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ORIGINAL PAPER

Ample active acoustic space of 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 attributes of the environment across which signals propagate, the environmental noise and the sensitivity of the intended receivers. *Eupsophus emiliopugini*, an anuran from the temperate austral forest communicates by means of an advertisement call of moderate intensity within the range for anurans. To estimate the range over which these frogs communicate effectively, we conducted measurements of call sound levels and of auditory thresholds to pure tones and to synthetic conspecific calls. The results show that *E. emiliopugini* produces advertisement calls of about 84 dB SPL at 0.25 m from the caller. The signals are affected by attenuation as they propagate, reaching average values of about 47 dB SPL at 8 m from the sound source. Midbrain multi-unit recordings show quite sensitive audiograms within the anuran range, with thresholds of about 44 dB SPL for synthetic imitations of conspecific calls, which would allow communication at distances beyond 8 m. This is an extended range as compared to *E. calcaratus*, a related syntopic species for which a previous study has shown to be restricted to active acoustic spaces shorter than 2 m. The comparison reveals divergent strategies for related taxa communicating amid the same environment.

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

Animals communicate over distances that depend on the power generated by the source, the physical properties of the environment through which signals propagate, ubiquitous environmental noise and the sensitivity of potential receivers (e.g., Bradbury and Vehrencamp 2011).

A number of studies have shown that acoustic communication in birds maximizes communication range, implying that signals have evolved adaptations for efficient transmission in populations from open or forested habitats (reviewed in Brumm and Naguib 2009). Studies on anuran sound communication have revealed remarkable adaptations for communicating in noisy stream environments using signals containing ultrasound components (Feng et al. 2006). However, other studies have shown that signal features and vegetation coverages of native environments are not related (Penna and Solís 1998; Kime et al. 2000; Penna et al. 2006), and the structure of the vocalizations is associated mainly with the systematic status of different taxa (Zimmerman 1983; Bosch and De la Riva 2004). Such lack of environmental correlates of signal structure suggests that anuran vocalizations are designed primarily to locate mates and competitors within limited breeding areas (Gerhardt and Huber 2002).

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Anurans produce advertisement calls exhibiting a remarkable diversity in their temporal and spectral structure, and also the sound levels of these signals differ considerably among species (Gerhardt and Huber 2002). Studies of thresholds of evoked vocal and auditory responses in anurans have shown that the distances over which different species can communicate are diverse, spanning from about 8 m, as measured in midwife toads *Alytes* communicating over ground (Penna et al. 2006, 2012) and neotropical frogs *Atelopus* from forest habitats (Boistel et al. 2011), up to distances of 60–70 m in frogs producing intense calls propagating over the water's surface (Boatright-Horowitz et al. 2000; Penna et al. 2008). These differences arise from the combined effects of amplitude of the signals, attenuation properties and/or noise profiles of the habitats and auditory thresholds of these anurans.

Studies comparing the effectiveness of sound communication in species sharing the same environment have not been addressed, to the best of our knowledge, although a number of studies characterizing assemblages of chorusing sympatric anuran species have reported a partitioning of the spectral and temporal structure of the calls which results in reducing overlap among heterospecific signals (e.g. Hödl 1977; Duellman and Pyles 1983; García-Rutledge and Narins 2001; Amézquita et al. 2011). A measure relevant for characterizing and comparing the efficiency of sound communication systems among different taxa is the active space, i.e., the distance over which emitters and receivers can potentially communicate. (Brenowitz 1982; Gerhardt and Huber 2002; Bradbury and Vehrencamp 2011).

