

The release call of *Rhinella bernardoi* (Anura: Bufonidae)

Eduardo A. Sanabria^{1,*}, and Lorena B. Quiroga^{1,2}

Abstract. *Rhinella bernardoi* belongs to the *Rhinella granulosa* group, of which species are characterized by explosive breeding events which make it difficult to obtain recordings of their advertisement call. As an alternative, it has been proposed that recordings of release calls can be used to differentiate between species. Here we describe the structure of the release call of *R. bernardoi*. Five males were stimulated by false axillary amplexus, upon which their release call was recorded for one minute. *Rhinella bernardoi* produces short and straight calls ($n = 11 \pm 2.3$). The duration of the call ranged between 2.2-5.2 ms. Pulse length ranged between 0.13-0.4 ms. Comparative studies of related species within the *Rhinella granulosa* group are needed to determine the taxonomic value of the release call.

Keywords. Argentina; Ethology; vocalization; Bufonidae.

Introduction

Amongst amphibians, vocalizations represent the most important form of communication, and play a major role in inter- and intra-specific interaction (Duellman and Trueb, 1986; Ryan, 1988; Pough et al. 2001). Concerning anurans, the call most commonly described is the advertisement call which is emitted by males during the breeding season. This call is usually emitted both to attract females for breeding, and to designate the territory of the caller (Duellman and Trueb, 1986; Salas et al. 1998; Pough et al. 2001; Wogel, Abrunhosa and Weber, 2004; Pimenta and Cross, 2004; Heyer and Carvalho, 2004). Conversely, the release call can be described as an agonistic signal emitted by male toads when they are amplexed by others males, which is usually accompanied by vibrations of the body (Duellman and Trueb, 1986). The release call may differ from the advertisement call in the number of pulses, frequency and duration (Pough et al. 2001). Furthermore, it has been described that the release calls of different species of Bufonid toads from North America differ significantly in their temporal structure, thus providing phylogenetic information (Sullivan and

Wagner, 1988; Sullivan and Lamb. 1988; Sullivan and Malmos, 1994). Difference in release calls was used by di Tada, Martino and Sinsch (2001) to separate species within the *Rhinella spinulosus* group.

Rhinella bernardoi is a recently described species (Sanabria et al. 2010) which belongs to the *Rhinella granulosa* group. This group has several particular natural history characteristics, such as a very short reproductive period which can be as short as two days (Gallardo, 1972; Yanosky, Mercolli & Dixon, 1997; Langone, 1999). These short breeding events make it difficult to obtain recordings of the advertisement call, particularly in arid regions where breeding activity is highly irregular. Therefore, the use of release calls is an alternative option to obtain appropriate variables to differentiate species within the *Rhinella granulosa* group. The aim of this study is to describe the structure of the release call of *R. bernardoi*, and to provide additional information about the relationship of call parameters with temperature and body size.

Materials and Methods

The study area is located 320 km northeast of the city of San Juan, Argentina, on National Highway 510. The dominant vegetation in the area includes *Deuterocohnia longipetala* (chaguar), *Larrea cuneifolia* and *L. divaricata* (jarillas), *Prosopis* sp. (Algarrobos), *Tipha dominguensis* (totoras) and *Bacharis salicifolia* (chilca dulce). This region is part of the Monte Desert which is characterized by an arid climate with a mean annual temperature of 17.3 °C, a mean maximal temperature of 25.7 °C, and a mean minimum temperature of 10.4 °C, with a mean annual rainfall of 89 mm during the summer months (Cabrera, 1976).

On January the 24th, 2008, we hand-captured five males of *R. bernardoi* and transported these to the laboratory at Universidad Nacional de San Juan. We simulated false axillary amplexus by

1 Laboratorio de Investigaciones Andrológicas de Mendoza, Instituto de Histología y Embriología de Mendoza, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Centro de Investigaciones en Ciencia y Técnica de Mendoza, CONICET, Mendoza, Argentina.

2 Departamento de Biología, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de San Juan. Avenida Ignacio de la Roza y Meglioli, 5400 San Juan, Argentina.

*Corresponding autor; e-mail: sanabria.eduardoa@gmail.com

Table 1. Descriptive statistics of call parameters. NPC (number of pulses per call), MPD (mean of pulse duration), AID (average interpulse duration), CL (Call length), DF (dominant frequency), DBC (distance between calls), R (Rate = Number of pulse / call length), EF (emphasized frequency), DB (decibel average in which the frequency is emphasized).

