

Severe constraints for sound communication in a frog from the South American temperate forest

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Abstract The efficiency of acoustic communication depends on the power generated by the sound source, the quality of the environment across which signals propagate, the environmental noise and the sensitivity of the intended receivers. *Eupsophus calcaratus*, an anuran from the temperate austral forest, communicates by means of an advertisement call of weak intensity in a sound-attenuating environment. To estimate the range over which these frogs communicate effectively, we conducted measurements of sound level and degradation patterns of propagating advertisement calls in the field, and measurements of auditory thresholds to pure tones and to natural calls in laboratory conditions. The results show that *E. calcaratus* produces weak advertisement calls of about 72 dB sound pressure level (SPL) at 0.25 m from the caller. The signals are affected by attenuation and degradation patterns as they propagate in their native environment, reaching average values of 61 and 51 dB SPL at 1 and 2 m from the sound source, respectively. Midbrain multi-unit recordings show a relatively low auditory sensitivity, with thresholds of about 58 dB SPL for conspecific calls, which are likely to restrict communication to distances shorter than 2 m, a remarkably short range as compared to other anurans.

Keywords Advertisement calls · Auditory thresholds · Frogs · Sound propagation · *Torus semicircularis*

Abbreviations

SPL Sound pressure level
RMS Root mean square
BTL Best threshold of the low-frequency region
BTH Best threshold of the high-frequency region
CFH Center frequency of the high-frequency region

Introduction

Animal communication implies transmission of signals from senders to receivers, and the range over which this exchange occurs effectively depends on the power generated by the source, the physical properties of the environment through which signals propagate, the quality and level of environmental noise and the sensitivity of the intended receivers (e.g., Bradbury and Vehrencamp 1998).

Acoustic signal designs in birds and primates have been reported to maximize communication range. Such adaptations imply that signals of species from different populations have features favoring transmission in their native habitats (e.g., Morton 1975; Shy 1983; Sorjonen 1986).

Studies on anuran sound communication have revealed remarkable adaptations for improving signal detection in frogs from noisy mountain stream environments, which communicate by means of signals shifted out of the spectral range of the background sound reaching the ultrasound range (Feng et al. 2006). However, relationships between signal features and vegetation coverage of native environments of the kind reported for birds and primates do not occur in different groups of anurans (Penna and Solís 1998;

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Kime et al. 2000; Penna et al. 2006). Such lack of optimization suggests that anuran vocalizations are designed primarily to locate mates and competitors within limited breeding areas (Gerhardt and Huber 2002).

Studies of auditory and behavioral thresholds of evoked vocal responses in anurans have shown that the distance over which different species can communicate are diverse. Anurans producing relatively intense calls propagating over the water's surface are likely to communicate up to distances of 60–70 m (Boatright-Horowitz et al. 2000; Penna et al. 2008). In contrast, midwife toads *Alytes* communicating over ground substrate in Mediterranean savannas (Penna et al. 2006; Penna et al. 2012) and neotropical frogs *Atelopus* from forest habitats (Boistel et al. 2011) are just able to communicate over distances of about 8 m. These restrictions originate in the low amplitude of the signals, attenuation properties or noise profiles of the habitats of these anurans.

Anurans from the South American temperate forest confront important constraints for sound communication. The bogs in which most native species breed have dense vegetation coverage, which imposes high attenuation rates on propagating acoustic signals (Penna and Solís 1998). Furthermore, some of the species inhabiting these environments produce high pitched, low-intensity calls and call from ground level (Penna and Veloso 1990), a condition that impairs propagation of high frequencies as compared to elevated perching sites (e.g., Gerhardt and Huber 2002).

Among the quiet-voiced anurans in this region are species of the *Roseus* group of the genus *Eupsophus*, which produce calls resembling soft, brief meows from inside burrows excavated in muddy and mossy substrates, generally in the vicinity of small creeks (Penna and Veloso 1990; Penna 2004; Márquez et al. 2005; Penna and Márquez 2007; Opazo et al. 2009). *Eupsophus calcaratus* is a species of this group for which different aspects of its vocal behavior have been studied. This frog breeds and vocalizes from mid winter through the beginning of spring at very low temperatures (Penna 2004; Penna et al. 2005a; Penna and Quispe 2007), and because auditory sensitivity depends inversely on temperature in anurans (e.g., Narins 2001), this environmental condition may imply a sensory constraint for communication in this species. In the present study, we conducted field measurements of sound levels of calls produced by individual males *E. calcaratus* at different distances from the sound source in natural settings, and carried out measurements of auditory thresholds by means of multi-unit auditory responses in the laboratory, to estimate the range over which these frogs communicate effectively. These combined measurements contribute an assessment of the adaptive status of the sound communication system of this terrestrial vertebrate amid seemingly substantial restrictions.

Methods

Field call recordings

Study site

The study was conducted during September and October 1996 and 1997, at the locality of La Picada (41°06'S, 72°30'W, altitude 820 m above sea level), within the Vicente Pérez Rosales National Park in southern Chile. The study site was a bog of volcanic substrate, where males of *E. calcaratus* call from inside small burrows along the borders of small streams or pools among vegetation composed mainly of mosses (*Rhacomitrium*), grasses (*Scyrpus* and *Myrteola*) and ferns (*Blechnum*). Previous studies on the vocal behavior of *E. calcaratus* have been carried out at this site (Penna 2004; Penna et al. 2005a; Márquez et al. 2005; Penna and Quispe 2007).

Air and substrate temperatures were measured after recording the calls of each animal with a thermocouple thermometer (Digi-Sense 8528-20) to the nearest 0.1 °C. For the animals that could be extracted from their burrows without altering the structure of these shelters, snout-vent length (SVL) was measured to the nearest millimeter by pressing the animal flat (ventrally) against a ruler, and weight was measured with a scale (Acculab pp 62) to the nearest 0.1 g.