Anurans from the South American temperate forest exhibit a diversity of call patterns and intensities in their vocalizations (Penna and Veloso 1990; Penna and Solís 1998). Two species of *Eupsophus* from this region overlap their distribution extensively and breed in the very same microenvironment. Males of *E. calcaratus* and *E. emiliopugini*, belonging to the Roseus and Vertebralis species group of this genus, respectively, call in bogs from inside flooded burrows where mating, oviposition and egg attendance take place. Breeding seasons differ in time between both species, since *E. calcaratus* is active from mid-winter to mid-spring (July–October, Penna 2004; Penna et al. 2005a, Penna and Quispe 2007) and *E. emiliopugini* breeds from mid-spring to early summer (October–December, Penna and Solís 1996, 1998; Penna et al. 2005a, Penna and Hamilton-West 2007), so that the vocal activity of the two species overlaps for about 1 month. We have recently reported that *E. calcaratus* exemplifies an extreme case of restriction in active space for sound communication since males produce a call of low amplitude and have relatively high auditory thresholds that constrain their active communication space to a radius shorter than 2 m (Penna et al. 2013). The advertisement call of *E. emiliopugini* is louder and lower pitched than the call of *E. calcaratus* and has a pulsed structure, in contrast with the tonal characteristic of the vocalization of the second species (e.g., Penna et al. 2005b; Penna and Hamilton-West 2007).

In the current study, we conducted field measurements of sound levels of calls produced by individual male *E. emiliopugini* 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 this terrestrial vertebrate communicates effectively. These combined measurements contribute to a comparative assessment of the sound communication system of this frog and the congeneric species studied previously. The contrasting results obtained reveal different strategies to communicate amid the very same environment, resulting in rather dissimilar acoustic active spaces between these two related taxa.

Methods

Field call recordings

Study site

The study was conducted during November and December 1996 and 1998, 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. emiliopugini* call from inside small burrows along the borders of small streams or pools among vegetation composed mainly of mosses (*Racomitrium*), grasses, (*Scirpus* and *Myrteola*) and ferns (*Blechnum*). Previous studies on the vocal behavior of *E emiliopugini* have been carried out at this site (Penna and Solís 1996, 1999; Penna et al. 2005b; Penna and Hamilton-West 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 p. 62) to the nearest 0.1 g.

Call recording procedures

Field measurements were carried out following the same procedures used by Penna et al. (2013), so a summarized account will be given here. Sound pressure levels (SPLs, dB re 20 μPa) of advertisement calls were measured with the microphone of a sound level meter (Bruel & Kjaer 2230) placed successively at distances of 0.25, 0.5, 1, 2, 4 or 8 m from the opening of a burrow occupied by a calling male. In most cases, the burrow opening was clearly visible among the mossy substrate, and recordings with the microphone placed at the burrow opening were also conducted. 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 & Kjaer 4230) was recorded with the microphone at the same recording level to provide a signal of known SPL value (93.8 dB SPL RMS) to calculate the SPLs of the calls recorded.

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: 20 Hz) and the variables measured were call duration (ms) and RMS amplitude (mV). Call duration and dominant frequency reported in the "Results" section correspond 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. Some of the calls recorded at 8 m from the emitters were embedded in noise and if needed, corrections were applied to subtract the background noise following Bradbury and Vehrencamp (1998, pp. 34–35). To do this, we measured the RMS amplitude of an interval containing just background noise contiguous to the calls measured.

Statistical analysis

Variation in sound pressure level (SPL) and dominant frequency of the calls at different distances from the emitter was 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.

Covariation between size and weight of the experimental subjects and dominant frequency of the calls and between air and substrate temperature and call duration were explored with Spearman's correlation ($P < 0.05$).

Laboratory midbrain recordings

Animal conditioning and experimental procedures

The methods employed for these recordings were the same used in Penna et al. (2013), so a summarized account is given here. The frogs were housed in terraria at 10 ± 1 °C temperature, under an inverted 12:12 light/dark cycle and fed with tenebrionid larvae once per week, and 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) and a hole was drilled into the underlying skull 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 50-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. The frog was placed on a Peltier plate at an average temperature of 10.3 °C (range 9.7–10.9 °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, calls and white noise were generated with Adobe Audition 3.0 on a Macintosh computer (MacBook). Pure tones having a duration of 250 ms and linear rise and fall ramps of 50 and 100 ms, respectively, were used to obtain audiograms. These tones were set at frequencies between 100 and 5,000 Hz, with 100-Hz steps between 100 and 3,000 Hz and 1,000-Hz steps between 3,000 and 5,000 Hz. The synthetic call was composed of 36 pulses having a 1,000-Hz carrier frequency, a duration of 7 ms and linear rise and fall ramps of 2 and 5 ms, respectively, and a 7-ms pulse repetition period. The duration and amplitude envelope of the synthetic call were the same as for the pure tones (Fig. 1).