	Mean	Range	SE
NPC	6,94	4,00	0,18
MPD	0,25	0,26	0,008
AID	0,18	0,38	0,01
CL	3,40	3,00	0,10
DF	1151,97	1490,60	63,64
DBC	9,80	12,60	0,39
TP	205,99	138,11	5,30
EF	1422,58	474,00	15,50
DB	-32,74	14,60	0,46

gently pushing individuals using thumb and forefinger (Leary, 1999) until release calls were emitted. We recorded each call for three minutes using a digital recorder (Olympus VN-2000). Additionally, we measured body temperatures of individuals with a digital thermometer TES 1312 (TES Electrical Electronic Corp., Taipei, Taiwan, $\pm 0.1^\circ\text{C}$), and snout vent length (SVL). Laboratory temperature during data collection was $25 \pm 2^\circ\text{C}$.

Sampling frequency was 44.1 kHz at a 16 bit resolution. The calls were digitized and subsequently analyzed using Syrinx software version 2.2b (Burt, 2001). We analyzed 10 calls, and measured 9 variables: number of pulses per call (NPC), mean of pulse duration (MPD), average interpulse duration (AID), Call length (CL), dominant frequency (DF), distance between calls (DBC), Rate = Number of pulse / call length (R), emphasized frequency (EF), decibel average in which the frequency is emphasized (DB). We used descriptive statistics (mean \pm standard error), to characterize each of the variables. In addition, we carried out Spearman regressions between the song variables, SVL, and body.

Results and discussion

During artificial amplexus stimulation, *R. bernardoii* produces a series of short, straight calls of variable number (mean 11 ± 2.3 calls, min = 7, max = 19.8). The wide variation found in the number of calls emitted may be due to pressure differences that can be exerted on the individual at the time of simulating amplexus. Martin (1971) noted that variations of pressure on the chest during artificial amplexus cause an increase in the number of calls emitted by frogs. The timing between calls was relatively constant amongst individuals, with a mean of 98 ± 4 ms. The release call is composed of a small pulse train containing 5 to 9 pulses per call with a downward modulation. We observed that the last pulse is distended in length relative to the rest of the pulses heard in the call (Fig. 1B). The call duration ranged between 2.2 and 5.2 ms. Pulse length range was bounded by a minimum of 0.13 and a maximum of 0.4 ms duration, with mean inter-pulses of 0.18 ± 0.01 ms (Table 1).

The results of Spearman regressions between the song variables, SVL and body temperature of analyzed individuals showed that body size correlated positively and significantly with the mean number of pulses, mean length of inter-pulses, length of the call, emphasized frequency and decibels to which the frequency is emphasized. Also, the dominant frequency, distance between calls and pulse rate were negatively and significantly correlated with body size. The body temperature of individuals exhibited a significant, positive correlation with the dominant frequency and the average duration of the pulses (Table 2). Therefore, the dependency of the call variables on temperature

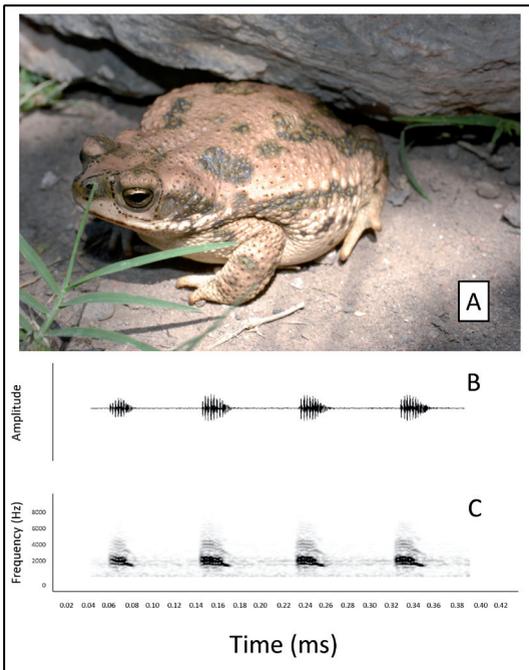


Figure 1. Release call of *R. bernardoii* (A) from Ischigualasto Provincial Park, Waveform (B) and spectrogram (C). Body temperature: 24.8°C .

Table 2. Spearman correlations between call variables versus body temperature and body size (SVL). For abbreviations see Table 1.

	LHC (mm)			Temperature (°C)		
	r	t(N-2)	p	r	t(N-2)	p
NPC	0.38	2.87	0.005	-0.23	-1.65	0.10
MPD	-0.13	-0.95	0.34	0.35	2.67	0.01
AID	0.52	4.23	0.0001	-0.17	-1.20	0.23
CL	0.56	4.79	0.00001	-0.16	-1.17	0.24
DF	-0.45	-3.51	0.0009	0.29	2.14	0.03
DBC	-0.35	-2.59	0.01	-0.08	-0.61	0.54
TP	-0.37	-2.83	0.006	-0.02	-0.18	0.85
EF	0.43	3.33	0.001	0.01	0.09	0.92
DB	0.24	1.72	0.09	0.27	1.99	0.05

and body size should be considered for comparative studies.

