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Relationship between body size and release call parameters in *Rhinella* species (Anura: Bufonidae), with the description of the release call of *Rhinella ocellata* (Günther, 1858)

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Abstract. A release call is a type of vocalisation emitted by anurans mainly to avoid either intra- or interspecific mating, during the breeding season. Due to the importance of these calls in the social context of anuran behaviour, we hypothesised that the release call parameters of *Rhinella* species should be associated with body size. We found a negative relationship between dominant frequency of the release calls and body size of *Rhinella* species. The release call of *R. ocellata* showed a harmonic structure composed of 1–4 pulsed notes and the call duration and dominant frequency had average lengths of 1.6 s and 1214 Hz, respectively. Acoustic signals can express information on the physical condition of their senders. Since release calls may reflect both phylogenetic relationships and individual or population differences, we emphasise the importance of describing and carrying out further studies on release calls.

Key words. Acoustic repertoire, body mass, *Cerrado*, dominant frequency, vocalization

Introduction

Anurans use acoustic signals to mediate mating, aggressive behaviour and antagonistic encounters during the breeding season (Wells, 2007; Toledo et al., 2015; Köhler et al., 2017). Among call parameters, dominant frequency and call duration play important roles in the social context of species, such as in territory defence and mate attraction (Wells, 2007; Toledo et al., 2015; Köhler et al., 2017). Some of these call parameters are also associated with the calling individual's body characteristics and may reflect the physical condition of the sender (Morais et al., 2012; Gambale and Bastos, 2014; Köhler et al., 2017). For example, body size can be accurately estimated by the frequency of the advertisement call (Bee and Gerhardt, 2001). This association has its physiological basis in the relationship between larynx size, vocal fold length, and body size (Wells, 2007). The relationship between dominant frequency and body size is the most commonly known pattern, while temporal parameters, such as call duration and pulse number, are correlated with the social context into which males are inserted (e.g., Lemes et al., 2012; Morais et al., 2012; Gambale and Bastos, 2014).

Although advertisement calls are the most common type of call studied (Köhler et al., 2017; Guerra et al., 2018), a wide variety of other call types are emitted by males in multiple social contexts (see Toledo et al., 2015). The function of some of these calls is still not well known, and few species have had their complete vocal repertoire described (e.g., Lemes et al., 2012; Morais et al., 2012; Guerra et al., 2018). Furthermore, few studies have evaluated the relation of these calls with abiotic parameters (e.g., air temperature and humidity) and body condition (e.g., body length and body mass) (Sanabria and Quiroga, 2012).

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The release call is an important acoustic signal related to anuran reproduction as it may be emitted by females or males when they are clasped by an undesirable partner, either conspecific or not, to avoid heterospecific mating or gratuitous energy expenditure (Wells, 2007; Toledo et al., 2015; Batista et al., 2017). This call differs between species and has proved to be phylogenetically informative (Brown and Littlejohn, 1972; Sullivan, 1989; Castellano et al., 2002). Release calls have received less attention in acoustic studies than advertisement call, but they have been used to diagnose species in the *Rhinella spinulosus* group (Di Tada et al., 2011).

The anuran family Bufonidae Gray, 1825 includes 52 widely distributed genera. The genus *Rhinella* Fitzinger, 1826 currently consists of 92 valid species that occur throughout the Neotropics, of which approximately 42 species are known, and divided into five species groups, in Brazil (Frost, 2019). The Ocellated Toad, *Rhinella ocellata* (Günther, 1858) is a small-sized terrestrial and nocturnal toad found in the *Cerrado* in the States of Bahia, Goiás, Mato Grosso, Minas Gerais, Pará, and Tocantins, as well as in *restinga* habitat in Maranhão State (Caldwell and Shepard, 2007; Matavelli et al., 2014; de Freitas et al., 2018). Since release calls may be subject to the same morphological restrictions (larynx size and vocal fold length) of the advertisement calls, are important in social context, and may be related to the phylogeny of some groups, we hypothesise that the release call parameters of *Rhinella* species are correlated to body size. Additionally, we describe the release call of *R. ocellata*.