Call recording procedures

Sound pressure levels (SPLs, dB re 20 µPa) of advertisement calls were measured with the microphone of a sound level meter (Brüel and Kjaer 2230) fitted with a 10-m extension cable and a foam windshield placed successively at distances of 0.25, 0.5, 1, 2 or 4 m from the opening of a burrow occupied by a calling male. When the burrow opening was clearly visible among the mossy substrate, recordings with the microphone placed at the burrow opening were also conducted. The distant microphone positions were chosen to allow comparisons with previous studies on propagation of playback calls of frogs from the southern temperate forest (Penna and Solís 1998). In addition, recordings with the microphone placed at a distance of 0.5 m from the experimental subject, but at a 90° angle relative to the measurement made at this distance on the propagation transect, were conducted.

The output of the sound level meter was fed into a channel of a cassette tape recorder (Sony TCD 5M), keeping the recording level constant throughout the recording session, and subsequently a 1-kHz tone from a sound calibrator (Brüel and Kjaer 4230) was recorded with the microphone at the same recording level to provide a signal of known SPL value [93.8 dB SPL root mean square

(RMS)] to calculate the SPLs of the calls recorded. This was particularly useful for calls recorded at the farthest distance (4 m), at which the signal SPL readings on the sound level meter display became uncertain because of low signal-to-noise ratios. For recordings at closer distances, SPL readings (integration time: fast, frequency weighting: linear) were noted manually to provide additional means of checking the accuracy of sound level measurements.

Signal analysis

Ten calls recorded for a given subject at a given microphone position devoid of interfering environmental sounds were chosen for analysis. Signal analysis was carried out with Raven 1.3 software (Cornell Lab of Ornithology, NY, USA; sampling rate 44,100 Hz, resolution 16 bits) and the variables measured were call duration (ms) and RMS amplitude (mV). Call duration reported in “Results” corresponds to calls recorded at 0.25 m, to assure the accuracy of measurements. SPLs were calculated from the ratio of these amplitudes to the calibration tone contained in the same recording. Calls recorded at 4 m from the emitters were embedded in noise and therefore corrections were applied to subtract the background noise following Bradbury and Vehrencamp (1998, p. 34–35). We measured the RMS amplitude of an interval containing just background noise contiguous to the calls measured. The square root of the sum of the squared amplitudes of both measurements was computed, and the difference between this value and the call amplitude actually measured was subtracted from the later value.

Measurements of the amplitudes of the harmonics in power spectra were done employing a custom automated analysis implemented with R (R Development Core Team 2012, <http://www.R-project.org/>) and the Seewave sound package analysis (Sueur et al. 2008; sampling rate 44,100 Hz, frequency resolution 86.13 Hz, Hanning window, 512 samples). The ‘spec’ function of this package was used to calculate the spectrum at the time corresponding to two-thirds (66.7 %) of the duration of each call, starting from call onset.

Variation in SPL and in the relative amplitude of harmonics at different distances from the emitter were evaluated with Friedman ANOVA ($P < 0.05$) and a posteriori comparisons were performed with Wilcoxon signed-ranks tests with Bonferroni corrections. Variation in SPL at a fixed distance (0.5 m) and different directions (0° and 90°) from the emitter was also evaluated with Wilcoxon signed-ranks tests. Ten calls recorded from each subject at each microphone position were used for these analyses. Average values of SPLs were calculated after transforming dB measurements to a linear scale and then converted back to dB.

Laboratory midbrain recordings

Animal conditioning and experimental procedures

The frogs were carried to the Laboratory of Neuroethology at the University of Chile in Santiago, Chile, where they were housed in terraria at 7 ± 1 °C temperature, under an inverted 12:12 light/dark cycle and fed with tenebrionid larvae once per week. The animals were used for neurophysiological recordings within 1 month of their arrival in the laboratory.

The subjects were anesthetized by immersion in a 0.4 % aqueous solution of MS-222 (Sigma). Under anesthesia, the skin on the dorsal surface of the head was incised and a hole was drilled into the underlying skull. Dura and pia membranes covering the brain were dissected away to expose the surface of the optic tectum. After surgery, animals were allowed to recover from anesthesia for 12–24 h. Then, the subjects were immobilized with an injection of *d*-tubocurarine chloride (3 µg/g body weight), placed on a Peltier plate (Cambion) and covered with light moist gauze to facilitate cutaneous respiration. The recordings were conducted in a sound-attenuated booth having walls and ceiling covered with 10-cm height foam wedges. Immobilization was maintained throughout the recording session with periodic injections of *d*-tubocurarine chloride, and lidocaine (2 %) was applied topically to the wound margins. A DC current of about 1 A was supplied to the Peltier plate to keep the frog’s body at 7.0 ± 1 °C, a temperature at which these frogs normally breed and call in their natural settings. Frog temperature was monitored with a miniature thermometer probe (Digi-Sense 8528-20) inserted in the cloaca.

Stimuli generation

Synthetic pure tones and white noise were generated with SigGen 1.3 software (Tucker-Davis Technologies). Pure tones having a duration of 250 ms and linear rise and fall ramps of 50 ms were used to obtain audiograms. These pure tones were set at frequencies between 100 and 5,000 Hz, with 100-Hz steps between 100 and 3,000 Hz, 200-Hz steps between 3,000 and 4,000 Hz, and 500-Hz steps between 4,000 and 5,000 Hz.