The stimuli amplitude was controlled with a programmable attenuator (PA4, Tucker-Davis Technologies),

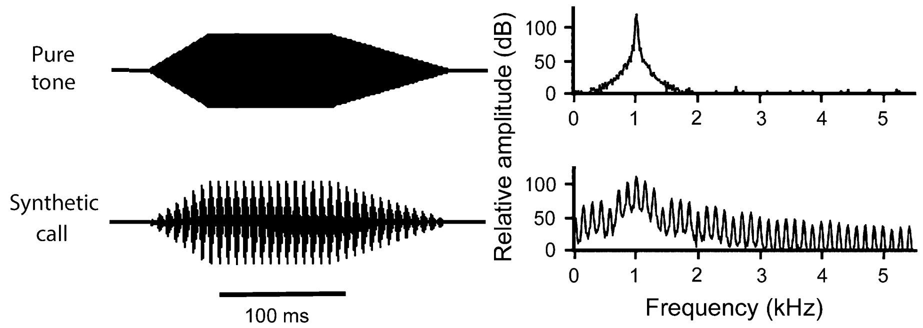

Fig. 1 1-kHz tone and synthetic advertisement call used as stimuli to obtain multi-unit midbrain auditory responses in males of *E. emiliopugini*

amplified (NAD C 320 BEE) and broadcasted with a two-way loudspeaker (Dynaudio BM 6, frequency response 38-20,000 Hz), positioned at 1 m in front of the 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 & 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 steps. 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 m diameter) driven with a hydraulic microdrive (Narishige MO-8), positioned on the dorsal surface of the optic tectum and advanced into the brain. For all subjects, recordings were obtained from both midbrain hemispheres.

The neural responses were passed through a pre-amplifier (Dagan 2400) and filtered between 100 and 3,000 Hz to obtain multi-unit recordings. Neural responses were monitored by means of an oscilloscope and a loudspeaker, and thresholds determined by audiovisual criteria. This methodology has been used in previous studies and yields reliable measurements of auditory thresholds in different anurans (Penna et al. 1990, 1992, 2008, 2013).

Recording procedures

A search stimulus consisting of a white noise burst having the same amplitude envelope structure as the pure tones was presented at an intensity of about 80 dB SPL RMS, 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 for the high-frequency region (CFH) was calculated from a weighted average of thresholds for frequencies of 600 Hz and higher 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 stadigraphs reconverted to dB SPL. Comparisons of BTL, BTH, CFH and thresholds for synthetic calls between both hemispheres were carried out with Wilcoxon signed-ranks tests ($P < 0.05$). The dependence of multi-unit audiograms' CFH on body size and weight was explored with Spearman's correlation ($P < 0.05$).

Thresholds to pure tones allow comparisons of the auditory sensitivity of *E. emiliopugini* with those of other species studied previously, and thresholds to the synthetic calls allow to estimate the active space, i.e., the distance over which this species communicates.

Comparisons of parameters measured for natural calls at different distances from the subjects in the field and of auditory thresholds measured in the laboratory between *E. emiliopugini* and *E. calcaratus* (Penna et al. 2013) were carried out with Mann–Whitney *U* tests ($P < 0.05$).

Results

Field call recordings

Advertisement calls of 10 males of *E. emiliopugini* were recorded. During recordings, air and substrate temperature averaged 7.2 °C (range 1.7–10.6 °C) and 9.2 °C (range 7.4–12.2 °C), respectively. Body length of 9 individuals captured after recording their calls averaged 50.4 mm (range 48–53 mm) and their body weight 12.7 g (range 11.1–16.2 g). Background noise levels measured directly with the sound level meter when no nearby frogs were calling averaged 31.2 dB SPL RMS (range 29.2–33.9 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 and also on choruses of *Batrachyla antartandica*, a species that overlaps its breeding activity with *E. emiliopugini*.

The burrows from which frogs called had different orientations relative to the horizontal plane: two were horizontal (0°), four were vertical (90°) and four had intermediate orientations (30°–45°). The total length of the burrows averaged 16.3 cm (range 9–34 cm) and the segment free from water averaged 8.2 cm (range 0–19 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 the shelters. Burrows of three subjects recorded in 1998 were occupied by calling males of *E. calcaratus* on the previous year.

