

SHORT COMMUNICATION

Novel observations on *Leptodactylus fuscus* (Anura: Leptodactylidae) mating behavior: a couple in acoustic interaction

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In most anuran reproductive behavior, the males are vocally active and females approach the males they have selected in silence (Wells 2007). However, in some species, females may call at low intensities in close-range interactions with males (Márquez and Verrel 1991, Schlaepfer and Figueroa-Sandí 1998, Forti *et al.* 2017a). Females may vocalize during courtship to signal acceptance, reveal their location to the male, and accelerate the encounter (Cui *et al.* 2010, Toledo *et al.* 2014, Preininger *et al.* 2016). Observations of reproductive behavior remain scarce for many anurans, and recordings of female calls are rare, even in well-known species (Preininger *et al.* 2016).

Leptodactylus fuscus (Schneider, 1799) is one such common species, having an extensive range across South America east of the Andes (Frost 2017). A genomic (mtDNA) study of many populations of this species revealed three well-differentiated lineages representing three different geographical regions (Camargo *et al.* 2006), as follow: (1) Central America, Guiana, and Amazonia; (2) Argentina and Bolivia; and (3) southeastern South America (Camargo *et al.* 2006). The reproductive biology of these clades of *L. fuscus* seems to be relatively plastic (Lucas *et al.* 2008).

As with many other leptodactylids, the calls of male *Leptodactylus fuscus* attract females of the species to subterranean mud chambers constructed by males (Crump 2015) where the eggs are deposited in a foam nest. The male may obstruct the opening to the cavity with his head (Martins 1988), and sometimes, the female seals

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the chamber with mud (Arzabe and Prado 2006). The embryos develop in their foam nest in the subterranean chamber, and after hatching, the exotrophic tadpoles are washed into ponds by floods (Crump 2015).

Herein, we describe an acoustic interaction between a mating pair of *Leptodactylus fuscus*. The vocalizations of both the male and female are described, along with the first observation of a male sealing the nest opening with mud. In addition, we analyze the individual variation in calls in a male and female *L. fuscus*.

Fieldwork was carried out in the municipality of Macapá, state of Amapá in northern Brazil (00°00'28.01" N, 51°05'12.64" W). We observed a reproductive interaction between a pair of *L. fuscus* using the focal-animal method (Altmann 1974). We extracted the vocalizations from a video made during the observation using a Cannon PowerShot SX60 HS digital camera. We used the software Adobe Audition 8.1.0 to convert the video extension (aac-mp4) to a readable sound file (wav) with a sampling rate of 44.1 kHz and 24-bit resolution. The video recording was deposited at Fonoteca Neotropical Jacques Viellard (access code FNJV 1000392). We used the call-centered approach of Köhler *et al.* (2017) to analyze 50 calls (31 emitted by the male and 19 emitted by the female). We applied a band filter (above 5400 Hz and below 260 Hz) to reduce background noise. Calls were normalized individually to -1 dB using the software Audacity 2.1.2 (Audacity Team 2017). All vocalizations were analyzed with Raven Pro 1.4 (Bioacoustic Research Program 2011) to measure the following acoustic properties: (1) call duration (ms); (2) number of pulses; (3) duration of ascendant frequency modulation (ms); (4) duration of descendent frequency modulation (ms); (5) time to maximum amplitude (ms); (6) minimum frequency (Hz); (7) peak dominant frequency (Hz); (8) maximum frequency (Hz); and (9) range frequency (as maximum subtracted by minimum) (Hz). Acoustic Variables 3 and 4 were only analyzed for females, because male calls have only

ascendant modulation, which is equal to call duration. We measured the spectral units in the spectrogram. Spectral measurements were obtained using a FFT (Fast Fourier Transform) of 1024 samples and 50% overlap, with Hann window type, hop size of 128 samples and grid spacing of 43.1 Hz. For check spectrograms we used DFT of 256 and dB filter of 248 Hz. We selected the calls using the waveform, and we used the following Raven functions to measure the acoustic properties: (1) Delta time (s) (for call duration); (2) Frequency 5% (Hz) (for the minimum frequency, ignoring 5% below the total energy in the selected call); (3) Frequency 95% (Hz) (for the maximum frequency, ignoring 5% above the total energy in the selected call); (4) Max frequency (Hz) (for the peak dominant frequency); and (5) Bandwidth 90% (for the range frequency, a band of frequency that includes 90% of the energy of the sound). "Max amplitude (u)" was used to determine the time to the maximum amplitude visualizing the power limits in the waveform. Figures were prepared with FFT of 256 samples, with 50% overlap in a Hann window. The power spectrum was generated in the software Goldwave v6.24, using the spectrum filter function.

