016	25.3	10308	10341
D. rubicundulus	2	-17.875487	-48.684175	06/03/2016	23	103011	10344
D. rubicundulus	3	-17.875487	-48.684175	05/03/2016	25.5	10307	10342
D. rubicundulus	4	-17.875487	-48.684175	06/03/2016	23	10313	10362
D. rubicundulus	5	-17.875487	-48.684175	05/03/2016	25.3	10309	10343
D. rubicundulus	6	-17.875487	-48.684175	06/03/2016	22.9	10310	10345
D. rubicundulus	7	-17.875487	-48.684175	06/03/2016	23	10312	10346
D. rubicundulus	8	-17.875487	-48.684175	06/03/2016	23	10314	10347
D. rubicundulus	9	-17.875487	-48.684175	18/02/2017	24.7	NA	10348
D. rubicundulus	10	-17.875487	-48.684175	18/02/2017	23.9	NA	10349
D. rubicundulus	11	-17.875487	-48.684175	18/02/2017	23.9	NA	10350
D. rubicundulus	12	-17.875487	-48.684175	18/02/2017	25.9	NA	10351
D. rubicundulus	13	-17.875487	-48.684175	18/02/2017	25.9	NA	10352
D. cruzi	1	-17.838824	-48.602381	09/02/2015	22.2	NA	10353
D. cruzi	2	-17.838824	-48.602381	09/02/2015	21.9	NA	10354
D. cruzi	3	-17.838824	-48.602381	17/01/2015	21.9	NA	10355
D. cruzi	4	-17.838824	-48.602381	17/01/2015	21.9	NA	10356
D. cruzi	5	-17.838824	-48.602381	17/01/2015	19.5	NA	10357
D. cruzi	6	-17.838824	-48.602381	17/02/2017	21.5	NA	10359

Taxa	Individual	Latitude	Longitude	Date	Temperature	Voucher	FonoZoo
D. cruzi	7	-17.838824	-48.602381	17/02/2017	21.2	NA	10360
D. cruzi	8	-17.838824	-48.602381	17/02/2017	21.2	NA	10361
D. cruzi	9	-17.838824	-48.602381	17/01/2015	21.9	NA	10358

*Individuals of *D. minutus* used for analysis of the call parameters along night.

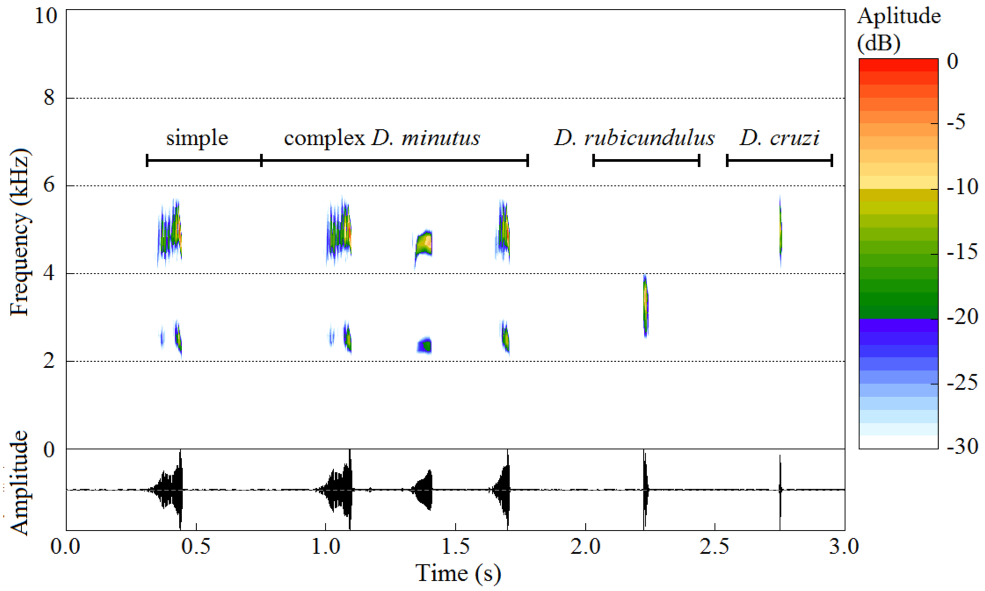
APPENDIX B - Results of the simple regression between the acoustic parameters and temperature. Statistically significant values ($p < 0.05$) are shown in bold

Species	Call parameter	Df	F	$R^2_{aj.}$	B	p	N
D. minutus	A note	103	0.001	-0.009	-0.079	0.966	21
	B note	103	0.838	-0.001	0.59	0.362	21
	C note	103	2.475	0.014	1.775	0.119	21
	Call rate simple	103	0.371	-0.006	-0.544	0.544	21
	Call rate complex	103	1.279	0.002	0.327	0.26	21
	Call rate total	103	0.005	-0.009	0.068	0.941	21
	Call Duration (s)	748	23.63	0.029	-0.005	**<0.01**	15
	Pulse Number	748	21.28	0.026	-0.993	**<0.01**	15
	Pulse Duration (s)	748	2.966	0.002	-0.001	0.085	15
	Bandwidth Frequency (Hz)	748	15.78	0.019	100.79	**<0.01**	15
	Peak Frequency (Hz)	748	34.31	0.042	69.02	**<0.01**	15
	Interval between calls (s)	748	2.755	0.003	-0.33	0.097	15
	Call rate	748	0.482	-0.001	0.022	0.488	15
	Duty cycle	748	0.069	-0.001	-0.012	0.792	15
D. rubicundulus	Call Duration (s)	648	58.39	0.081	-0.001	**<0.01**	13
	Pulse Number	648	6.27	0.008	-0.019	**0.01**	13
	Pulse Duration (s)	648	47.13	0.066	-0.001	**<0.01**	13
	Bandwidth Frequency (Hz)	648	10.85	0.015	0.041	**<0.01**	13
	Peak Frequency (Hz)	648	11.01	0.015	-22.54	**<0.01**	13
	Interval between calls (s)	648	2.305	0.002	-0.117	0.129	13
	Call rate	648	0.327	-0.001	-0.625	0.567	13
	Duty cycle	648	4.291	0.005	0.066	**0.03**	13
D. cruzi	Call Duration (s)	448	10.16	0.02	0.000	**<0.01**	9
	Pulse Number	448	41.97	0.084	0.104	**<0.01**	9
	Pulse Duration (s)	448	22.18	0.045	-0.001	**<0.01**	9
	Bandwidth Frequency (Hz)	448	0.196	-0.002	-0.005	0.658	9
	Peak Frequency (Hz)	448	0.552	-0.001	-6.767	0.458	9
	Interval between calls (s)	448	1.362	0.001	-0.029	0.244	9
	Call rate	448	3.095	0.005	1.253	0.079	9
	Duty cycle	448	8.842	0.017	0.001	**0.03**	9

APPENDIX C - Call parameters of the three *Dendropsophus* species analysed. Measurements are presented as the mean ± standard deviation (minimum–maximum) and/or mode (for pulse number)

Call parameters	*D. minutus* (15 males/800 calls)	*D. rubicundulus* (13 males/650 calls)	*D. cruzi* (9 males/450 calls)
Call Duration (s)	0.132±0.017 (0.081—0.179)	0.023±0.004 (0.002—0.038)	0.010±0.004 (0.004—0.019)
Pulse Number	27 (17—35)	5 (2—9)	2 (1—6)
Pulse Duration (s)	0.005±0.001 (0.003—0.007)	0.005±0.001 (0.003—0.008)	0.004±0.001 (0.001—0.009)
Bandwidth Frequency (Hz)	2993.50±389.38 (2156.2—4921.90)	796.09±317.51 (375—2282.50)	791.17±215.19 (301.50—1593.50)
Peak Frequency (Hz)	5008.55±183.04 (4734.40—5512.50)	3421.28±180.58 (2670.10—4218.80)	4780.82±221.24 (3962.10—5211)
Interval between calls (s)	3.858±3.01 (0.013—31.45)	1.156±2.028 (0.160—30.742)	0.885±0.898 (0.239—10.164)
Call rate	11.28±4.78 (3.50—34.00)	63.93±28.82 (7.5—21.5)	43.744±217.354 (14.5—84.5)
Duty cycle	0.054±0.046 (0.004—0.285)	0.039±0.031 (0.001—0.134)	0.016±60.010 (0.001—0.052)

APPENDIX D - Spectrogram and oscillogram of the calls of *Dendropsophus minutus*, *D. rubicundulus* and *D. cruzi*



Spectrogram and oscillogram of the calls of *Dendropsophus minutus* (simple — composed by one note A; complex call — composed by notes A, B and C; air temperature = 24.3 °C), *D. rubicundulus* (air temperature = 25.5 °C) and *D. cruzi* (air temperature = 21.6 °C). Sound figures were produced using Seewave package version 1.6.4 (Sueur et al. 2008) of the R platform (R Development Core Team 2017). Seewave settings employed were: window name (Fourier transform window) = Hanning; window length = 256 samples; overlap = 80%.