Natural calls used as stimuli were recorded during October 1996 using a cassette tape recorder (Sony TCD 5M) and a directional microphone (AKG CK8) placed at distances of about 25 cm from the burrow openings. The calls were judged to encompass the range of variation of the frequency and amplitude of the second and third harmonic of this signal in the population studied (Penna 2004; Penna and Quispe 2007). The second and third harmonics measured at the midpoint of the calls were 1,227 and

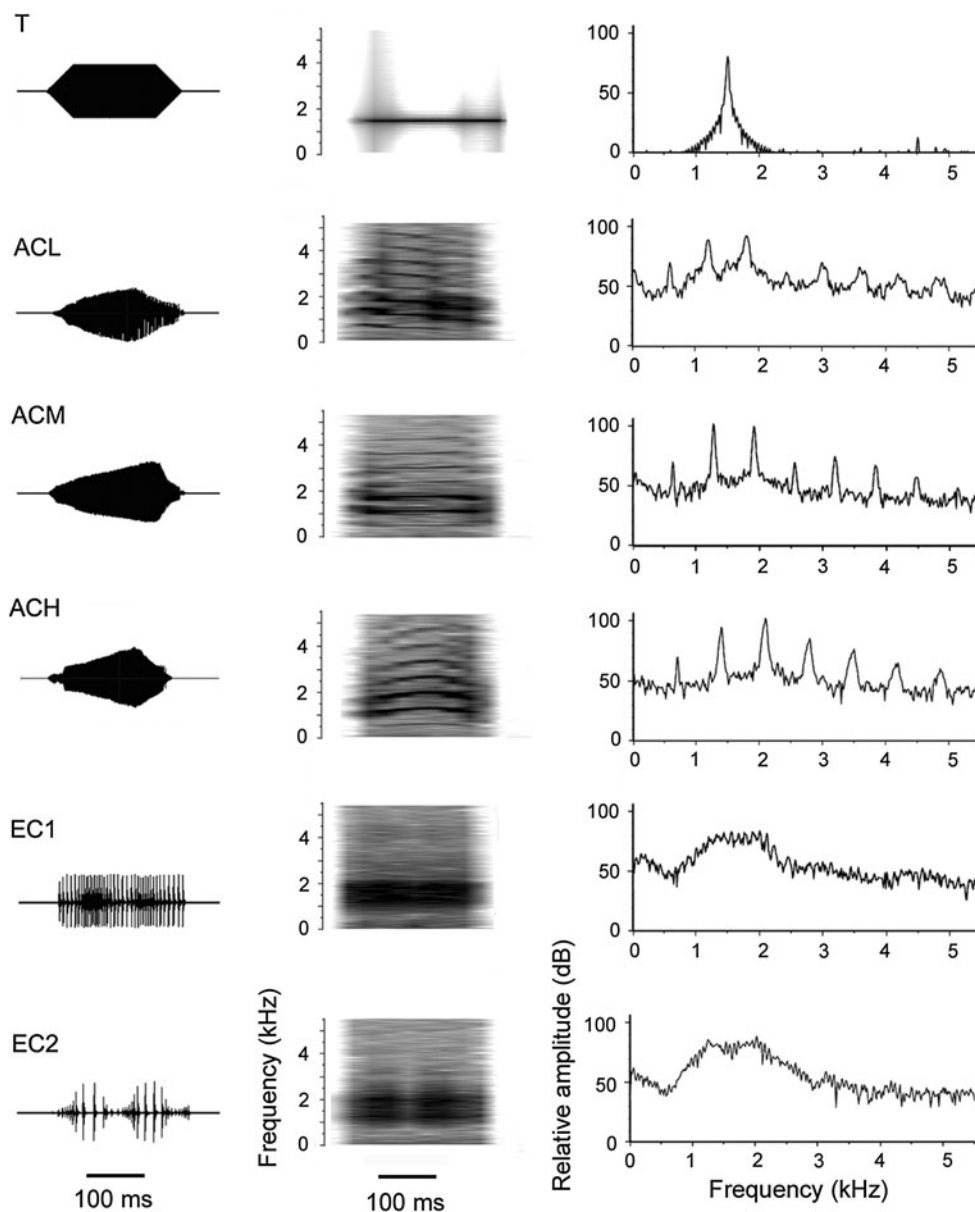


Fig. 1 Tone and natural calls used as stimuli to obtain multi-unit midbrain auditory responses in males of *E. calcaratus*. The natural calls were recorded in the field study site at substrate temperatures of

6.3–9.8 °C. *T* pure tone, *ACL* low frequency advertisement call, *ACM* mid frequency advertisement call, *ACH* high frequency advertisement call, *EC1* encounter call 1, *EC2* encounter call 2

1,841 Hz for the low-frequency call, 1,271 and 1,917 Hz for the mid-frequency call, and 1,400 and 2,100 Hz for the high-frequency call. The relative amplitude of the second and third harmonic was different for the three calls: the amplitude of the second harmonic was 3 dB above, 1.5 dB below and 8.4 dB above the amplitude of the third harmonic, for the low-, mid- and high-frequency calls, respectively. Call duration was 248, 251 and 242 ms for the low-, mid- and high-frequency calls, respectively. The encounter calls 1 and 2 were 250-ms segments of longer vocalizations composed of pulses of similar amplitude and

of amplitude-modulated pulses, respectively. Both signals had broadband spectra lacking a harmonic structure with energy concentrated between about 1 and 2.5 kHz. These stimuli are shown in Fig. 1.

The stimuli amplitude was controlled with a programmable attenuator (PA4, Tucker-Davis Technologies), amplified (NAD C 320 BEE) and broadcast with a two-way loudspeaker (Dynaudio BM 6, frequency response 38–20,000 Hz), positioned at 1 m in front of experimental subject. At the beginning of each experimental session, the SPLs of the pure tones of frequencies 100–5,000 Hz

delivered at a constant electrical peak amplitude were measured with the microphone of a sound level meter (Brüel and Kjaer 2230) positioned 2–3 mm above the subject's head. The absolute SPLs of auditory thresholds were determined during the subsequent experimental session by varying the attenuation in 3-dB step. The frequency response of the system was within ± 6 dB in the 100–5,000 Hz range.

Multi-unit responses from the Torus semicircularis were recorded with custom-made gross glass-insulated tungsten electrodes (75 μ m diameter). The electrode was attached to a hydraulic microdrive (Narishige MO-8), positioned on the dorsal surface of the optic tectum and advanced into the brain. The large size of the electrode relative to the optic lobe facilitated a regular positioning of the electrode on the brain surface at about the intersection of the limit between the mid and caudal third and the limit between medial and lateral half of the hemisphere. This placement consistently yielded the largest auditory responses; recordings for all subjects were made at this single location.