To analyze call variation within individuals, we calculated the coefficient of variation of each acoustic property with the following equation: $CV = (\text{Standard Deviation}/\text{Mean}) \times 100$ (value in percentage).

We found a male calling near a cavity opening at 20:36 h, on 03 March 2017 (Figure 1A). After a few minutes, we located a female emitting a sequence of calls (described further) at the entrance of another cavity, less than 1 m from the male. Following this acoustic interaction, the male entered the nearest cavity, and the female approached and followed him. After spawning, the pair left the cavity, and the male sealed the nest opening with mud. One day later, we checked the chamber and found the foam nest (Figure 1B).

The male calls are whistles composed of a single, non-pulsed note characterized by a

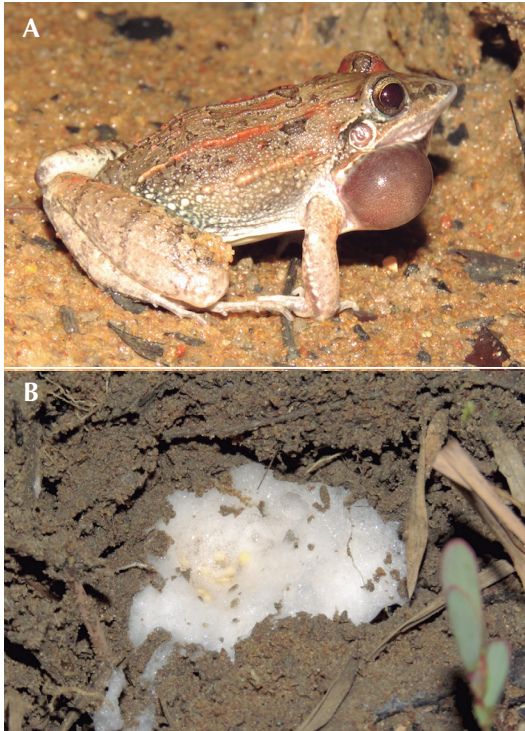


Figure 1. (A) A male *Leptodactylus fuscus* vocalizing during acoustic interaction with the female. (B) Details of the foam nest inside the subterranean chamber used by the pair of *L. fuscus*.

remarkable ascendant frequency modulation. The female calls are composed of a single note that comprises two pulses. The first pulse has an ascendant frequency modulation of 26 ms (range, 15–32 ms; $N = 19$), whereas the second has an accentuated descendent frequency modulation of 20 ms (range, 12–28 ms; $N = 19$) at the end of the call (Figure 2). The male calls are longer and have a higher frequency range than those of the female. Call properties of the male and female are compared in Table 1.

With the exception of minimum frequency, acoustic properties of the female calls are more variable than those of the male (Figure 3). In both sexes, spectral properties varied less than temporal properties, with the exception of frequency range in the female calls. Such pattern with temporal properties varying more than spectral properties seems to be conserved in male calls among several anuran species (Forti *et al.* 2010, 2015, 2016, 2017b, 2018).

Female calls are known for other leptodactylids, such as *Leptodactylus syphax* Bokermann, 1969 and *L. troglodytes* Lutz, 1926 (Silva *et al.* 2008, Kokubum *et al.* 2009). Dimorphic acoustic differences, such as the variation in call duration and range frequency in *L. fuscus*, occur in many other species in at least 11 anuran families

Table 1. Properties of male and female calls emitted during a courtship interaction of *Leptodactylus fuscus* in the municipality of Macapá, state of Amapá in northern Brazil. Values presented as mean \pm SD (range).

Acoustic properties	Male call ($N = 31$)	Female call ($N = 19$)
Range frequency (Hz)	1513 \pm 55 (1378–1594)	825 \pm 212 (430–1205)
Minimum frequency (Hz)	1173 \pm 55 (1077–1249)	1154 \pm 53 (1034–1249)
Peak dominant frequency (Hz)	2374 \pm 93 (2153–2498)	1467 \pm 143 (1163–1680)
Maximum frequency (Hz)	2685 \pm 69 (2541–2799)	1979 \pm 183 (1594–2326)
Call duration (ms)	223 \pm 11 (204–259)	63 \pm 8 (38–72)
Number of pulses	Non-pulsed	2
First pulse duration (ms)	-	26 \pm 6 (15–32)
Second pulse duration (ms)	-	20 \pm 4 (12–28)
Time to maximum amplitude (ms)	103 \pm 21 (66–146)	42 \pm 9 (13–53)