Material and Methods

Data compilation.—We compiled data on release call parameters from the scientific literature using the Google Scholar search engine. The search terms used included ‘release call’ OR ‘release vocalization’ AND ‘*Rhinella*’ OR ‘*Chaunus*’ OR ‘*Bufo*’ OR ‘Toad’. We considered only toad species that are currently classified in the genus *Rhinella* according to Frost (2019). Additionally, we searched for other studies that were cited in the surveyed literature but were not recovered in the initial bibliographic search (e.g., Brown and Guttman, 1970; Zimmerman and Bogart, 1988). We extracted the following metadata from each study: (i) species name, (ii) call type (pulsed or unpulsed), and (iii) any additional available call parameters (Table 1). The snout–vent length (SVL) of the species was obtained from the studies found in the search (release call descriptions) or in studies of natural history and taxonomy (e.g., Jofré et al., 2005; Narvaes and Rodrigues, 2009; Haddad et al., 2013).

Data analysis.—We performed a linear regression analysis to determine whether dominant frequency and call duration of the release calls were related to the SVL of *Rhinella* species (Table 1). Before performing the analyses, all variables were tested for homoscedasticity and normal distribution, and log-transformed. Release calls of *R. dapsilis* (Myers and Carvalho, 1945) were not used in the analysis as these showed an atypically high dominant frequency (4810 ± 350 Hz; Zimmerman and Bogart, 1988), which is up to twice as high as that in all other species (representing an outlier in the analysis). The analysis was performed with the ‘vegan’ package (Oksanen et al., 2013) in R version 3.3.3 (R Development Core Team, 2016).

Recording and call analysis.—We found two males of *R. ocellata* (Fig. 1) calling on the ground from the edge of a dam on 21 October 2015, in Caldas Novas Municipality, Goiás State, Brazil (17.8837ºS, 48.6837ºW). We simulated false axillary amplexus in the toads by positioning them on the ground and gently pressing their lateral body parts using thumb and forefinger until release calls were emitted. The SVL of each male was measured using digital callipers (accurate to the nearest 1 mm). Temperature was taken using a digital thermohygrometer (to the nearest 0.1°C).

Recordings were obtained using a Marantz PMD 660 recorder coupled with a Sennheiser ME66/K6 directional microphone. Calls were recorded at 44.1 kHz with 16-bit resolution and saved in .wav audio file format. Call parameters were assessed using Raven Pro

Figure 1. Adult male *Rhinella ocellata* photographed while calling in Caldas Novas Municipality, Goiás State, central Brazil.

Table 1. Release calls traits of *Rhinella* species. See materials and methods section for call parameter acronym definitions. *Call described in this study.