The neural responses were passed through a pre-amplifier (Dagan 2400) and filtered between 100 and 3,000 Hz for multi-unit recordings. Neural responses were monitored by means of an oscilloscope and a loudspeaker, and thresholds determined by audiovisual criteria. Auditory responses were considered to cease when no audible neuronal discharge occurred for two of three consecutive presentations of a pure tone at a given level. During the recording sessions, measurements conducted independently by different experimenters were always highly coincident, never differing by more than a 3-dB step, the minimum amplitude interval used throughout the study. This methodology has been used in previous studies and yields reliable measurements of auditory thresholds in different anurans (Penna et al. 1990, 1992, 2008). In addition, recordings of evoked potentials were performed to provide objective threshold measurements. To obtain evoked potential recordings, the high- and low-pass filter settings of the Dagan pre-amplifier were switched to 1 and 1,000 Hz, respectively, with the tungsten electrode positioned at the same recording site as for multi-unit recordings from a given subject. The stimuli presentation and data acquisition were done in an automated mode with Biosig 3.01 software (Tucker-Davis Technologies).

Recording procedures

A search stimulus consisting of a white noise burst having the same envelope structure as the pure tones was presented at an intensity of about 80 dB RMS SPL, while the electrode was lowered in the brain to determine the depth at which the strongest auditory response was evoked. Stimuli were presented at a rate of 0.5 stimulus/s.

The lowest threshold observed in the low-frequency region of enhanced sensitivity corresponded in all subjects recorded to the 100 or 200-Hz tone and this was termed the best threshold of the low-frequency region (BTL). The lowest threshold in the high-frequency region of enhanced sensitivity corresponded to different tone frequencies in individual audiograms and was termed the best threshold of the high-frequency region (BTH). A center frequency of the high-frequency region (CFH) was calculated from a weighted average of thresholds for frequencies between 1,000 and 5,000 Hz that were within 6 dB above the BTH. To calculate the CFH of an individual audiogram, each of the frequencies considered was multiplied by the sound pressure (N/m^2) corresponding to the difference between the SPL at the BTH and the SPL at that frequency, the products summed and divided by the sum of SPLs.

Statistical analysis

To obtain average thresholds of multi-unit responses at each of the frequencies tested, threshold values of individuals in dB SPL were converted to N/m^2 and the resulting stadiographs reconverted to dB SPL. The dependence of multi-unit audiograms' CFH on body size and weight was explored with Spearman correlation ($P < 0.05$).

To explore the variation in thresholds to the natural call stimuli, BTL and BTH, Friedman ANOVA ($P < 0.05$) and a posteriori comparisons with Wilcoxon signed-ranks tests with Bonferroni corrections were applied. Thresholds to pure tones allow comparisons of the auditory sensitivity of *E. calcaratus* with those of other species studied previously, whereas thresholds to natural calls allow the estimation of active space, i.e., the distance over which this species communicates.

Results

Field call recordings

Advertisement calls of ten males of *E. calcaratus* were recorded. During recordings, air and substrate temperature averaged 4.7 °C (range 2.5–7.4 °C) and 7.3 °C (range 6.3–9.8 °C), respectively. Body length of five individuals captured after recording their calls averaged 36.2 mm (range 34–38 mm) and their body weight 4.9 g (range 4.6–5.2 g). Background noise levels measured directly with the sound level meters when no nearby frogs were calling averaged 38.0 dB SPL RMS (range 31.4–47.2 dB SPL RMS). The abiotic component corresponded mainly to running water of nearby small creeks and the biotic component originated basically on distant (>about 5 m) conspecific chorusing.

The burrows from which frogs called had different orientations relative to the horizontal plane: three were horizontal (0°), two were vertical (90°) and five had intermediate orientations (30° – 45°). The total length of the burrows averaged 16.2 cm (range 6–34 cm) and the segment free from water averaged 7.4 cm (range 0–13 cm). Two burrows were flooded after heavy rains on the nights when measurements were carried out and the resident frogs called at the burrows' openings, with their heads out of these shelters. The subjects called spaced from their nearest calling neighbors at distances averaging 1.0 m (range 0.32–2.7 m).

Calls recorded 0.25 m in front of calling males had an amplitude of 71.7 dB SPL RMS (range 64.1–80.9 dB SPL RMS). Measurements using the peak time weighting were also carried out at 0.5 m to allow comparisons with measurements of other frogs from the southern temperate forest in a former study using these settings (Penna and Solís 1998). The value obtained for *E. calcaratus* in the present study was 72.2 dB SPL peak (range 68.8–76.5 dB SPL peak). Calls recorded at 0.25 m had an average duration of 231 ms (range 176–304 ms), and the first, second and third harmonics were 785 Hz (732–827 Hz), 1,471 Hz (1,335–1,575 Hz) and 2,173 Hz (1,981–2,338 Hz). The second and third harmonics had the largest amplitude in the calls of 4 and 5 subjects, respectively. This spectral analysis was not applied to one of the subjects because it had a sub-harmonic structure. For four individuals for which the burrow entrance was clearly visible, call recordings and SPL measurements at this position were also conducted. Calls of an individual recorded at different distances from the source are shown in Fig. 2.

Neither fundamental frequency nor call duration were related to air temperature ($R_s < -0.539$, $P > 0.05$ for all comparisons). The small sample of individuals captured ($N = 5$) precluded the exploration of relationships of the acoustic variables and body size or weight.

Call SPLs decreased progressively with distance from the sound source, as shown in Fig. 3a and Table 1. One subject was not recorded at 1 m because of technical problems. For three subjects, calls at 4 m were not detectable from the background noise due to the proximity of running water or interfering conspecific background chorusing at this position of the microphone. For the recordings obtained at this distance for the other seven subjects, the signal-to-noise ratio was on average 12.1 dB (range 4.3–13.6 dB) and corrections to subtract the background noise were on average 0.6 dB (range 0.1–1.6 dB).