8 NIGHTLY CALLING ACTIVITY IN ANURAN COMMUNITIES OF THE CERRADO, BRAZIL

RESUMO

Anuros que vivem em comunidades tropicais ricas em espécies e que utilizam as vocalizações como a principal forma de comunicação podem experimentar competição temporal de nicho acústico. Para evitar a sobreposição de nicho e minimizar a interferência acústica, as espécies devem apresentar diferenças no período de atividade. Neste estudo, dados sobre fenologia de cinco comunidades de anuros foram utilizados para verificar os efeitos de fatores climáticos, e diferenças diárias e sazonais no padrão de atividade de chamada de 19 espécies. Houve um pico de atividade acústica entre a segunda e a terceira hora após o pôr-do-sol, com uma diminuição gradual da atividade até o nascer do sol. As comunidades de anuros foram altamente influenciadas por fatores climáticos e mostraram um padrão circadiano, com sobreposição de nicho temporal na atividade acústica durante a noite. No entanto, avaliadas individualmente, as espécies apresentaram diferentes períodos de máxima atividade de vocalização. Espécies filogeneticamente mais próximas (congêneres), que apresentaram uma aparente sobreposição nos picos de atividade, podem exibir diferenças no comportamento de vocalização, nos locais de oviposição ou nos parâmetros acústicos. As variáveis climáticas atuam como fatores impulsionadores para determinar a atividade acústica e a reprodução de anuros em uma escala diária e que as espécies podem usar diferentes estratégias para evitar a sobreposição no nicho acústico e facilitar a coexistência.

Palavras-chave: partição acústica; reprodução; sobreposição de nicho temporal; bioacústica; fenologia.

ABSTRACT

Anurans living in species-rich tropical communities and using vocalizations as the main form of communication may experience temporal acoustic niche competition. In order to avoid niche overlap and to minimize acoustic interference, the species should present differences in the calling period. Herein, data on phenology of five anuran communities were used to verify the effects of climatic factors, seasonal and daily differences in calling activity pattern of 19 species. There was a peak of calling activity of species in between the second and third hour after sunset, with a gradual decrease in activity until sunrise. Anuran communities were highly influenced by climatic factors and showed a pattern of circadian cycle with temporal niche overlap in calling activity throughout the night. However, different species showed different periods of maximum calling activity. Closely related species with an apparent overlap in the vocalization peaks may present differences in calling behavior, oviposition sites or acoustic parameters. Climatic variables act as driver factors to determine calling activity and reproduction of anurans at daily scale and that species may use different strategies to avoid overlap in the acoustic niche and to facilitate the coexistence.

Key-words: acoustic partitioning; breeding; temporal niche overlap; bioacoustic; phenology.

8.1 INTRODUCTION

The acoustic niche integrates a spatial dimension (microhabitat used for calling), a temporal dimension (time of day when calling takes place; Llusia *et al.* 2013), and a call structure dimension (physical features of the advertisement call; Sinsch *et al.* 2012). In species-rich anuran communities, it is expected that performed acoustic niches be narrower and with little overlap to minimize acoustic interference, and thus facilitating mating of conspecifics by acoustic resource partitioning (Duellman & Pyles 1983, Protázio *et al.* 2015, Heard *et al.* 2006). Moreover, species saturation in a community should lead to a more or less even distribution of niche breadth (Tilman 2004). Thus, anuran species may adopt different calling strategies to avoid acoustic niche overlapping during breeding period.

Calling activity is an important indicator of the daily and seasonal patterns of anuran reproduction (Wells 2007). The timing, intensity and duration of calling and reproductive activities are mainly influenced by environmental factors (Wells 2007, Llusia, Márquez, Beltrán, Moreira, *et al.* 2013, Protázio *et al.* 2015, Heard *et al.* 2015, Ximenez & Tozetti 2015). Abiotic factors, such as temperature, humidity and precipitation (Márquez 1992, Saenz *et al.* 2006, Llusia, Márquez, Beltrán, Benítez, *et al.* 2013), pond hydroperiod (Jakob *et al.* 2003, Cayuela *et al.* 2012), barometric pressure (Oseen & Wassersug 2002), and light intensity (Almeida-Gomes *et al.* 2007) are known to modulate phenology of anuran calling. In some species, calling activity can also vary along altitudinal and latitudinal gradients (Morrison & Hero 2003, Tessarolo *et al.* 2016), or even due the presence of a phylogenetic closely related species reproducing in the same breeding site (Duellman & Pyles 1983, Marquez *et al.* 1993). Amphibians of different climatic zones can follow different breeding strategies due the variation in predictability of environmental factors (Llusia, Márquez, Moreira, *et al.* 2013, Llusia, Márquez, Beltrán, Benítez, *et al.* 2013). Species may vary their calling activity over the season and throughout the day (Protázio *et al.* 2015, Heard *et al.* 2015, Ximenez & Tozetti 2015). On a daily scale, there are daylight, crepuscular and nocturnal species (Almeida-Gomes *et al.* 2007, Wells 2007, Farina & James 2016). Therefore, a combination of social, environmental and climatic variables can act as synergistic effects, influencing the reproductive activity of the species.

Determining the factors that regulate the onset and intensity of reproduction can improve our understanding of the evolution of this key life-history trait. Moreover, since anuran amphibians are ectothermic organisms with permeable skin and largely aquatic reproduction (Duellman & Trueb 1994, Wells 2007), this comprehension has important practical applications for predicting how populations may respond to global change (Chambers *et al.* 2013, Llusia, Márquez, Beltrán, Benítez, *et al.* 2013). When evaluating calling activity through phenology it is possible to evaluate the adaptive capacity of species, their resilience, and this information may be valuable for the management of threatened species (Chambers *et al.* 2013).

Studies on phenological trends may thus provide significant evidence of the effects of anthropogenic impacts on biota (Beaumont *et al.* 2015), and shed light on the constraints on species adaptation (Chambers *et al.* 2013). Although studies dealing with this question in tropical anuran communities were largely conducted on a seasonal scale, little is known about factors that may act on a daily scale. Herein we studied the phenology (timing and duration), intensity (relative abundance), and effects of weather conditions and temporal factors on the nightly calling activity of anuran communities from Cerrado, Central Brazil. We addressed the following questions: (i) Are there daily and seasonal differences in calling activity patterns among species?; (ii) Is there temporal overlap in the calling activity of the species and communities?; (iii) Do time, temperature and relative humidity influence the calling activity of the study species?

8.2 MATERIAL AND METHODS

8.2.1 Sampling area

The calling males of anuran amphibians were monitored in five water bodies within the municipality of Caldas Novas, State of Goiás, Brazil (Table 1; Figure 1). Habitat features were similar in all the study sites, all permanent water bodies with presence of vegetation on the edges and connected with forested areas. The vegetation of the region is characterized by the presence of phytophysiognomies of the Cerrado, including a range of savannas (*cerrado sensu stricto*, *campo sujo*, *campo de murundus*) and forest (*cerradão* and *mata galeria*), as well as areas dominated by pastures (sensu Eiten 1978).

The soil type is red latossol and neosol with flat relief, and the flora is represented by herbs, with predominance of grasses, and trees varying from 3 to 5 meters in height (Silva *et al.* 2002, Lima *et al.* 2010). The shortest distance among the sampled sites was 1 km (Sites 3 and Site 4) and the farthest distance was 11.5 km (Site 1 and Site 2). The local climate is tropical, classified as Aw in the Köppen classification system, with two well-defined seasons (wet summer and dry winter; Peel *et al.* 2007). The dry season, in the colder months, extends from May to September and the rainy season, in the hotter months, from October to April. Annual mean temperature is 24.8°C, annual mean of relative humidity reach 60% and annual mean rainfall reaches 137.8 mm (from period 2007–2016; INMET 2017).

Table 1. Coordinates and area of the sites sampled in the municipality of Caldas Novas, State of Goiás, Brazil.