Species (Reference)	Call type	CD (s)	NN	ND (s)	DF (Hz)	IBC (s)	Pulses/s	Pulses/call	PD (s)	IBD (s)
***Rhinella crucifer* group**										
R. abei (Batista et al., 2017)	Pulsed	1.620 ± 0.679 (0.585–2.906)	2 (2–8)	0.206 ± 0.366 (0.058–0.161)	1214.10 ± 37.12 (1125.9–1378.1)	–	–	5 (4–71)	0.007 ± 0.002 (0.003–0.013)	0.006 ± 0.004 (0.001–0.021)
***Rhinella granulosa* group**										
R. azarai (Guerra et al., 2011)	Unpulsed	0.029 ± 0.034 (0.002–0.260)	1	0.029 ± 0.034 (0.002–0.260)	2097 ± 174 (1736–2431)	0.406 ± 1.008 (0.084–9.024)	75.7 ± 86.3 (3.8–500)	–	–	–
R. azarai (Guerra et al., 2011)	Pulsed	0.038 ± 0.019 (0.009–0.091)	1	0.038 ± 0.019 (0.009–0.091)	2143 ± 156 (1910–2431)	0.247 ± 0.466 (0.051–5.462)	137 ± 44.7 (23.5–222.2)	4.8 ± 2.4 (2–14)	0.008 ± 0.008 (0.001–0.058)	0.005 ± 0.003 (0.001–0.025)
R. bergi (Guerra et al., 2011)	Unpulsed	0.015 ± 0.003 (0.011–0.023)	1	0.015 ± 0.003 (0.011–0.023)	2712 ± 93 (2587–3061)	0.479 ± 0.637 (0.120–2.900)	69.5 ± 12.6 (43.5–90.9)	–	–	–
R. bernardoi (Sanabria and Quinoga, 2012)	Pulsed	0.034 (0.022–0.052)	1	0.034 (0.022–0.052)	1151.97	0.098 ± 0.004	–	(5–9)	0.003	–
R. dorbignyi (Guerra et al., 2011)	Pulsed	0.471 ± 0.200 (0.195–0.775)	1	0.471 ± 0.200 (0.195–0.775)	2109 ± 82 (2005–2252)	0.452 ± 0.482 (0.168–1.876)	47.5 ± 15.1 (19.9–68.5)	23.6 ± 14.7 (7–49)	0.005 ± 0.005 (0.001–0.028)	0.019 ± 0.013 (0.002–0.087)
R. fernandezae (Guerra et al., 2011)	Pulsed	0.206 ± 0.065 (0.155–0.321)	1	0.206 ± 0.065 (0.155–0.321)	1937 ± 26 (1897–1961)	0.238 ± 0.040 (0.167–0.292)	54.5 ± 10.7 (36.9–64.5)	10.7 ± 1.5 (10–14)	0.011 ± 0.004 (0.006–0.026)	0.009 ± 0.011 (0.001–0.048)
R. mirandaribeiroi (Vieira et al., 2014)	Pulsed	0.730 ± 0.550 (0.130–2.050)	15.9 ± 13.7 (6–53)	0.012 ± 0.010 (0.006–0.029)	2434.4 ± 126.9 (2192–2596)	–	–	2.5 ± 0.7 (1–4)	0.005 ± 0.004 (0.002–0.039)	–
***R. margaritifera* group**										
R. dapsilis (Zimmerman and Bogart, 1988)	Pulsed	0.08 ± 0.01 (0.07–0.11)	1	0.08 ± 0.01 (0.07–0.11)	4810 ± 350 (4220–5250)	0.13 ± 0.1 (0.06–0.36)	55.42 ± 9.56 (44.78–71.27)	(1–4)	0.003 ± 0.001	–
*Rhinella ocellata**	Pulsed	0.315 ± 0.270 (0.086–1.105)	1 ± 0.99 (1–4)	0.106 ± 0.043 (0.014–0.184)	1101.563 ± 86.888 (750–1312.5)	0.872 ± 0.533 (0.439–3.815)	42.94 ± 18.05 (12.38–76.09)	5 ± 4.59 (4–21)	0.013 ± 0.012 (0.005–0.153)	0.016 ± 0.007 (0.001–0.038)
***R. marina* group**										
R. arenarum (Di Tada et al., 2001)	Unpulsed	0.036 (0.005–0.196)	1	0.036 (0.005–0.196)	804 (653–1256)	–	–	1	–	–
R. arenarum (Di Tada et al., 2001)	Pulsed	0.034 (0.007–0.228)	1	0.034 (0.007–0.228)	979 (703–1206)	–	187.5 (97.6–375)	6 (2–23)	0.003 (0.002–0.007)	0.002 (0.001–0.006)
R. arenarum (Di Tada et al., 2001)	Pulsed	0.352 (0.072–0.792)	1	0.352 (0.072–0.792)	854 (703–1256)	–	39.2 (30.8–92.8)	16 (3–28)	0.013 (0.006–0.025)	0.014 (0.008–0.017)
R. arenarum (Brown and Guttman, 1970)	–	–	–	–	–	–	45.3 (42.5-62.5)	–	–	–
R. icterica (Batista et al., 2017)	Pulsed	0.237 ± 0.338 (0.026–1.113)	5 (3–7)	0.237 ± 0.338 (0.026–1.113)	786.87 ± 105.20 (689.1–1205.9)	–	–	1 (1–33)	0.022 ± 0.012 (0.010–0.044)	0.014 ± 0.004 (0.008–0.019)
R. jimi (Garda et al., 2010)	–	–	1	–	600.59 ± 51.71 (516.8–689.1)	–	–	(2–5)	–	–

Table 1. Continued.