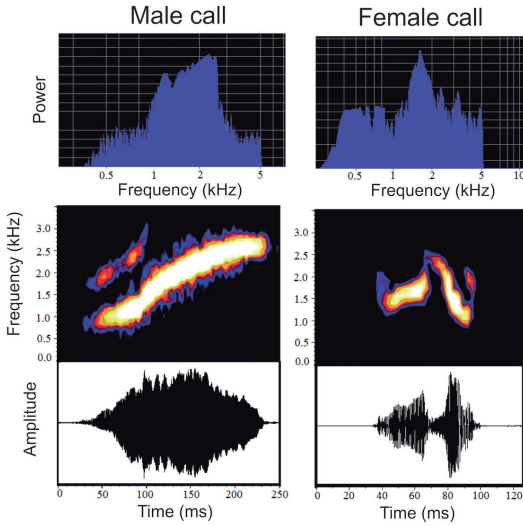


Figure 2. Power spectrum (above), spectrogram (middle) and oscillogram (below) of calls used by a pair of *Leptodactylus fuscus* in the municipality of Macapá, state of Amapá in northern Brazil. Spectrogram generated with FFT of 256 samples and 50% window overlap. Air temperature: 26.8°C.

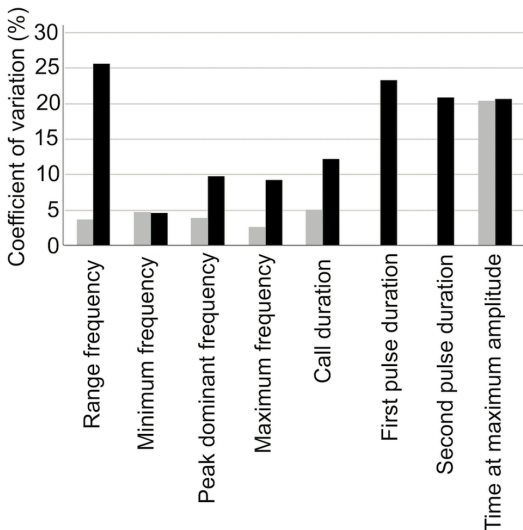



Figure 3. Coefficients of variation in call properties of individual frogs. Gray bars = male calls, black bars = female calls.

(Preininger *et al.* 2016). Among the factors that may underlie these differences are body size (with females usually being larger than males), vocalization social function, presence of vocal sac that aids in call transmission (only in males), and laryngeal size and complexity (males with larger laryngeal structures than females) (Duellman and Trueb 1986, Monnet and Cherry 2002, Wells and Schwartz 2007, Wilkins *et al.* 2013, Toledo *et al.* 2014, Preininger *et al.* 2016). Females are larger than males in *L. fuscus* (Carvalho *et al.* 2008), which may explain the fact that calls of females have lower frequencies than those of males.

Usually, males produce species-specific advertisement calls for attracting conspecific mates (Duellman and Trueb 1986, Ryan and Rand 1993); this may contribute to the lower variability in acoustic properties of males relative to females. Acoustic properties involved in reproductive isolation usually are stereotyped (Gerhardt 1991, Gerhardt and Huber 2002). The large variability in female calls may be functionally significant; thus, female calls are a receptivity signal used only in close-range interactions (Márquez and Verrel 1991, Bosch 2002, Preininger *et al.* 2016) and may not have a species-recognition function. Indeed, in the case described here, the female call seems not to be a source for reproductive isolation, but instead a signal emitted to advertise immediate availability for a candidate male during courtship. However, our data are insufficient for generalizations; additional samples from other individuals and playback experiments are needed to confirm such hypothesis.

The behavior involving parents sealing the nest openings with mud after oviposition is known for six species of the *Leptodactylus fuscus* Group. The sex responsible for the sealing is unknown in *L. camaquara* Sazima and Bokermann, 1978, *L. cunicularius* Sazima and Bokermann, 1978, and *L. mystacinus* (Burmeister, 1861) (Arzabe and Prado 2006, Oliveira-Filho and Giaretta 2008). In *L. fuscus* and *L. bufonius* Boulenger, 1894 females have been observed

sealing the chamber (Arzabe and Prado 2006, Lucas *et al.* 2008, Crump 2015), whereas males do it in *L. troglodytes* (Kokubum *et al.* 2009). Our record is the first report of this behavior by a male *L. fuscus*. Lucas *et al.* (2008) found that the northern and southeastern clades of the *Leptodactylus fuscus* Group differ in reproductive behavior; thus, we cannot be sure that the novel behavior described here is exhibited for all lineages, or it may be specific for the northern clade. However, we have determined that both parents are able to close the nest with mud in *L. fuscus*. Such behavior should be considered a fundamental form of parental care that may help protect eggs from predation, parasites, and desiccation (Arzabe and Prado 2006, Crump 2015).

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