The variation of call SPL with distance was analyzed for microphone positions between 0.25 and 2 m from the subjects, due to the low number of cases at distances 0 and 4 m. Call SPL varied significantly with distance from

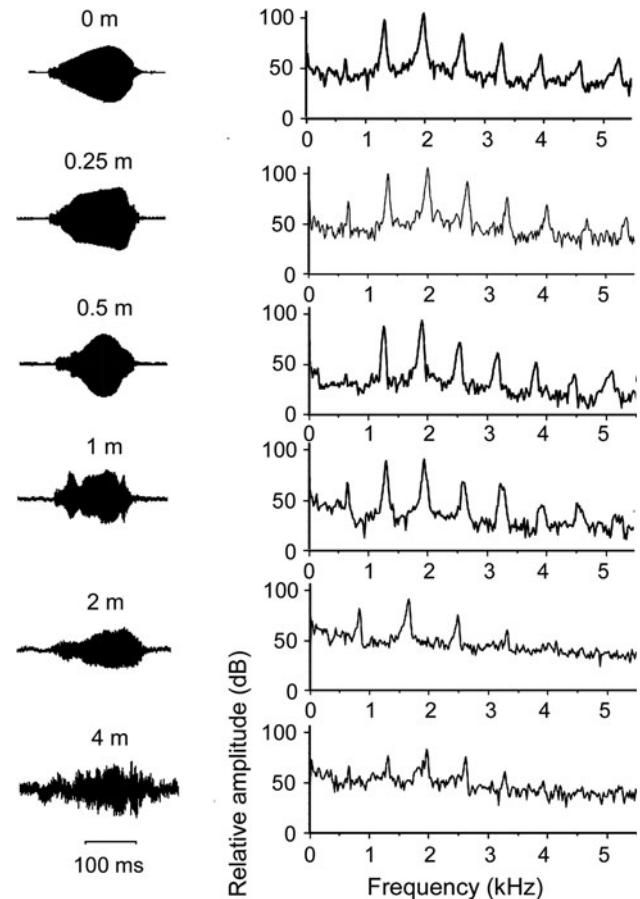


Fig. 2 Oscillograms (left column) and power spectra (right column; 3,171 points, 20 Hz bandwidth) of calls of an *Eupsophus calcaratus* male recorded at different distances from its burrow opening (air temperature 4.5°C , substrate temperature 6.6°C)

the sound source (Friedman ANOVA: $\chi^2_3 = 23.13$, $P < 0.0001$; Fig. 3a) and results of multiple comparisons show significant differences for most comparisons (Table 2). Call SPLs measured at the same distance (0.5 m) and at 90° relative to the burrow opening did not differ from those measured at 0° , averaging 61.1 dB SPL RMS (range 54.3–66.9 dB SPL RMS) and 62.3 dB SPL RMS (range 53.4–66.6 dB SPL RMS), respectively (Wilcoxon signed-rank test: $Z = 1.183$, $P = 0.23672$; Fig. 3b).

Because SPL values were obtained from different calls measured at different distances, transmission losses could not be calculated with accuracy from these data to compare them with losses measured in former studies (Penna and Solís 1998; Penna et al. 2006, 2012). However, to gain an idea of the restrictions imposed by the environment on propagation of the vocalizations, values predicted by spherical spreading were calculated with the equation: spherical transmission loss (dB) = $20 \log$ [far distance (m)/0.25 (m)], and this value was subtracted from the actual transmission loss, i.e., the decrease in average SPL from

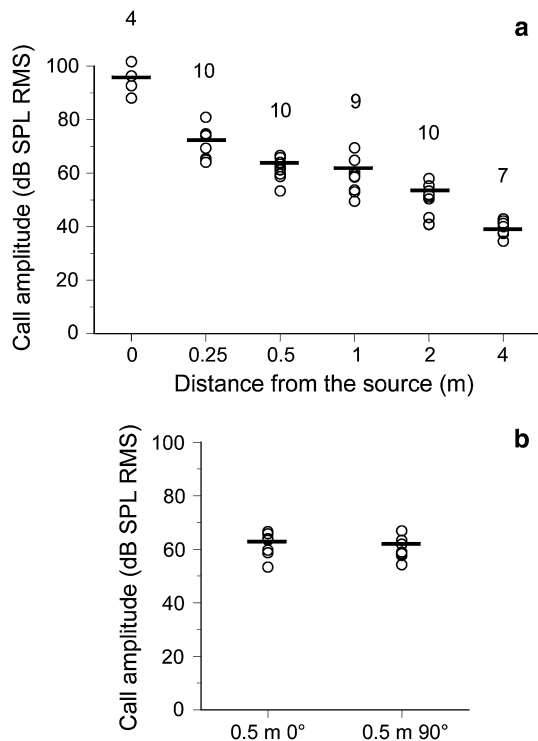


Fig. 3 **a** Sound pressure levels of advertisement calls recorded at different distances from males of *Eupsophus calcaratus*. Circles indicate averages for 10 calls of a given male at a given distance and horizontal lines indicate averages calculated for SPLs of the calls of all males measured at a given distance from the sound source. Numbers above symbols indicate animals sampled at each distance. **b** Sound pressure levels of calls of males of *E. calcaratus* ($N = 7$) recorded at a distance of 0.5 m and at 0° and 90° relative to burrow openings

Table 1 Sound pressure levels of advertisement calls at different distances from males of *Eupsophus calcaratus* (averages with ranges in parenthesis)

Distance (m)	Number of males	Call amplitude (dB SPL RMS)	Excess attenuation (dB)
0	4	96.1 (88.0–101.6)	
0.25	10	71.7 (64.1–80.9)	
0.5	10	62.3 (53.4–66.6)	3.4
1	9	60.6 (49.5–69.4)	−0.9
2	10	51.3 (40.8–58.0)	2.4
4	7	39.9 (34.6–42.9)	7.8

Measurements at 0 m correspond to recordings obtained with the microphone placed at burrow openings. Excess attenuations were calculated from the average SPLs at 0.25–4 m (see text)

0.25 m from the loudspeaker to the corresponding farther distances. Positive and negative values indicated that the sound attenuated at higher and lower rates, respectively, relative to the SPLs predicted by spherical transmission loss for each distance (positive or negative excess attenuation). The values obtained are listed in Table 1 and show a tendency to increase with distance from the sound source.

