Release and distress calls of *Rhinella abei* (BALDISSERA, CARAMASCHI & HADDAD, 2004), and *Rhinella icterica* (SPIX, 1824)

Vocalization is the main form of communication in anurans. These animals produce many different types of sounds which have specific meanings in the social context they are used in (WELLS et al. 2007; TOLEDO et al. 2014). The advertisement call is the best known and thus, most frequently described and used call type in taxonomic studies (Wells et al. 2007; GAMBALE & BASTOS 2014; BATISTA et al. 2015). However, other calls, such as release calls, play important roles in anuran biology. Release calls are emitted by males or females when another frog, either conspecific or not, attempts to mate with them (WELLS et al. 2007; TOLEDO et al. 2014). Like advertisement calls, release calls may vary between species and help distinguish between closely related taxa (BROWN & LITTLEJOHN 1972; GRENAT & MARTINO 2013). Distress call is a defensive scream call emitted by males, females, juveniles, newly metamorphosed as well as larvae of anurans when grasped by potential predators (WELLS 2007;

NATALE et al. 2011; TOLEDO et al. 2014). Loud and explosive notes characterize the distress call, which distinguishes it from other vocalizations (DUELLMAN & TRUEB 1994). Distress calls can be emitted with mouth open or closed (TOLEDO & HADDAD 2009). Calls are stereotyped and also help to identify differences among species (CARVALHO et al. 2013). In the present note, the authors describe the release and distress calls of *Rhinella abei* (BALDISSERA, CARA-MASCHI & HADDAD, 2004) (of the *Rhinella crucifer* group) and *Rhinella icterica* (SPIX, 1824) (of the *Rhinella marina* group).

Five male individuals of *R. abei* and three of *R. icterica* were spotted on September 9, 2014, and September 23, 2015. Specimens were found calling from the edge of water bodies, near an Araucaria Forest remnant in the Atlantic Rain Forest domain in the municipality of Campo Largo (25°30' 26.90" S, 49°22'35.88" W, 905 m above sea level), Paraná state, southern Brazil. The frogs were caught, positioned sitting on the ground, and squeezed in the lateral abdominal region behind the forelimbs, simulating an amplexus. This procedure caused the males to emit release calls. When the males were squeezed the same way lifted off the ground, they emitted distress calls. Recordings were obtained using Marantz[®] PMD 660 and Tascam[®] Dr-40 digital recorders connected to a Sennheiser® ME66/K6 directional microphone. Calls were recorded at 44.1 kHz with 16-bit resolution. Bioacoustic traits were analyzed using Raven Pro 64 1.5 software from the Cornell Lab of Ornithology (BIOACOUSTICS RESEARCH PRO-GRAM 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. Oscillogram and spectrogram figures were produced 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 DEVELOP-MENT CORE TEAM 2016). Voucher specimens are housed at the zoological collection of Universidade Federal de Goiás (ZUFG), Goiás state and the herpetological collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro state, Brazil (R. abei: ZUFG 9884; ZUFG 9885; R. icterica: MNRJ 89526; MNRJ

89527). The following acoustic parameters were analyzed: call duration, note duration, note number, internote interval, pulse number, pulse duration, inter-pulse interval, dominant frequency of the call, and lower and upper frequency of the call (see BATISTA et al. 2015; FORTI et al. 2015). Parameters were measured from 87 calls of R. abei and 68 calls of *R. icterica*. Measurements are presented as the mean (or mode) \pm standard deviation (minimum - maximum). Call description and terminology follow TOLEDO et al. (2014). Voucher individuals were measured (snout-vent length) using a digital caliper (to the nearest 0.1 mm) and the temperature was taken using a digital thermohygrometer (to the nearest 0.01 °C).

Average snout-vent length (SVL) of *R*. *abei* males recorded was 60.01 ± 2.64 mm (57-64.02 mm; N = 5) and mean air temperature was 16.7 ± 0.82 °C (15.0 - 17.6 °C, N = 5). Three types of release call and one of distress call were identified. Release call "A" was emitted by five males of R. abei and consists of a single pulsed note (Table 1; Fig. 1A). Release call "B" was emitted by three males and consists of a series of pulsed notes (Table 1; Fig. 1B). In the release call "B" the first $(0.366 \pm 0.158 \text{ s},$ 0.077-0.531 s, N = 10) and last (0.355 ± 0.093 s, 0.077-0.208 s, N = 6) notes of the call have longer duration in relation to the middle notes $(0.058 \pm 0.039 \text{ s}, 0.031 - 0.187)$ s, N = 15), which comprise the notes between the first and last notes of the call. Release call "C" was emitted by one male and has a harmonic structure. This call presented three well-defined harmonics (Table 1; Fig. 1C). When emitting release calls, all males of R. abei made small body vibrations. Distress calls were emitted by four males of R. abei as a loud scream of short duration and harmonic structure (Table 1; Fig. 1D).