Species (Reference)	Call type	CD (s)	NN	ND (s)	DF (Hz)	IBC (s)	Pulses/s	Pulses/call	PD (s)	IBD (s)
***R. spinulosa* group**										
R. spinulosa (Di Tada et al., 2001)	Unpulsed	0.078 (0.009–0.187)	1	0.078 (0.009–0.187)	1055 (704–1381)	–	–	–	–	–
R. spinulosa (Di Tada et al., 2001)	Pulsed	0.578 (0.223–0.290)	1	0.578 (0.223–0.290)	1080 (954–1306)	–	46.5 (33.1–48.7)	28 (9–46)	0.012 (0.009–0.018)	0.01 (0.002–0.013)
R. spinulosa (Brown and Guttman, 1970)	–	–	–	–	–	–	71.4 (65–77.4)	–	–	–
R. achalensis (Di Tada et al., 2001)	Unpulsed	0.048 (0.011–0.187)	1	0.048 (0.011–0.187)	954 (603–1231)	–	–	–	–	–
R. achalensis (Di Tada et al., 2001)	Pulsed	0.455 (0.058–0.878)	1	0.455 (0.058–0.878)	929 (829–1080)	–	61.8 (48.8–86.2)	28 (4–56)	0.015 (0.005–0.019)	0 (0–0.01)
R. limensis (Di Tada et al., 2001)	Unpulsed	0.047 (0.013–0.184)	1	0.047 (0.013–0.184)	728 (603–979)	–	–	–	–	–
R. limensis (Di Tada et al., 2001)	Pulsed	0.052 (0.017–0.114)	1	0.052 (0.017–0.114)	854 (653–954)	–	164.7 (115.4–233.3)	9 (2–20)	0.004 (0.002–0.006)	0.003 (0.002–0.003)

64 1.5 software from the Cornell Lab of Ornithology (Bioacoustics Research Program, 2014). We applied a window size of 256 samples, 75% overlap, hop size of 64 samples, DFT of 512 samples, and Hamming window type to produce the spectrograms. Figures of oscillogram and spectrogram were obtained using TuneR 1.0 (Ligges et al., 2013) and Seewave 1.7.3 (Sueur et al., 2008) packages for R version 3.3.3 (R Development Core Team, 2016), with settings of window name = Hanning, window length = 256 samples, and overlap = 90%. We measured the following acoustic parameters: call duration (CD), number of notes per call (NN), note duration (ND), interval between calls (IBC), number of pulses emitted per second (pulses/s), number of pulses per call (pulses/call), pulse duration (PD), interval between pulses (IBP), and call dominant frequency (DF). Parameters were measured from 40 calls of the two *R. ocellata* males (20 release calls for each individual). Measurements are presented as mean ± standard deviation (SD) and also providing the minimum–maximum size range. Terminology and call description follow Toledo et al. (2015). Voucher specimens are housed at the Coleção Zoológica da Universidade Federal de Goiás (ZUFG 10302, 10303), Goiás State, Brazil. Voucher recordings are available at the FonoZoo, Museo Nacional de Ciencias Naturales, Madrid, Spain (FonoZoo 10318, FonoZoo 10319).

Results

We found a correlation between dominant frequency of release calls and SVL in *Rhinella* species ($b = -1.421$; $R^2_{adj} = 0.67$; $F = 27.83$; $P < 0.01$; Fig. 2). Call duration was not related to body size ($P > 0.05$).

The release call of *Rhinella ocellata* presents a harmonic structure (1–6 well-defined harmonics) and is composed of 1–4 pulsed notes (Table 1; Fig. 3). The SVLs of the recorded males were 42.76 mm and 40.39 mm. The air temperature at the recorded time was 22.7°C. While emitting release calls, the two individuals of *R. ocellata* vibrated their bodies. Syntopic frog species include *R. diptycha* (Cope, 1862), *Boana albopunctata* (Spix, 1824), *B. paranaiba* (Carvalho et al., 2010), *B. raniceps* (Cope, 1862), *Dendropsophus minutus* (Peters, 1872), and *D. cruzi* (Pombal and Bastos, 1998).

Discussion

As hypothesised, small-sized *Rhinella* species have release calls with higher dominant frequencies. Thus, the dominant frequency of the release calls is directly related to body size, a well-known relationship in the

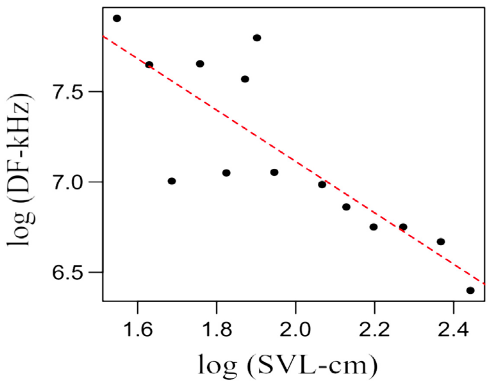

Figure 2. Linear regression analysis (regression equation 'y = 9.956 – 1.421x', P < 0.01) between dominant frequency of the release calls and SVL for *Rhinella* species.

advertisement calls of many anuran species (Morais et al., 2012; Gambale and Bastos, 2014; Köhler et al., 2017). Sanabria and Quiroga (2012) also found a relationship between some parameters of the release calls and body size for *R. bernardoi*. Toledo and Haddad (2009) and Santana et al. (2013) found a negative relationship between the dominant frequency of the distress calls and SVL in hylid species. Besides, as release calls are important in a reproductive context (Toledo et al., 2015), males may discriminate different sympatric species by these calls when they occur together at the same breeding site.