Sampled sites	Latitude	Longitude	Altitude (m)	Area (m^2)
1	-17.838824	-48.602381	720	3328
2	-17.867109	-48.706792	692	3697
3	-17.818536	-48.646447	754	406
4	-17.828148	-48.651263	763	1256
5	-17.888145	-48.689534	644	3140

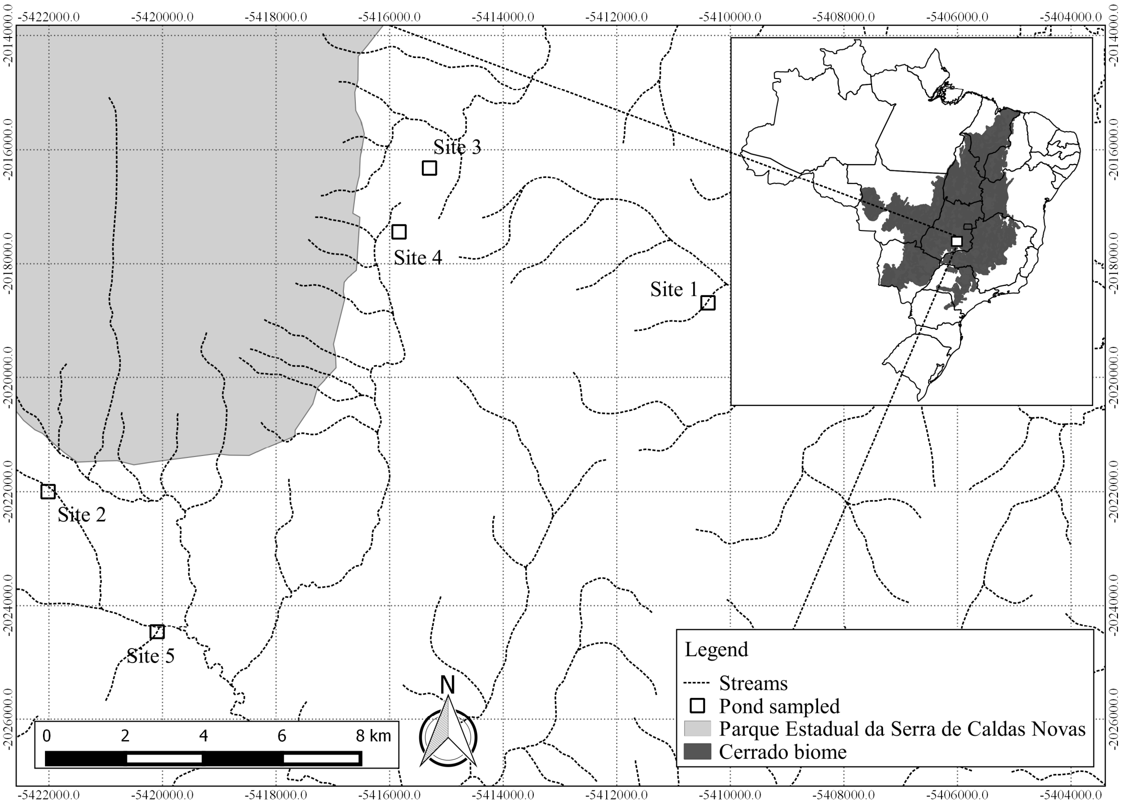


Figure 1. Map showing the five sampling sites (municipality of Caldas Novas, Goiás, Brazil).

8.2.2 Sampling techniques

We conducted a series of acoustic surveys to count the number of calling males in each study site. Anurans were sampled once per month, during six consecutive months, between October 2014 and March 2015, with a total of 30 sampling days for the monitoring period. Field work began always one hour before sunset (nearly 18:00 hrs) and finished after sunrise (nearly 07:00 hrs), and consisted in a single sampling every hour, so that a total of fourteen samplings were carried out per day (14 hours per day/site sampled), with a total sum of 420 sampling hours. The time of sunset and sunrise was obtained from the Interactive National Observatory Yearbook (Observatório-Nacional 2014), following the convention adopted by the reference text Supplementary Explanatory Astronomical Ephemeris and Nautical Almanac (Urban & Seidelmann 2012). Sampling method was standardized, always carried out by the same researcher (VG) and with the same protocol for each visit. It was based on acoustic search by walking slowly along all extension of the water body, counting the number of all calling males of each species (Heyer *et al.* 1994). Approximately every five meters, the researcher made a stop for about a minute to hear any calling male that was not registered, and to ensure that all males from each species were counted. The researcher took between thirty to fifty minutes during each auditory search, depending on the size of the sampled site. To count the individuals of *Barycholos ternetzi*, a species of direct development that reproduces in the leaf litter, a 25-meter transect was also carried through the forested environment (*mata galeria*). To confirm the identification of species, vocalizations were recorded and deposited in the sound library (FonoZoo) of the Museo Nacional de Ciencias Naturales, Madrid (Appendix A).

Air temperature and relative humidity at approximately 10 cm above the ground were registered using a digital termohygrometer Incoterm (to the nearest 0.1°C and 1% humidity). Individuals of every species were collected, euthanized with 5% lidocaine, fixed in 10% formalin solution and later stored in 70% alcohol. Vouchers specimens are deposited in the Zoological Collection of the Federal University of Goiás (ZUFG) (Appendix A).

8.2.3 Statistical analysis

Three types of analyzes, based on different datasets, were performed to identify: (1) daily patterns in the calling activity of species and communities; (2) temporal overlap in the calling activity of species and communities; (3) influence of time and climatic factors on calling activity of the studied species. First, temporal patterns of nightly calling activity at species and community levels were identified using circular statistical analyses (Zar 1999) with ORIANA 4.0 software, particularly Rayleigh's test (Kovach 2004). Two hourly response variables were applied to each level of analysis: (1) abundance and (2) presence/absence of each taxon for the species-level analysis; and (1) total abundance and (2) species richness for the community-level analysis. The hour of the night was converted to angles varying from 0º to 330º (0º being midnight) and associated with each response variable. The primary parameters considered for this analysis were: (1) mean vector (μ), which corresponds to the mean period when most species were active or there was the highest activity rate of a given species; (2) circular standard deviation (SD); and (3) the *r* vector, which corresponds to the mean of the data concentration around the circle (day), ranging from 0 (dispersed data) to 1 (aggregated data in the same direction). A significant result of Rayleigh's test ($p < 0.05$) indicates that data were not uniformly distributed and the presence of a significant mean angle or hour of the night (Kovach 2004) in which calling activity of the studied species and communities reach their peak.

To assess temporal niche overlap among species within the studied communities, we used the data base of hourly abundance of anuran species per site. The basis of each analysis was the relative number of calling individuals for each anuran species during each hour interval. This analysis was performed first for all species sampled and later for species of the same genus, namely *Dendropsophus*, *Boana* and *Leptodactylus*. We performed an analysis for all sampled time and then we divided by periods: 1 - October and November, 2 - December and January, 3 - February and March. Overlap was quantified as the average of all pair-wise overlap values calculated via the Czechanowski index (Feinsinger *et al.* 1981), following the analytical methods of Castro-Arellano *et al.* (2010). Null distributions of overlap values were generated using the randomization algorithm Rosario, which was designed specifically for using with interval data, in which the order of categories is important (Castro-Arellano *et al.* 2010). Rosario maintains the shape of the empirical activity distributions (i.e., temporal autocorrelation) for each species in the randomly generated matrices by shifting entire activity patterns a random number of intervals. For each analysis, overlap indices were calculated for 10,000 randomly generated matrices of temporal activity patterns. Significance was determined by comparing each empirical value to its associated null distribution. Analyses of niche overlap were conducted as two-tailed tests, which may detect a higher overlap in temporal niche than expected by chance alone (temporal coincidence) or a lower overlap than expected by chance alone (temporal avoidance). Coincidence in temporal activity was indicated by significantly more overlap than expected by chance. Segregation in temporal activity was indicated by significantly less overlap than expected by chance. Simulations for overlap in temporal activity were conducted with the TimeOverlap program (Castro-Arellano *et al.* 2010; program available for download at http://hydrodictyon.eeb.uconn.edu/people/willig/Research/activity%20pattern.html).

To examine the calling activity of anuran communities along the night, two response variables were considered: (1) species richness and (2) species diversity (Shannon index; Shannon & Weaver 1949). These variables were hourly calculated per site and sampling day and the Shannon index was estimated from the hourly abundance of each species recorded. A Generalized linear mixed-effects model (hereafter GLMM) was performed for each response variable (richness and species diversity). In both models, the sampled time (14 hours), air and water temperatures (°C) and relative air humidity (%) were used as fixed factors. Air and water temperature, and humidity were z-transformed. We also treated each site and days as random effects to address the potential non-independence (spatial and temporal correlation). First, to investigate the relationship between species richness, time along the night and abiotic factors (temperature and humidity) a first GLMM was set using binomial error structure and the logit link function. Such analysis was conducted assuming that all sites had equal chance to contain the same species number (19 spp.). Then we coded the response variable as a two-column matrix, being the first column the number of recorded species and the second one the number of absent species. For this model we separated anuran communities in three seasons: beginning season - first two months (October and November); middle season - two middle months (December and January); and end season - last two months (February and March). The season period (three levels) was also used as fixed factor. Second, to verify how diversity (Shannon index) varied along the night, a second GLMM was set using restricted maximum likelihood function.