Currently, comparative material from other species within the *Rhinella granulosa* group is lacking, as the current description of the release call of *R. bernardoi* is the first among its members. Therefore, the description and analyses of closely related species are highly needed to assess the taxonomic value of this character.

Acknowledgements. We thank Elena Espejo for housing and logistical support, and Florencia Cano, Rodrigo Lopez and Viviana Orozco for field assistance. The comments of Pablo Grenat helped to improve a previous version of this manuscript. Special thanks to Denita Week for correcting the English grammar of the manuscript. We thank the provincial fauna office of San Juan for permission to conduct our research. This research was partially supported by a post-graduate fellowship from CONICET awarded to EAS.

References

- Burt, J. (2001): Syrinx bioacoustical software. website: www.syrinxpc.com. (Accessed on December, 2011).
- Cabrera, A.L. (1976): Enciclopedia Argentina de Agricultura y Jardinería, Tomo II, Regiones Fitogeográficas Argentinas. Editorial ACME S.A.C.I.
- di Tada, I., Martino, A., Sinsch, U. (2001): Release vocalizations in neotropical toads (*Bufo*): ecological constraints and phylogenetic implications. *Journal of Zoological Systematics and Evolutionary Research* **39**: 13-23.
- Duellman, W.E., Trueb, L. (1986): *Biology of amphibians*. New York, McGraw-Hill Press.
- Gallardo, J.M. (1972): Anfibios de la provincia de Buenos Aires; observaciones sobre su ecología y zoogeografía. *Ciencias e Investigación* **28**: 3-14.
- Heyer, R.W., Carvalho, C.M. (2004): The enigmatic advertisement call of *Eleutherodactylus ramagii* (Amphibia: Anura: Leptodactylidae). *Amphibia-Reptilia* **21**: 117-121.
- Langone, J.A. (1999): Anfibios de aguas corrientes, departamento de Canelones, Uruguay (Amphibia). *Anales Museo Nacional de Historia Natural*, Pub. extra. **48**: 1-16.
- Martin, W.F. (1971): Mechanics of sound production in toads of genus *Bufo*: passive elements. *Journal of Experimental Zoology* **176**: 273-293.
- Penna, M.V., Veloso, A. (1981): Acoustical signals related to reproduction in the spinulosus species group of *Bufo* (Amphibia, Bufonidae). *Canadian Journal of Zoology* **59**: 54-60.
- Pimenta, V.S.B., Cruz, C.A.G. (2004): The tadpole and advertisement call of *Physalaemus aguirrei* Bokermann, 1996 (Amphibia, Anura, Leptodactylidae). *Amphibia-Reptilia* **25**: 197-204.
- Pough, F., Andrews, R., Cadle, E., Crump, M., Zivitzky, A., Wells, R. (2001): *Herpetology*, 2nd. Edition. New Jersey, Princeton Hall Press.

- Salas, N., Zavatierra, M., di Tada, I., Martino, A., Bridarolli, M. (1998): Bioacoustical and etho-ecological features in Amphibian communities of southern Cordoba province (Argentina). *Cuadernos de Herpetología* **12**: 37-46.
- Sanabria, E., Quiroga, L., Arias, F. Cortez, R. (2010): A new species of *Rhinella* (Anura: Bufonidae) from the Provincial Park of Ischigualasto, Argentina. *Zootaxa* **2396**: 50-60.
- Sullivan, B. K., Lamb, T. (1988): Hybridization between the toads *Bufo microcapus* and *Bufo woodhousii* in Arizona: variation in the release call and allozymes. *Herpetologica* **44**: 325-333.
- Sullivan, B.K., Malmos, K.B. (1994): Call variation in the Colorado river toad (*Bufo alvarius*): behavioral and phylogenetic implications. *Herpetologica* **50**: 146-156.
- Sullivan, B.K., Wagner, W.E. Jr. (1988): Variation in advertisement call and release call, and social influence on calling behaviour in the gulf coast toad (*Bufo valliceps*). *Copeia* **1988**: 1014-1020.
- Wogel, H., Abrunhosa, P., Weber, L. (2004): The tadpole, vocalizations and visual displays of *Hylodes nasus* (Anura: Leptodactylidae). *Amphibia-Reptilia* **25**: 219-227.
- Yanosky, A.A., Mercolli, C., Dixon, J.A. (1997): Field ecology of the pygmy toad *Bufo pygmaeus* (Anura: Bufonidae), in north-eastern Argentina with notes on sympatric sibling species of the granulosis group. *Bulletin of the Maryland Herpetological Society* **33**: 66-77.