Table 2 Multiple comparisons among sound pressure levels of advertisement calls measured at four distances (0.25–2.0 m) from males of *Eupsophus calcaratus*

Distances (m)	Z	P
0.25–0.5	2.67	0.00767*
0.25–1.0	2.55	0.01086
0.25–2.0	2.67	0.00767*
0.5–1.0	1.48	0.13864
0.5–2.0	2.67	0.00767*
1.0–2.0	2.67	0.00767*

Friedman ANOVA: $\chi^2_3 = 23.13$, $P < 0.0001$, Wilcoxon signed-ranks tests, with Bonferroni correction

* Significant values ($P < 0.0083$)

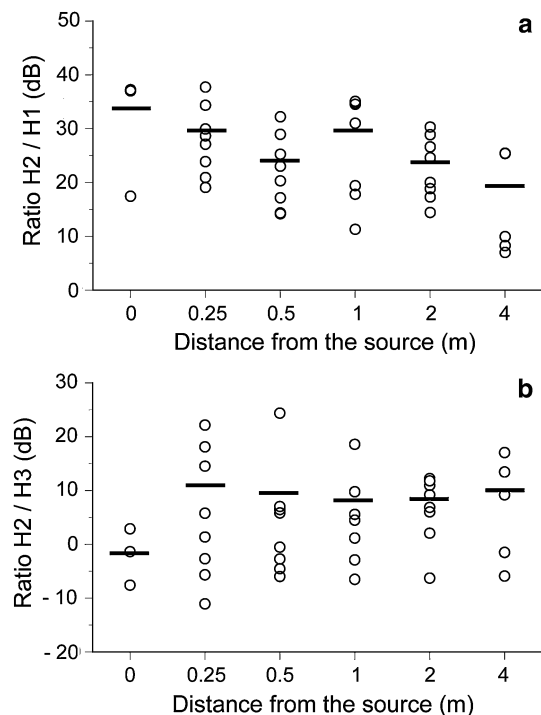


Fig. 4 Amplitude ratios of harmonics in calls of *E. calcaratus* recorded at different distances from burrow openings **a** ratio harmonic 2/harmonic 1, **b** ratio harmonic 2/harmonic 3. Symbols and numbers are as in Fig. 3

The relative amplitude of harmonics at different distances from the source was also explored. Two individuals for which calls recorded at certain distances had a sub-harmonic spectral structure were excluded from this analysis. The ratio amplitude second harmonic:amplitude first harmonic of the advertisement calls in the eight individuals analyzed showed a tendency to decrease with distance from the sound source; however, this variation did not reach a level of significance (Friedman ANOVA: $\chi^2_3 = 4.37$, $P = 0.22405$; Fig. 4a). The ratio amplitude second harmonic:amplitude third harmonic amplitude did not show

Table 3 Thresholds to pure tones and natural calls as measured by multi-unit recordings in the midbrain of males of *Eupsophus calcaratus* ($N = 13$)

Stimulus	Auditory thresholds (dB SPL RMS)
BTL	48.9 (25.1–57.9)
BTH	52.1 (43.2–57.8)
ACL	59.3 (46.5–66.0)
ACM	57.6 (46.8–62.1)
ACH	57.9 (47.7–57.9)
EC1	55.5 (47.4–60.4)
EC2	56.7 (50.0–60.5)

Averages, with ranges in parenthesis

BTL best threshold in the low-frequency region, *BTH* best threshold in the high-frequency region, *ACL* low-frequency advertisement call, *ACM* mid-frequency advertisement call, *ACH* high-frequency advertisement call, *EC1* encounter call 1, *EC2* encounter call 2

significant variation with distance either (Friedman ANOVA: $\chi^2_3 = 1.11$, $P = 0.77363$; Fig. 4b).

Laboratory midbrain recordings

The stimuli used to evoke neural responses are displayed in Fig. 1. The 13 males used for recordings had an average weight of 4.3 g (range 3.3–5.5 g) and an SVL of 35 mm (range 32–38 mm). The cloacal temperatures at which recordings were conducted averaged 7.2 ± 1.5 °C. Audiograms showed regions of enhanced sensitivity at a low-frequency range, between 0.1 and 0.5 kHz, and at a high-frequency range, between about 1.0 and 2.5 kHz. The BTL reached minima values averaging 48.9 dB SPL (range 25.1–57.9 dB SPL), and occurred at the lower frequencies tested (0.1 or 0.2 kHz), whereas BTH averaged 52.1 dB SPL (range 43.2–57.8 dB SPL). The frequencies at which BTH occurred ranged from 1.4 to 2.8 kHz. The center frequency for the high-frequency region (CFH) averaged 1.8 kHz (range 1.3–2.5 kHz) and was inversely correlated with body weight of the experimental subjects, but the relationship did not reach the significance level for body length (Spearman correlation: $R_s = -0.560$, $P < 0.05$ and $R_s = -0.546$, $P > 0.05$, respectively). Average values of BTL and BTH are listed in Table 3 and an average audiogram is shown in Fig. 5a.