GLMMs were fitted in R (R Core Team 2017) using the functions *lmer* and *glmer* of the package *lme4* (Bates *et al.* 2013). The R package ‘‘lme4’’ provides the ability to fit negative binomial and Poisson error distributions with or without zero-inflation. Both of these distribution families and their variants have been shown to work best for count data (Lindén & Mäntyniemi 2011, Bates *et al.* 2013). We established a model inference by full-null model comparisons using a likelihood-ratio test with the R-function *anova* (Dobson 2002, Forstmeier & Schielzeth 2011). Null models were fitted using only the random effects and control fixed factors and then compared to the full models by applying maximum likelihood (Bolker *et al.* 2009). To determine the significant effect of individual predictors, P-values were based on likelihood-ratio tests of the full model using the R-function *drop1* (R Core Team 2017).

Before performing analyses, we conducted graphical data exploration to check for normality and homogeneity by visually inspecting probability plots (Q-Q plots) and the residuals plotted against fitted values (Bolker *et al.* 2009, Zuur *et al.* 2010). Multicollinearity was assessed with Generalized Variance Inflation Factors (GVIF; Fox & Monette 1992, Field *et al.* 2013) in R with the function *vif* (Fox & Weisberg 2011). The GVIF was performed from a standard linear model excluding the random effects and reveled absence of collinearity between fixed factors (<2.5 in all cases). Regression diagnostics confirmed the absence of multicollinearity, autocorrelation and significant departures from normality in all final models. For all analyses, signification was set at a *p*-value < 0.05.

8.3 RESULTS

We monitored nightly calling activity of 19 anuran species in the five communities (Table 2). Circular statistical analysis revealed a nightly peak of total abundance (Rayleigh=9668.30, μ=334.69°, SD=41.14°, r=0.77, p<0.01) and species richness (Rayleigh=1008.04, μ=347.12°, SD=51.40°, r=0.70, p<0.01) in the anuran communities (Fig. 2), with most individuals and species calling in the time frame between one (~20:00 h) and three hours (~23:00 h) after sunset. After this period, calling activity typically decreased following a progressive drop until dawn (~06:00 h; Fig. 3). Species with nocturnal activity showed activity peaks ranging from 20:00 to 01:00 h (Table 3; Appendix B). Only *Pseudopaludicola mystacalis* commonly exhibited diurnal habits, with a rapid fall in calling activity after sunset. At species level, slightly differences were found between the circular analysis using abundance and that using the presence/absence data (see Table 3 and Appendix C). The peak of calling activity of the species was almost identified one hour later by the former case than the latter case.

Table 2. Seasonal patterns of anuran amphibians in vocalization activity in the municipality of Caldas Novas, State of Goiás, Brazil.

Species	Oct	Nov	Dec	Jan	Feb	Mar	Sites
Strabomantidae							
Barycholos ternetzi (Miranda-Ribeiro, 1937)	■	■	■				1, 2, 3, 4, 5
Hylidae							
Dendropsophus cruzi (Pombal and Bastos, 1998)	■	■	■	■	■	■	1, 2, 4, 5
Dendropsophus nanus (Boulenger, 1889)		■	■	■	■	■	2, 4, 5
Dendropsophus minutus (Peters, 1872)	■	■	■	■	■	■	1, 2, 4, 5
Dendropsophus rubicundulus (Reinhardt and Lütken, 1862)	■	■	■	■	■		1, 2, 4, 5
Boana albopunctata (Spix, 1824)	■	■	■	■	■	■	1, 2, 3, 4, 5
Boana paranaiba (Carvalho, Giaretta, and Facure, 2010)		■	■	■	■		2, 5
Boana lundii (Burmeister, 1856)	■	■	■	■	■	■	1, 2, 3, 4, 5
Boana raniceps (Cope, 1862)	■	■	■	■	■	■	1, 2, 4, 5
Scinax fuscovarius (Lutz, 1925)	■	■	■	■	■	■	1, 2, 4, 5
Pseudis bolbodactyla Lutz, 1925	■	■	■	■	■		1, 2, 4, 5
Phylomedusidae							
Pithecopus hypochondrialis (Daudin, 1800)	■	■	■	■	■	■	1, 2, 3, 4, 5
Leptodactylidae							
Pseudopaludicola mystacalis (Cope, 1887)	■	■	■	■	■	■	1, 2, 5
Physalaemus cuvieri Fitzinger, 1826	■	■	■	■	■	■	1, 2, 3, 4, 5
Leptodactylus latrans (Steffen, 1815)	■	■	■	■	■		1, 2, 4, 5
Leptodactylus labyrinthicus (Spix, 1824)	■	■	■	■	■		1, 2, 3, 4, 5
Leptodactylus podicipinus (Cope, 1862)	■	■	■	■	■	■	1, 2, 3, 4, 5
Leptodactylus fuscus (Schneider, 1799)	■	■	■	■	■		1, 2, 5
Microhylidae							
Elachistocleis cesarii (Miranda-Ribeiro, 1920)	■	■	■	■	■	■	1, 2, 5

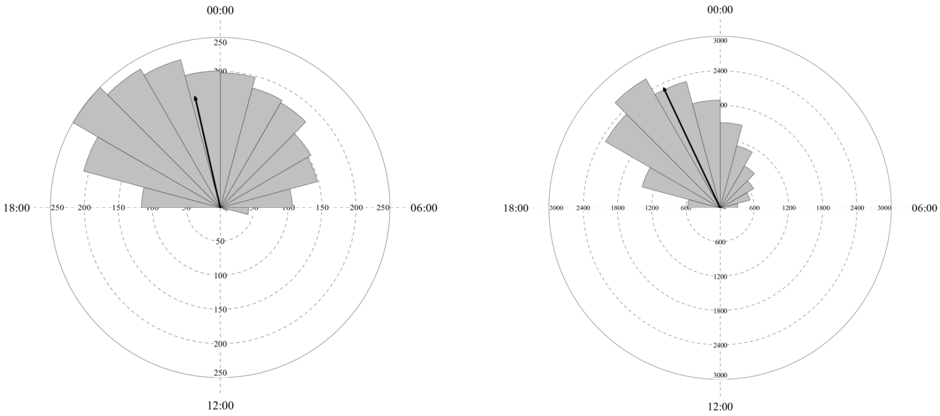


Figure 2. Rose-diagram of the circular analysis for species richness (*r* = 0.70) and total abundance (*r* = 0.77) of anuran species exhibiting calling activity along the night in the studied anuran communities (Caldas Novas, Goiás, Brazil). The arrow depicts the mean vector length ®, which indicates the concentration of species exhibiting calling activity along the hours of the night.

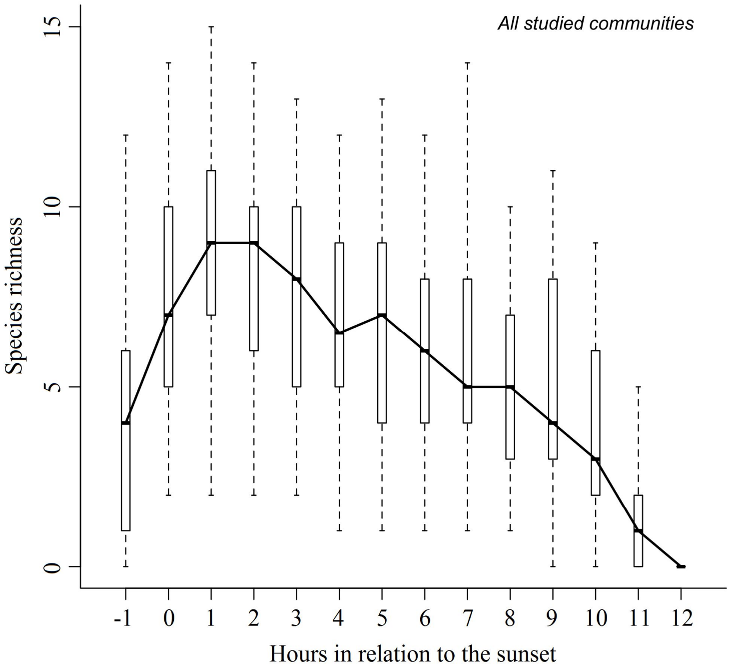


Figure 3. Average species richness along the night in the five studied anuran communities (municipality of Caldas Novas, State of Goiás, Brazil). Zero represents the sunset. Central horizontal bars represent medians, box heights represent the standart deviation, and whiskers represent maximum and minimum values.