Rhinella icterica specimens recorded had an average SVL of 96.73±24.81 mm (68.09–111.50 mm, N = 3) and mean air temperature was 18.01±0.70 °C (17.20– 18.41 °C, N=3). The authors identified two types of release call and one of distress call. The release call type "A" was emitted by all males of *R. icterica* and consists of a single pulsed note (Table 1; Fig. 2A). The release call type "B" was emitted by two males and consists of a series of pulsed notes (Table 1; Fig. 2B). While emitting release calls the males of *R. icterica* made small body vibrations. The distress call of *R. icterica* was emitted by three males and consisted of a single note with a harmonic structure (Table 1; Fig. 2C).

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Rhinella abei showed a greater release call repertoire than R. icterica. Such repertoire was composed of single and compound calls. There are no release and distress call descriptions for any other species in the *Rhinella crucifer* group. In the *Rhinella marina* group, the release call is only known for *Rhinella jimi* (STEVAUX, 2002) (GARDA et al. 2010). The release call repertoire of R. *icterica* is more extensive than *R. jimi* and the pulse number of the notes is higher. However, the dominant frequency is similar and overlaps in both species. A male of *R*. abei and six males of R. icterica were evaluated by TOLEDO et al. (2009). However, they did not emit distress calls. In the present work all males that were handled emitted release and/or distress calls. All individuals tested responded to the handling with a series of vibrations, similar with the muscular contractions produced by R. mirandaribeiroi (GALLARDO, 1965) (VIEIRA et al. 2014). Vibrations displayed by some anuran species may be important to initiate dismounting of males (WELLS 2007). Such behavior is commonly found among Rhinella species (BLAIR 1947; RENGEL 1948; VIEIRA et al. 2014). The release and distress calls recorded by R. abei and R. icterica were emitted with mouths closed as noted in other bufonids (WEBER 1978).

Some of the functions of release calls are to avoid breeding energy expenditure with a misleading amplexus, to warn conspecifics of predator presence and/or to frighten (or surprise) auditively oriented predators, or even to attract a secondary predator capable of interfering in the predatory process (Hödl & Gollmann 1986; TOLEDO et al. 2014). However, a case of amplexus between a male of R. icterica and a male of the invasive bullfrog Lithobates catesbeianus (SHAW, 1802), indicated that release calls emitted by the amplected male of R. icterica were not effective to prevent the interspecific amplexus (THEIS & CALD-ART 2015). Future researches using experi-



Fig. 1: Release and distress calls of *Rhinella abei* (BALDISSERA, CARAMASCHI & HADDAD, 2004) from Campo Largo, Paraná state, southern Brazil. A, B, C – release call types, D – distress call. Upper graphs: Audiospectrograms. Lower graphs: Oscillograms. Air temperature at time of recording – 17.6 °C. Water temperature – 20.0 °C. Male SVL – 64.2 mm. Unvouchered specimen.



Fig. 2: Release and distress calls of *Rhinella icterica* (SPIX, 1824) from Campo Largo, Paraná state, southern Brazil. A, B – release call types, C – distress call. Upper graphs: Audiospectrograms. Lower graphs: Oscillograms.
Air temperature at time of recording – 17.6 °C. Water temperature – 17.2 °C. Male SVL – 110.6 mm. Unvouchered specimen.