Thresholds of responses to natural call stimuli averaged about 55–59 dB SPL, and were on average higher than the BTL and BTH (Table 3; Fig. 5b). A Friedman ANOVA applied to the thresholds for the five natural calls and BTL and BTH yielded significant differences ($\chi^2_6 = 46.46$, $P < 0.00001$). Multiple comparisons with the Wilcoxon signed-rank test using Bonferroni correction ($P < 0.0024$) showed that BTL was significantly lower than thresholds for the three natural advertisement calls and the encounter

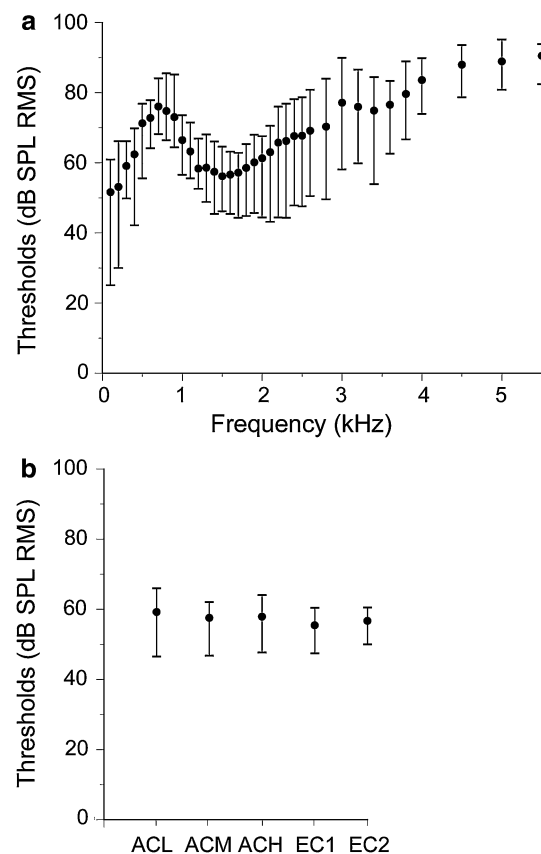


Fig. 5 Thresholds of multi-unit midbrain auditory responses in males of *E. calcaratus*: **a** audiograms obtained with pure tones, **b** responses to three different advertisement calls and to two encounter calls. Circles and bars indicate averages and ranges, respectively. *T* pure tone, *ACL* low-frequency advertisement call, *ACM* mid-frequency advertisement call, *ACH* high-frequency advertisement call, *EC1* encounter call 1, *EC2* encounter call 2

call 2 ($P < 0.00237$ for the 4 comparisons), and BTH was significantly lower than thresholds for the three natural advertisement calls and the two encounter calls ($P < 0.00237$ for the 5 comparisons). No significant differences occurred among thresholds for the natural calls and between BTL and BTH ($P > 0.00577$ for all comparisons).

Recordings of evoked potentials obtained for the same electrode positions as for multi-unit recordings showed a close concordance between both procedures. The recordings of evoked potentials showed a slow wave having a biphasic shape with maximum positive and negative peaks occurring at about 50 and 130 ms, respectively, with an amplitude difference between both deflections of about 20 mV for SPLs about 20 dB above threshold (Fig. 6). Amplitude difference of baseline recordings when no stimulus was presented was about 2 mV for the times at which the positive and negative peaks occurred. Therefore, an amplitude of 5 mV was considered as the threshold level of auditory evoked potential. Thresholds obtained

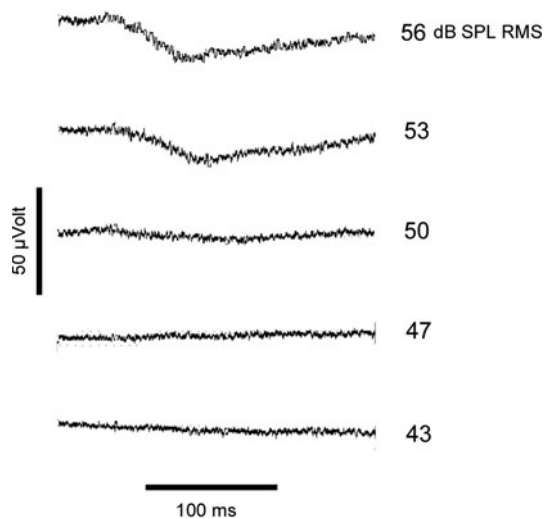


Fig. 6 Recordings of auditory evoked potentials from the Torus semicircularis of a male of *E. calcaratus* in response to a 250-ms pure tone of 1,900 Hz. Each trace represents an average for 15 presentations of the stimulus. Numbers indicate sound pressure levels of the stimuli. Threshold level: 50 dB SPL

with evoked potentials and with multi-unit responses obtained with audiovisual criteria were compared for a sample of 50 measurements in different individuals for frequencies close to the CFH of the audiograms. Results showed that a coincidence in threshold criteria occurred in 25 cases (50 %); in 22 (44 %) and 3 (6 %) of cases, the thresholds were 3 and 6 dB below those measured with audiovisual criteria, respectively. Recordings of evoked potentials in response to a pure tone presented at different SPLs are shown in Fig. 6.

Discussion

Results of this study show that SPLs of the advertisement calls of *E. calcaratus* decrease progressively with distance from the source. A drop of about 20 dB occurs from the burrow opening to 0.25 m, the minimum external distance measured. At 4 m from the source, the signal amplitude drops to levels close to the background noise; measurements of 3 out of 10 individuals could not be carried out because they fell within the background noise level at this location.

Transmission losses for distances doubling their magnitude, starting at 0.25 m from the sound source cannot be calculated with accuracy from these data, since they correspond to vocalizations occurring at different times and subjected to variation in intensity at the sound source. Simultaneous recordings of calls with two or more microphones placed at different distances are needed to calculate excess attenuations to compare these data with

other studies. However, in spite of this limitation, our data show a general similarity with measurements of a recent study on propagation of vocalizations of Iberian toads *Alytes* (Penna et al. 2012), in which simultaneous recordings at different distances from calling males showed excess attenuations increasing from about 0 dB at the closest distance of 1 m to about 5 dB at 4 m from the sound source. Results from the present study also bear a general resemblance with data from a study of propagation of pre-recorded calls of different anurans broadcast with a loudspeaker at the site where the present study was conducted, showing excess attenuations between about 0 and 10 dB at 4 m, depending on the species (Penna and Solís 1998; Fig. 3).