The temporal niche overlap of the anuran communities was greater than expected by chance (coincident activities), considering all periods (Czechanowski Index = 0.14, p<0.001) and each period separately (Period 1, Czechanowski Index = 0.14, p=0.01; Period 2, Czechanowski Index = 0.17, p<0.01; Period 3, Czechanowski Index = 0.13, p<0.01). For congenery species no segregated or coincident activities were found, namely *Dendropsophus*, *Boana* and *Leptodactylus* (p-value for Czechanowski > 0.05). Thus, no pattern of coincidence or avoidance in the calling activity throughout time was found for sympatric congener species.

Table 3. Results of the circular statistical analysis of calling activity of each anuran species (in abundance) sampled in the communities in the municipality of Caldas Novas, Goiás, Brazil. Rayleigh = "z" value for the uniformity of Rayleigh's test; µ (°) = value of the mean vector, in degrees, accompanied by the corresponding month and season; SD (°) = circular standard deviation, in degrees; r = length of the vector.

Total abundance	**Circular statistical analysis**			
Species	**Rayleigh**	**µ (°)**	**SD (°)**	***r***
Strabomantidae				
B. ternetzi	160.50*	22:08 (332.23°)	03:41 (55.41°)	0.63
Hylidae				
D. cruzi	2344.95*	22:24 (336.17º)	02:17 (34.36°)	0.83
D. nanus	379.27*	22:00 (330°)	02:26 (36.73°)	0.81
D. minutus	79.04*	21:00 (315°)	02:53 (43.32°)	0.75
D. rubicundulus	198.15*	21:32 (323.17°)	02:00 (30.07°)	0.87
B. albopunctata	2426.63*	22:33 (338.32°)	02:34 (38.69°)	0.8
B. paranaiba	28.54*	23:22 (350.58°)	03:08 (47.04°)	0.71
B. lundii	288.83*	22:07 (331.91°)	02:33 (38.437°)	0.8
B. raniceps	44.83*	22:03 (330.80°)	03:15 (48.944°)	0.69
S. fuscovarius	402.21*	21:16 (319.13°)	01:23 (20.99°)	0.93
P. bolbodactyla	89.23*	21:06 (316.57°)	02:48 (42.07°)	0.76
Phylomedusidae				

Total abundance	**Circular statistical analysis**			
Species	**Rayleigh**	**μ (°)**	**SD (°)**	***r***
P. hypochondrialis	1116.65*	23:58 (359.58°)	02:45 (41.37°)	0.77
Leptodactylidae				
P. mystacalis	701.21*	20:01 (300.25°)	02:05 (31.46°)	0.86
P. cuvieri	1396.19*	21:16 (319.03°)	02:14 (33.54°)	0.84
L. latrans	47.54*	22:03 (330.76°)	02:43 (40.83°)	0.78
L. labirinthycus	159.4*	22:24 (336.08°)	03:00 (45.08°)	0.73
L. podicipinus	303.81*	01:01 (15.37°)	02:58 (44.67°)	0.74
L. fuscus	223.72*	22:42 (340.58°)	03:36 (54.01°)	0.64
Microhylidae				
E. cesarii	89.23*	21:13 (318.39°)	02:24 (36.20°)	0.819

*p<0.01

Overall, both the full models were different from the null models (binomial likelihood-ratio test: $\chi^2 = 28.99$, df = 3, p < 0.01; Poison likelihood-ratio test: $\chi^2 = 14.69$, df = 1, p < 0.01), revealing that richness and diversity was significantly influenced by fixed factors (Table 4). Hour had a negative significant effect on anuran richness (z=-0.232, P<0.01) and diversity (t=-0.210, P<0.01), and period influenced significantly the richness of anuran species on communities throughout the reproductive season (Table 4). Also, air and water temperature had a negative significant effect on anuran richness (z=-0.383, P<0.01; z=-0.176, P<0.01) and diversity (*t*=-0.311, P<0.01; *t*=-0.162, P<0.01), and humidity had a positive significant effect on anuran richness (z=0.265, P<0.01) and abundance (*t*=0.179, P<0.01; Table 4).

Table 4. Output from general linear mixed-effects model testing the effects of abiotic variables, period and time on richness and abundance (Shannon Diversity Index) of anuran communities in Cerrado, Brazil. Model with binomial error structure and identity link function included two fixed factors: hour and period; while the model with Poisson error structure and identity link function included only hour as fixed factor. Both models included three covariates: air and water temperature and relative humidity (z-transformed); and two random factors: sampled site and sampled day.

	Estimate	**Standard error**	***z*-value**
Richness			
Binomial (glmer)			
(Intercept)	0.615	0.265	2.322*
hour	-0.232	0.023	-9.912*
periodp2	0.300	0.269	1.116*
periodp3	-0.356	0.277	-1.283*
z.tair	-0.383	0.052	-7.421*
z.TH2O	-0.176	0.049	-3.558*
z.um	0.265	0.043	6.148*
Shannon index			
(lmer)			***t* value**
(Intercept)	3.276	0.392	8.364*
hour	-0.211	0.028	-7.604*
z.Tair	-0.311	0.086	-3.623*
z.TH2O	-0.162	0.077	-2.115*
z.Um	0.179	0.070	2.557*

P<0.001*

8.4 DISCUSSION

Nightly monitoring of anuran communities studied revealed that calling activity was highly influenced by both nightly and seasonal periods as well as weather conditions, particularly temperature and relative humidity, showing a characteristic circadian pattern and a significant temporal niche overlap. The peak of calling activity at community level was identified between the first and third hour after sunset, with a subsequent progressive decrease until sunrise, when most species ceased to call.

Few studies have previously examined the daily variations in calling activity of anuran communities in tropical regions (e.g. Cardoso & Haddad 1992, Duellman & Pyles 1983, Bevier 1997, Moreira & Barreto 1997). Studying calling activity along the night in several species, Bevier (1997) found that calling rates declined throughout the evening, and most species stopped calling shortly after midnight (~five hours after sunset). The depletion of energy reserves may contribute to this decrease (Brepson *et al.* 2013), since sound production in anurans is highly costly in relation to metabolic rates and oxygen consumption (Wells & Taigen 1989, Wells 2007). Moreover, in prolonged breeders (sensu Wells 1977), some calling males may also reduce or cease calling activity to save energy reserves for consecutive nights (Castellano & Gamba 2011). Nevertheless, factors other than energetic costs have also been evoked to explain this temporal activity pattern, such as the variation in the timing of females of each species arriving at the breeding site (Bevier 1997, Dias *et al.* 2017).

As found by other studies in tropical regions, most species of the community exhibit preferably nocturnal activity (Duellman & Pyles 1983, Bevier 1997, Moreira & Barreto 1997). After sunset, air temperature typically decreases and air relative humidity increases, making the night period most suitable for the activity of animals with permeable skins, such as amphibians. Furthermore, during the night, anurans may avoid visually oriented predators, such as diurnal bird species (Vitt & Caldwell 2013), and also do not compete for the acoustic space with other animal groups (Farina & James 2016). As most species in the Cerrado have nocturnal habits, besides using different call parameters and/or calling sites to avoid competition for the acoustic niche, males may adjust the period of emission of their advertisement calls to avoid overlap (Grafe 1996, Martínez-Rivera & Gerhardt 2008). Moreover, females may present evolutionary adaptations to identify conspecific males in noisy choirs (Vélez *et al.* 2013), with a large number of species vocalizing at the same time.