Rhinella icterica			Rhinella abei			Tay	kon	are j and PD - the c	
C	в	A	D	C	в	A	Call		Ta preser C); di - puls call.
$0.061 \pm 0.031 \\ (0.029 - 0.122) \\ N = 18$	$\begin{array}{c} 0.237{\pm}0.0338\\ (0.026{-}1.113)\\ N=37 \end{array}$	$0.855 \pm 0.251 \\ (0.477 - 1.145) \\ N = 9$	$\begin{array}{c} 0.109 {\pm} 0.198 \\ (0.017 {-} 0.615) \\ N = 15 \end{array}$	$0.659 \pm 0.037 (0.616 - 0.682) N = 3$	$1.620{\pm}0.679 \\ (0.585{-}2.906) \\ N{=}10$	$\begin{array}{c} 0.442{\pm}0.150\\ (0.072{\text{-}}0.680)\\ N=27 \end{array}$	CD (s)		ble 1: Acoustic nted as mean ± S istress call (D). e duration; Inot
1 1 1	5 (3-7) N = 37				$2 \\ (2-8) \\ N=10$	1 1 1	NN		paramete SD (or m <i>Rhinella</i> – inter-no
1 1 1	$\begin{array}{c} 0.237 \pm 0.338 \\ (0.026 \text{-} 1.113) \\ N = 37 \end{array}$	1 1 1	111	1 1 1	$0.206\pm0.366 \\ (0.058-0.161) \\ N = 42$	1	ND (s)		rs of the releas ode – for parar <i>icterica</i> : releas ote interval; Ipi
$\begin{pmatrix} 1 \\ (1-2) \\ N = 18 \end{pmatrix}$		33 (17-36) N=9	111	(11-21) N=3	5 (4-71) N=42	$31 \\ (11-82) \\ N=27$	PN		e and distr neters pres e calls (A ul – inter-p
0.045 ± 0.025 (0.016-0.075) N = 8	$\begin{array}{c} 0.022 \pm 0.012 \\ (0.010 - 0.044) \\ N = 35 \end{array}$	0.018 ± 0.019 (0.009-0.030) N = 145	111	$\begin{array}{c} 0.008 \pm 0.001 \\ (0.006 - 0.012) \\ N = 3 \end{array}$	$\begin{array}{c} 0.007 \pm 0.002 \\ (0.003 \text{-} 0.013) \\ N = 42 \end{array}$	$\begin{array}{c} 0.007{\pm}002\\ (0.003{-}0.012)\\ N{=}135 \end{array}$	PD (s)		ress calls of <i>Rhin</i> senting only integ and B); distress sulse interval; DF
1 1 1	$\begin{array}{c} 0.284{\pm}0.057\\ (0.117{-}0.389)\\ N{=}29 \end{array}$	1 1 1		111	$0.215 \pm 0.056 \\ (0.142 - 0.395) \\ N = 32$	1 1 1	Inot (s)	Acoustic p	<i>ella abei</i> (BALD) gers numbers), r call (C). CD – c ⁷ – dominant fre
0.008 ± 0.003 (0.005-0.011) N = 4	$\begin{array}{c} 0.014 \pm 0.004 \\ (0.008 \text{-} 0.019) \\ N = 35 \end{array}$	$\begin{array}{c} 0.011 \pm 0.003 \\ (0.006 \text{-} 0.018) \\ N = 45 \end{array}$		0.004 ± 0.002 (0.001-0.007) N = 15	0.006 ± 0.004 (0.001-0.021) N = 131	$\begin{array}{c} 0.004{\pm}0.004\\ (0.001{-}0.040)\\ N{=}135 \end{array}$	Ipul (s)	arameters	ISSERA, CARAMA ange and N (nur call duration; NN quency; MaxF –
746.51 \pm 132.11 (689.10-1205.90) N = 18	786.87 \pm 105.20 (689.10-1205.90) N = 37	$650.81 \pm 75.977 (516.80 - 689.10) N = 9$	$1194.40{\pm}102.25 (1033.60{-}1378.10) N = 15$	$(1205.90 \\ (1205.90-1205.90) \\ N=3$	$1214.10\pm 37.12(125.9-1378.1)N = 42$	$1156.01\pm68.08(1033.6-1205.9)N = 27$	DF (Hz)		SCHI & HADDAD, 20 nber of calls analyz N – note number; N - highest frequency
$1512.10{\pm}274.34(861.30{-}2067.20)N = 18$	$1569.02 \pm 324.29 (1205.90 - 2239.50) N = 37$	$1684.38 \pm 392.63 \\ (1205.90-2239.50) \\ N = 9$	$\begin{array}{c} 1538.90{\pm}322.08\\ (941.72{\text{-}}143.64)\\ N{=}15 \end{array}$	$(1378.10 \\ (1378.10-1378.10) \\ N = 15$	$1533.98 \pm 189.52 (1378.10-1894.90) N = 42$	$(1033.60-1205.90) \\ N = 27$	MaxF (Hz)		104), and <i>R. icterica</i> red). <i>Rhinella abei</i> : D – note duration; F of the call; MinF – 1
507.23 ± 124.96 (172.30-689.10) N = 18	$\begin{array}{c} 428.34{\pm}138.\\ (172.30{-}516.80)\\ N=37 \end{array}$	$\begin{array}{c} 401.97 \pm 172.25 \\ (516.80 \hbox{-} 172.30) \\ N = 9 \end{array}$	941.72 \pm 143.64 (689.10-1205.90) N = 15	976.17 \pm 99.48 (861.30-1033.60) N = 15	$824.40{\pm}775.17(344.50{-}1033.60)N = 42$	$863.23{\pm}143.57 (516.80{-}1033.60) N = 27$	MinF (Hz)		(SPIX, 1824). Data release calls (A, B ³ N – pulse number; owest frequency of

ments could be conducted in order to better understand the role of release and distress calls. The taxonomic status of some bufonid species is problematic due their similar external morphology and on account of hybridization zones between sympatric species such as e.g., *Rhinella ornata* (SPIX, 1824), and *R. abei* (THOMÉ et al. 2012). Basic natural history information is fundamental to evaluate the process shaping the ecology of the species and to differentiate the species-specific characters of important taxonomic value.

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