The amplitude of the calls of *E. calcaratus* reaches at 2 m values of about 51 dB SPL, which are below the 58 dB SPL auditory thresholds for conspecific advertisement calls measured with multi-unit recordings in the auditory midbrain. According to this combined evidence, male frogs would be able to interact vocally within distances shorter than 2 m. In concordance with this assumption, the subjects studied in the field were spaced at distances from their nearest neighbors averaging 1 m. Playback experiments of evoked vocal responses as conducted for a related species, *E. emiliopugini* (Penna et al. 2005b), are needed to determine the correspondence between auditory and behavioral thresholds in *E. calcaratus*. In another Chilean anuran, *Pleurodema thaul*, a close correspondence between auditory thresholds and thresholds for evoked vocal responses has been measured (Penna et al. 2008). The occurrence of a similar relationship in *E. calcaratus* would facilitate sound communication amid the considerable restrictions that this anuran confronts in nature.

The radiation pattern of vocalizations by males of *E. calcaratus* is not directional, a result concordant with measurements reported for other anurans (Narins and Hurley 1982; Wells and Schwartz 1982; Penna et al. 2012). In two of these frogs, directional patterns observed occasionally were associated with the foliage structure of calling sites (Narins and Hurley 1982; Wells and Schwartz 1982). Directional patterns have been reported for some anuran species (Gerhardt 1975; Passmore 1981); however, amplitude differences among directions in the horizontal plane are relatively small, typically below 3 dB.

The limited directionality of signal broadcast in anurans contrasts with the strong directional patterns of emission reported for birds (e.g., Larsen and Dabelsteen 1990; Robisson 1991; Brumm 2002; Patricelli et al. 2007). Directionality in birds is related to displays directed toward visually detected partners or contenders (Patricelli et al. 2007). In contrast with birds, the call radiation patterns of burrowing frogs are constrained by the structure of their

refugia. In addition, a former study has shown that males of *E. calcaratus* are not sensitive to the direction from which incoming calls are broadcast, responding with similar call rates to synthetic conspecific stimuli reproduced with loudspeakers positioned at angles differing in 90° relative to the burrow's position (Penna and Quispe 2007). The lack of directionality in signal emission and in evoked vocal responses would promote the occurrence of communication networks in which different modalities of interactions coexist (e.g., McGregor 2005), thus maintaining the persistence of chorusing activity in breeding aggregations of this anuran.

The relatively complex spectral composition of the call of *E. calcaratus*, with discrete harmonics having amplitude ratios susceptible to change with distance from the sound source could provide cues for assessing distances of the emitter from the receivers as occurs in birds (e.g., Naguib et al. 2008). However, although there is a tendency to a decrease in the amplitude ratio of the first and second harmonic, further measurements with simultaneous recordings at different distances are needed to look for significant spectral differences in propagated signals of this anuran.

The auditory sensitivity in the high-frequency range is tuned to a frequency intermediate between the averages for the main spectral components of the call of *E. calcaratus*, the second and third harmonics. This mismatch points to a relatively similar importance of both spectral peaks for signal detection. Congruent with this assessment is the invariance of the amplitude ratio between these two harmonics over distance from the sound source, which implies that the two main spectral peaks of signals of callers located at maximum detectable distances from receivers would be detected with similar sensitivities. However, resonance of burrows, where males of this species call from, also affects the spectra of incoming signals, increasing the amplitude of the second relative to the third harmonic (Penna 2004). This effect would imply that the auditory sensitivity is tuned to frequencies higher than the main spectral component of the advertisement call of *E. calcaratus* as it reaches male receptors in their calling posts. If females' audiograms are similar to those of males, their phonotactic behavior would not be affected by the burrow effect on call spectra, since females are likely to orient to calling males in chorusing aggregations by approaching from across the swamp surface.

The audiograms of *E. calcaratus* showing minimum thresholds to pure tones slightly above 50 dB RMS SPL are in the upper limit of those measured in previous studies in other anurans, spanning from 30 to 50 dB RMS (e.g., Penna et al. 1990, 1992, 2008). These relatively high thresholds could be determined in part by the low temperatures at which this species breeds and at which the

auditory recordings were conducted, since this environmental factor increases auditory thresholds in anurans (Hubl and Schneider 1979; Mohnke and Schneider 1979; Narins 2001). Studies exploring long-term adaptations of auditory processing are needed to determine how auditory sensitivity is affected by temperature in *E. calcaratus*.

Auditory thresholds to pure tones in the high-frequency range are lower than thresholds for natural calls having their main spectral components in this frequency domain. The higher thresholds measured for the vocalizations are likely to result from the spectral composition of these signals, containing various harmonics over which the total energy of the signals is distributed, as compared to pure tones having all the energy concentrated in a single frequency. However, the complex nature of the frequency-modulated advertisement calls and of the amplitude-modulated encounter calls precludes a more precise comparative analysis of thresholds for natural stimuli versus tone thresholds, such as spectrum level.

Overall, our study shows that *E. calcaratus* confronts considerable disadvantages for sound communication in its native environment. The amplitude of the advertisement call, with an average of about 72 dB SPL peak, at 0.5 m is lower than those measured in other anurans of the temperate forest using this time weighting (Penna and Solís 1998). The attenuation properties of the physical environment are comparable to those of other terrestrial habitats, and the auditory sensitivity is in the upper limit measured for other anurans. All these factors add up to restrict the communication range likely to a radius shorter than 2 m, a distance considerably closer than those calculated for other anurans (e.g., Boistel et al. 2011; Penna et al. 2008, 2012). This is likely an extreme case of vocalizations designed primarily to locate mates and competitors within limited breeding areas, as proposed by Gerhardt and Huber (2002) for anuran calls, in contrast with the long-distance design often found in signals of birds and primates.

A partial compensation for the drawbacks in the communication of *E. calcaratus* is the amplification of incoming vocalizations inside burrows where males call from (Penna 2004). The low environmental noise of biotic origin present at the breeding sites (Penna et al. 2005a) also contributes to facilitate the detection of the weak signals of this anuran. Another likely compensatory mechanism is the vocal activation that this frog exhibits in the presence of natural abiotic noises (Penna et al. 2005a), which may counteract the difficulties imposed by such ubiquitous interferences.

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