Although we have not verified an overall temporal pattern of segregation or coincidence in niche overlap in species of the same genus, it was possible to notice that calling activity along the night differ substantially among species. At a specific level, each presented a small variation in the peak of calling activity. During the reproductive season, anuran species may avoid niche overlap by selecting different calling sites within water body and being active at different hours of the day (Bridges & Dorcas 2000). Between the four *Dendropsophus* and *Leptodactylus* sympatric species recorded, each one showed a different activity peak, indicating a difference in temporal niche of calling. *Boana lundii* and *B. raniceps* seem to overlap in calling activity peak. However, both species have marked differences in calling sites and even in egg laying location: while *B. lundii* call in higher calling sites generally inside gallery forest (*veredas*) and lay eggs in pans made by males near lotic or lentic environments, *B. raniceps* vocalize on shrubs close to lentic environments, where it deposits its eggs directly into the water. Species that vocalize in sympatry such as *Boana albopunctata* and *B. paranaiba* may show variation in some acoustic parameters, avoiding acoustic niche overlap (Vieira *et al.* 2016). Male calling activity may also vary along latitudinal or altitudinal gradients because of variation in meteorological factors and environmental triggers (e.g. Lemckert & Mahony 2008, Llusia, Márquez, Beltrán, Benítez, *et al.* 2013). Various factors potentially influence male behavior, including energy reserves, predation risk, cost of calling, probability of finding food, distribution of male energy states in the population, and probability of surviving to another breeding season (McCauley *et al.* 2000, Wells 2007).

Overall, timing and duration of anuran calling activity are highly dependent on environmental cues (Protázio *et al.* 2015, Heard *et al.* 2015, Ximenez & Tozetti 2015). As ectotherms, temperature is the main abiotic factor to determine calling activity in many anuran species (Saenz *et al.* 2006, Wells 2007, Lowe *et al.* 2016). Water temperature is important for species that call from or near the water (e.g. Gambale & Bastos 2014, Oseen & Wassersug 2002). Air relative humidity is also relevant due the sensitivity of anurans to desiccation, especially for species that lay their eggs out of water, such as *Phitecopus hypocondrialis* (in leaves above water; Dias *et al.* 2017), or those with direct development, such as *Barycholos ternetzi* (eggs in leaf-litter of the forest floor; Caramaschi & Pombal-Jr 2001). Thus, both temperature and relative humidity modulate calling activity and reproduction of most anuran species, and thereby alterations in climate regimes related to climate change might affect adversely their acoustic communication and reproduction (Blaustein *et al.* 2010, Llusia, Márquez, Beltrán, Benítez, *et al.* 2013, Mainwaring *et al.* 2017).

Anuran reproduction in the Cerrado biome is cyclical and seasonal, increasing in spring or early summer when rain is abundant, and decreasing throughout the seasons, with most species having completed their reproduction (Moreira & Barreto 1997, Oda *et al.* 2009, Kopp *et al.* 2010). Our results corroborate with this pattern, with an increase of species during the second studied period (December and January) and then a decrease in the third one (February and March). The high temporal acoustic niche overlap verified at community level suggests an absence of competition in acoustic space or that species avoid overlap using different calling sites and physical proprieties of the call (Duellman & Pyles 1983, Protázio *et al.* 2015). Although anurans living in tropical species-rich communities may experience temporal and spatial competition, species call in chorus due several reasons. Choruses usually are formed in areas with suitable environmental conditions (physical resources) required by females and offspring, and occur during seasonal and daily periods that are favorable for courtship and reproduction (Gerhardt & Huber 2002, Wells 2007). Species that aggregate in chorus increase the attraction of mates, though also increase competition among males and the attraction of acoustically- oriented predators (Tuttle & Ryan 1981). Choruses are thus focal points for sexual selection (Gerhardt & Huber 2002, Wells 2007).

Temporal patterns of species richness and diversity were influenced by similar environmental factors. In studies based on species surveys without standardization and ignoring the time of activity of the species that may potentially occur in a given area may result in low detection probabilities of focal species, leading investigators to the false conclusion that some species are rare or absent (Heard *et al.* 2006, Schmidt & Pellet 2009). The use of different sampling techniques can also influence species detection (Da Silva 2010, Mascarenhas *et al.* 2016, Madalozzo *et al.* 2017). A recent automatic recording technique (Passive Acoustic Monitoring, PAM) has been widely used to sample and measure the phenology of acoustically active animals (Acevedo & Villanueva-Rivera 2006, Madalozzo *et al.* 2017). Although PAM has become a standard technique, it has some limitations, such as not being able to estimate the total number of individuals of certain species (e.g. Pellet *et al.* 2007), specially in tropical anuran communities, which contain species with high abundance and some of them with very similar calls. We verified that the use of species richness may be as efficient as other diversity index based on abundance data, thus, it is possible to reinforce the use and importance of automated digital recording equipment for species recording, especially due to the lower cost, less time spent in the field and high effectiveness in detecting species through long sampling periods without human interference (Madalozzo *et al.* 2017).

In this study, we verified that the circadian rhythm of calling activity in anuran communities of the Cerrado are highly influenced by climatic factors such as temperature and relative air humidity, and that there is no segregation pattern of calling activity along night. However, species showed different peaks of calling activity and different calling and oviposition sites, and together with species-specific call parameters, these differences may allow the partition of the acoustic niche avoiding sound masking interference (Duellman & Pyles 1983, Wells 2007). To better understand phenological patterns in species activity other factors such as photoperiod (Jaeger & Hailman 1981), social factors (Höbel 2017) and anthropogenic noise (Vélez *et al.* 2013) deserve further study. We also show that the best hours to sample anuran communities in Cerrado would be between two and three hours after sunset in order to ensure maximum detection probabilities for anuran species in these communities. Our results highlight the influence of sampling time in species detection. Phenology studies allow a better understanding of how species would respond to future climate changes (e.g. Klaus & Lougheed 2013), in addition to improving the knowledge of the biology and ecology of each species.

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APPENDIX A - Anuran species with recorded calls at FonoZoo (Museo Nacional de Ciencias Naturales, Madrid, Spain) and voucher individuals at Coleção Zoológica da Universidade Federal de Goiás (ZUFG, Goiânia, Brazil)

Family	Species	FonoZoo	Voucher ZUFG
Craugastoridae	*Barycholos ternetzi* (Miranda-Ribeiro, 1937)	10059	9596
Craugastoridae	*Barycholos ternetzi* (Miranda-Ribeiro, 1937)	10060	9614
Hylidae	*Dendropsophus cruzi* (Pombal and Bastos, 1998)	10070	9579
Hylidae	*Dendropsophus cruzi* (Pombal and Bastos, 1998)	10071	9580
Hylidae	*Dendropsophus minutus* (Peters, 1872)	10100	—
Hylidae	*Dendropsophus minutus* (Peters, 1872)	10101	—
Hylidae	*Dendropsophus nanus* (Boulenger, 1889)	10112	—
Hylidae	*Dendropsophus nanus* (Boulenger, 1889)	10113	—
Hylidae	*Dendropsophus rubicundulus* (Reinhardt and Lütken, 1862)	10124	10562
Hylidae	*Dendropsophus rubicundulus* (Reinhardt and Lütken, 1862)	10125	10563
Hylidae	*Boana albopunctata* (Spix, 1824)	10141	—
Hylidae	*Boana albopunctata* (Spix, 1824)	10142	—
Hylidae	*Boana lundii* (Burmeister, 1856)	10165	10511
Hylidae	*Boana lundii* (Burmeister, 1856)	10166	—
Hylidae	*Boana raniceps* (Cope, 1862)	10183	9539
Hylidae	*Boana raniceps* (Cope, 1862)	10184	10506
Hylidae	*Scinax fuscovarius* (Lutz, 1925)	10256	9592
Hylidae	*Scinax fuscovarius* (Lutz, 1925)	10257	9601
Phyllomedusidae	*Pithecopus azureus* (Cope, 1862)	10206	9604
Phyllomedusidae	*Pithecopus azureus* (Cope, 1862)	10207	9605
Leptodactylidae	*Leptodactylus fuscus* (Schneider, 1799)	10187	9557
Leptodactylidae	*Leptodactylus fuscus* (Schneider, 1799)	10188	9557
Leptodactylidae	*Leptodactylus labyrinthicus* (Spix, 1824)	10197	—
Leptodactylidae	*Leptodactylus* cf. *podicipinus*	10204	—
Leptodactylidae	*Physalaemus cuvieri* Fitzinger, 1826	10226	9563
Leptodactylidae	*Physalaemus cuvieri* Fitzinger, 1826	10227	9565
Leptodactylidae	*Physalaemus nattereri* (Steindachner, 1863)	10239	9594

Family	**Species**	**FonoZoo**	**Voucher ZUFG**
Leptodactylidae	*Physalaemus nattereri* (Steindachner, 1863)	10241	10501
Leptodactylidae	*Pseudopaludicola mystacalis* (Cope, 1887)	10247	10531

APPENDIX B - Results of the circular statistical analysis of the calling activity of each anuran species (presence/absence) sampled in the communities in the municipality of Caldas Novas, Goiás, Brazil