Release calls of *Rhinella* species clearly differ among species in some acoustic parameters, and this is particularly important because this type of call avoids breeding energy expenditure with a misleading amplexus (Toledo et al., 2015). Moreover, release calls are important to distinguish species (Di Tada et al., 2011), as they may vary between individuals and can also be used to compare populations (Brown and Littlejohn, 1972; Sullivan, 1989). Although these calls have received considerably less attention than advertisement calls, they may indicate important characteristics of individuals, such as the body size (Sanabria and Quiroga, 2012). Leary (2001) demonstrated that release calls may converge due sympatry in bufonid species, probably as an example of convergent character displacement.

The release call of *R. ocellata* has higher values of call duration, pulse number, pulse duration, and lower values of dominant frequency than values found for *R. dapsilis* (Table 1). Although release calls of most *Rhinella* species have just one note (Guerra et al., 2011; Vieira et al., 2014), in *R. ocellata* the note number ranged from 1–4, in *R. miranderibeiroi* from 6–53 (Vieira et al., 2014), in *R. abei* from 2–8, and in *R. icterica* from 3–7 pulsed notes (Batista et al., 2017). The body vibrations seen

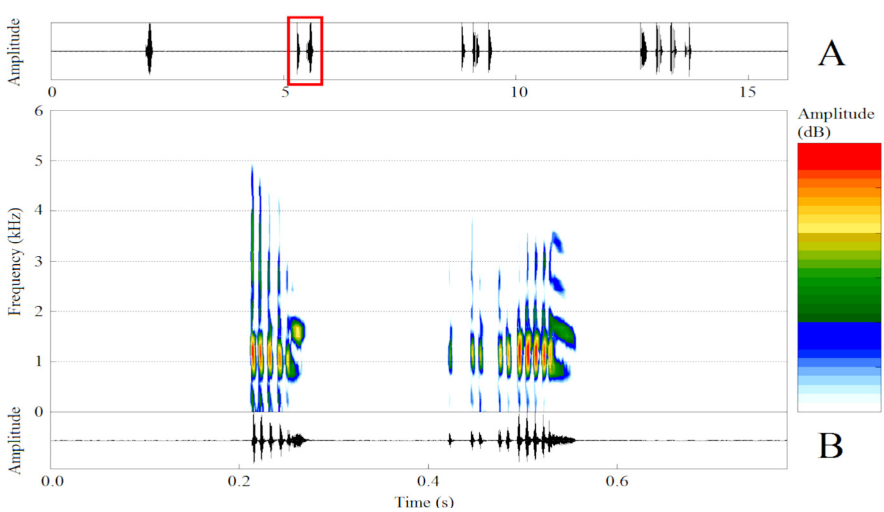

Figure 3. Release call of *R. ocellata*. (A) Waveform of a series of four calls with different note numbers (one, two, three, and four); (B) Spectrogram (above) and respective oscillogram (below) of the second call (two notes) highlighted by a red outline (air temperature = 22.7 °C; air humidity = 99%; SVL = 42.76 mm). Voucher toad specimen (ZUFG 10302). Voucher call recording (FonoZoo 10319).

in *R. ocellata* while emitting release calls is a typical feature of *Rhinella* species (Vieira et al., 2014; Batista et al., 2017) and may be important to initiate dismounting of males (Wells, 2007).

There is a considerable number of cryptic anuran species awaiting description, especially among sympatric bufonids which have similar external morphology and live in hybridisation zones (Brown and Guttman, 1970; Thomé et al., 2012). Some species of the *R. granulosa* group have a very short reproductive period (Yanosky et al., 1997), making it difficult to record advertisement calls. Thus, release calls could be an alternative option to obtain acoustic variables for species differentiation. Furthermore, acoustic traits play an important role for inferring phylogenetic relationships among species in integrated molecular and morphological studies (Brown and Littlejohn, 1972; Sullivan, 1989; Goicoechea et al., 2010; Di Tada et al., 2011).

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