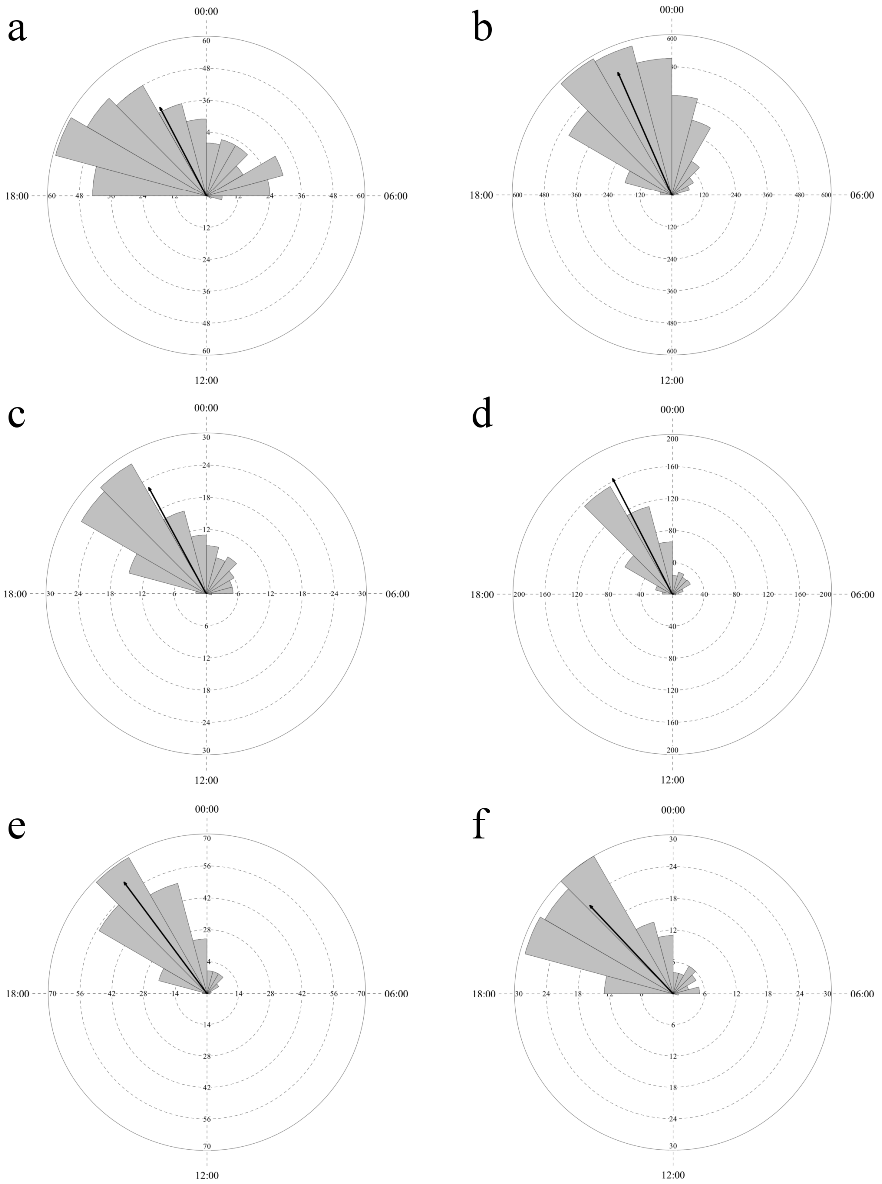
Rayleigh = "z" value for the uniformity of Rayleigh's test; µ (°) = value of the mean vector, in degrees, accompanied by the corresponding month and season; SD (°) = circular standard deviation, in degrees; R = length of the vector.

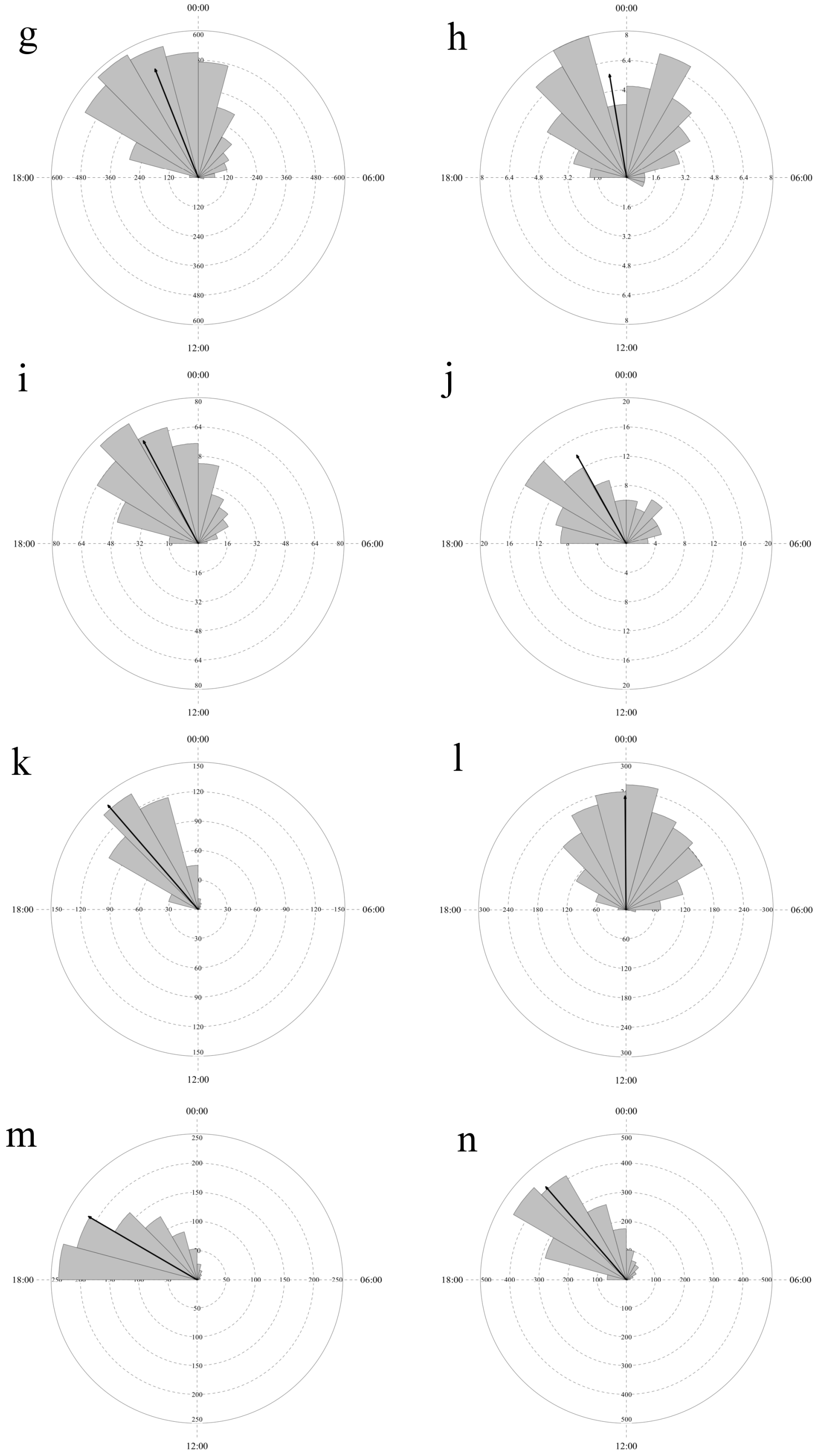
Presence/Absence	**Circular statistical analysis**			
Species	**Rayleigh**	**µ (°)**	**SD (°)**	***R***
Strabomantidae				
B. ternetzi	44.53	23:34 (353.52°)	03:52 (58.21°)	0.6
Hylidae				
D. cruzi	88.09*	23:00 (345°)	03:28 (52.04°)	0.66
D. nanus	27.02*	23:00 (345°)	03:22 (50.63°)	0.68
D. minutus	34.73*	23:09 (347.35°)	03:21 (50.27°)	0.68
D. rubicundulus	34.25*	22:35 (338.90°)	02:49 (42.25°)	0.76
B. albopunctata	112.60*	23:45 (356.47°)	03:34 (53.69°)	0.64
B. paranaiba	14.84*	23:58 (359.52°)	03:28 (52.18°)	0.66
B. lundii	92.27*	22:41 (340.342°)	02:59 (44.789°)	0.74
B. raniceps	27.99*	22:26 (336.67°)	03:08 (47.09°)	0.71
S. fuscovarius	42.86*	22:08 (332.25°)	02:09 (32.39°)	0.85
P. bolbodactyla	27.76*	22:00 (330°)	03:35 (53.78°)	0.64
Phylomedusidae				
P. hypochondrialis	107.35*	00:07 (1.866°)	03:17 (49.47°)	0.69
Leptodactylidae				
P. mystacalis	47.12*	21:23 (320.796°)	03:07 (46.86°)	0.72
P. cuvieri	117.28*	22:36 (339.01°)	03:17 (49.45°)	0.69
L. latrans	17.11*	22:21 (335.34°)	03:05 (46.42°)	0.72
L. labirinthycus	58.92*	23:02 (345.62°)	03:16 (49.15°)	0.69
L. podicipinus	73.30*	00:28 (7.00°)	03:16 (49.15°)	0.69
L. fuscus	48.01*	23:33 (353.43°)	03:45 (56.28°)	0.62
Microhylidae				
E. cesarii	43.15*	21:33 (323.39°)	02:58 (44.55°)	0.74

*$p<0.01$

APPENDIX C - Rose-diagram of the circular analysis and mean vector length (*r*), indicated by the arrow vector, for calling activity along the night of each species sampled in the municipality of Caldas Novas, Goiás, Brazil

a) *Barycholos ternetzi*; b) *Dendropsophus cruzi*; c) *D. minutus*; d) *D. nanus*; e) *D. rubicundulus*; f) *Pseudis bolbodactyla*; g) *Hypsiboas albopunctatus*; h) *B. paranaiba*; i) *B. lundii*; j) *B. raniceps*; k) *Scinax fuscovarius*; l) *Phitecopus hypocondrialis*; m) *Pseudopaludicola mystacalis*; n) *Physalaemus cuvieri*; o) *Leptodactylus labyrinthicus*; p) *L. latrans*; q) *L. fuscus*; r) *L. podicipinus*; s) *Elachistocleis cesariii*.

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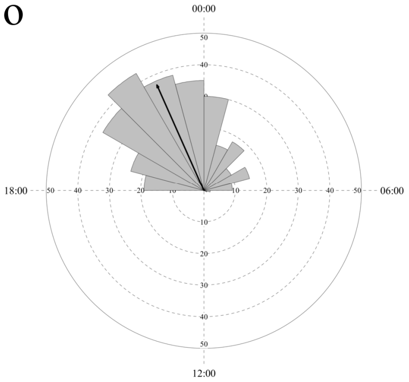
o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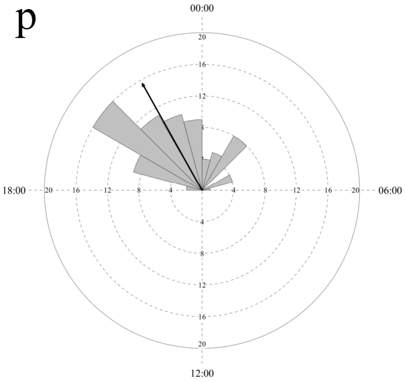
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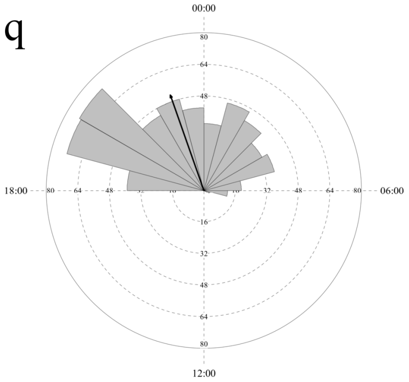
q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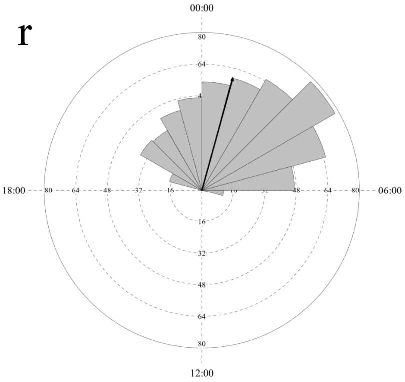
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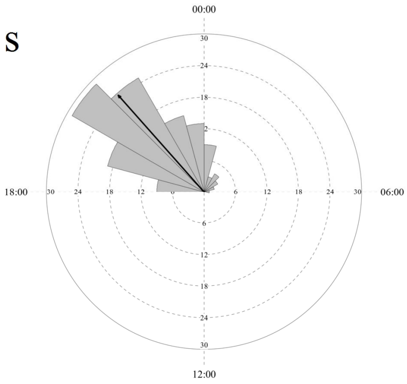
s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9 CONSIDERAÇÕES FINAIS

Existe uma grande lacuna de conhecimento sobre os cantos de anúncio dos anfíbios anuros do Brasil. Sabendo que os cantos são atributos importantes para espécies que utilizam os sinais acústicos como principal forma de comunicação intra e interespecífica, essa lacuna pode afetar avanços nos estudos sobre a ecologia e evolução deste grupo. No entanto, observamos que existe um aumento exponencial no número de descrições. O estudo da bioacústica tem se tornado mais frequente não só porque existem mais pesquisadores interessados neste tema, mas também porque os equipamentos de gravação e analise de sons tem se tornado mais acessíveis. É necessário enfatizar que outros dados (funcionais) de história natural das espécies, além dos cantos, como o tamanho das desovas, sítio de canto, dieta, e comportamentos defensivos também precisam ser descritos e estudados.

Como os cantos são indicadores fieis da identidade das espécies, conhecer as vocalizações além de ser um fator essencial para a taxonomia também é útil em estudos de monitoramento das comunidades. Além disso, algumas espécies podem apresentar um repertório vocal complexo e a descrição dos diferentes tipos de cantos pode ajudar a compreender melhor o comportamento social, reprodutivo e também fatores evolutivos. Estudos recentes têm demonstrado que cantos de soltura e de perigo possuem um sinal filogenético. Portanto, as vocalizações podem ser variáveis importantes ao estudar como as relações interespecíficas e o ambiente podem ter influenciado mudanças nas espécies ao longo do tempo.

Assim como já registrado em outros estudos, a maioria dos parâmetros temporais dos cantos de anúncio de *P. centralis* e *B. jaguariaivensis* foram dinâmicos, enquanto os parâmetros espectrais foram estáticos. Os valores dos parâmetros do canto sempre maiores que 1 para a relação do coeficiente de variação intra e interindividual (CV_B/CV_W) pode indicar que as duas espécies utilizam o canto para reconhecimento individual. Ao estudar a variação intra e interindividual nos parâmetros do canto podemos compreender melhor os fatores relacionados ao reconhecimento individual e específico, assim como os comportamentos relacionados a defesa e manutenção do território pelos machos e também a escolha de parceiros para a reprodução.

Investigando as tendências temporais na atividade acústica de três espécies simpáticas de *Dendropsophus* nós encontramos que existe uma dinâmica social complexa no comportamento vocal das espécies. Os machos podem apresentar mudança na atividade acústica em detrimento do estabelecimento do território ou ainda devido a fatores fisiológicos (demanda energética). Já a modificação dos parâmetros do canto pode estar relacionada a chegada das fêmeas nos sítios reprodutivos. No entanto, essa hipótese precisa ser confirmada com uma abordagem experimental. Estudos futuros também devem investigar as adaptações fisiólogas de diferentes grupos de espécies (por ex. reprodutores explosivos e prolongados) para identificar mecanismos determinantes da atividade acústica em escalas diária e sazonal. Além disso, também verificamos que as três espécies de *Dendropsophus* se diferem em relação aos parâmetros do canto, ao sitio de canto e ao período de máxima atividade de vocalização, portanto, não existe sobreposição do espaço acústico, o que indica a existência de múltiplos mecanismos para manter o isolamento reprodutivo.

A nível de comunidade a atividade acústica de anfíbios anuros do Cerrado apresentou um padrão circadiano, sendo influenciada principalmente por fatores climáticos (temperatura e umidade). Em geral, as comunidades apresentaram um pico de atividade das espécies entre a primeira e a terceira hora após o por do sol, com uma gradual diminuição ao longo da noite. Embora as exista uma alta sobreposição temporal da atividade acústica das espécies a nível de comunidade, avaliando cada uma individualmente parece existir segregação em relação ao pico máximo de atividade. As espécies podem usar diferentes estratégias para evitar a sobreposição no nicho acústico e facilitar a coexistência. Futuros estudos deverão avaliar os padrões de atividade acústica em escalas mais amplas com o objetivo de entender melhor os processos estruturadores da variação fenológica das espécies entre diferentes ecossistemas ou nos extremos de suas distribuições geográficas.