Calls recorded 0.25 m in front of calling males had an average amplitude of 83.7 dB SPL RMS (range 76.0–88.8 dB SPL RMS), an average duration of 243 ms (range 193–299 ms) and an average dominant frequency of 1,001 Hz (range 711–1,206 Hz). Call duration was not related to air or substrate temperature ($R_s = 0.030$, $P > 0.05$ and $R_s = -0.134$, $P > 0.05$, respectively). For the nine individuals captured, no significant relationships among the acoustic variables and body size or weight occurred ($R_s < 0.487$, $P > 0.05$ for all cases). Calls of an individual recorded at different distances from the source are shown in Fig. 2.

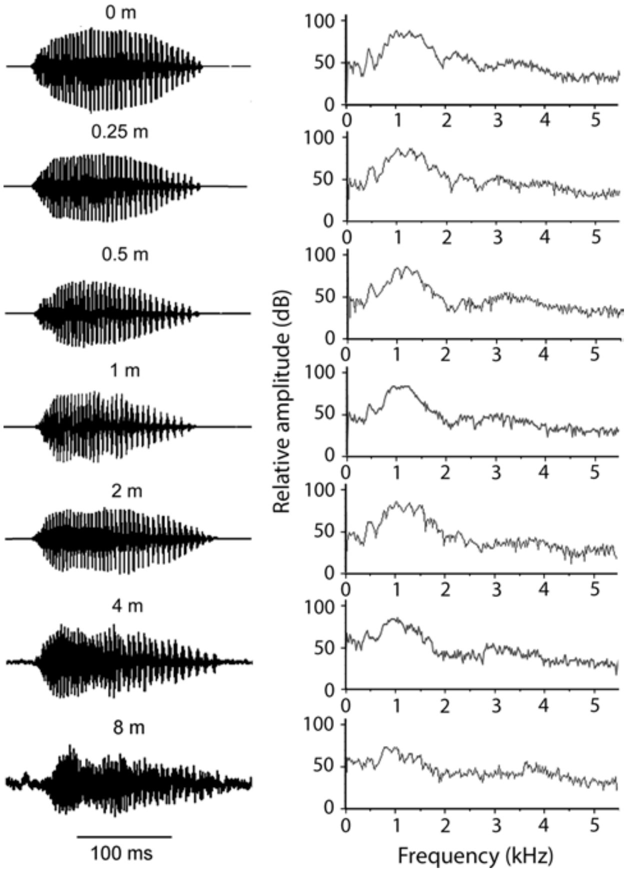

Fig. 2 Oscillograms (*left column*) and power spectra (*right column*; 3,171 points, 20 Hz bandwidth) of calls of an *Eupsophus emiliopugini* male recorded at different distances from its burrow opening (air temperature: 5.5 °C, substrate temperature: 10.2 °C)

Call SPLs decreased progressively with distance from the sound source, as shown in Fig. 3a and Table 1. One subject was not recorded at 0 m because the burrow opening was not clearly identified among the mossy substrate. For three subjects, calls at 8 m were not measured because one frog ceased calling and for the other two the proximity of conspecific chorusing at this location of the microphone interfered with recordings and measurements. For the recordings obtained at this distance for the other seven subjects, the signal to noise ratio was above 10 dB for six frogs (average: 17.3 dB, range 9.8–22.2 dB) and corrections to subtract the background noise were therefore not applied, following Hassal and Zaveri (1988). For one individual, the signal to noise ratio was 4.4 dB and a subtraction of 1.6 dB to the signal amplitude measured was applied.

The variation of call SPL with distance was analyzed for microphone positions between 0.25 and 4 m from the subjects, due to the lower number of cases at distances 0 and 8 m. Call SPL varied significantly with distance from the sound source (Friedman ANOVA: $\chi^2_4 = 38.56$, $P < 0.0001$; Fig. 3a) and results for multiple comparisons show marginally 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 75.3 dB SPL RMS (range 71.0–78.2 dB SPL RMS) and 77.1 dB SPL RMS (range 71.9–81.6 dB SPL RMS), respectively (Wilcoxon signed-rank test: $Z = 1.481$, $P = 0.13864$; 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

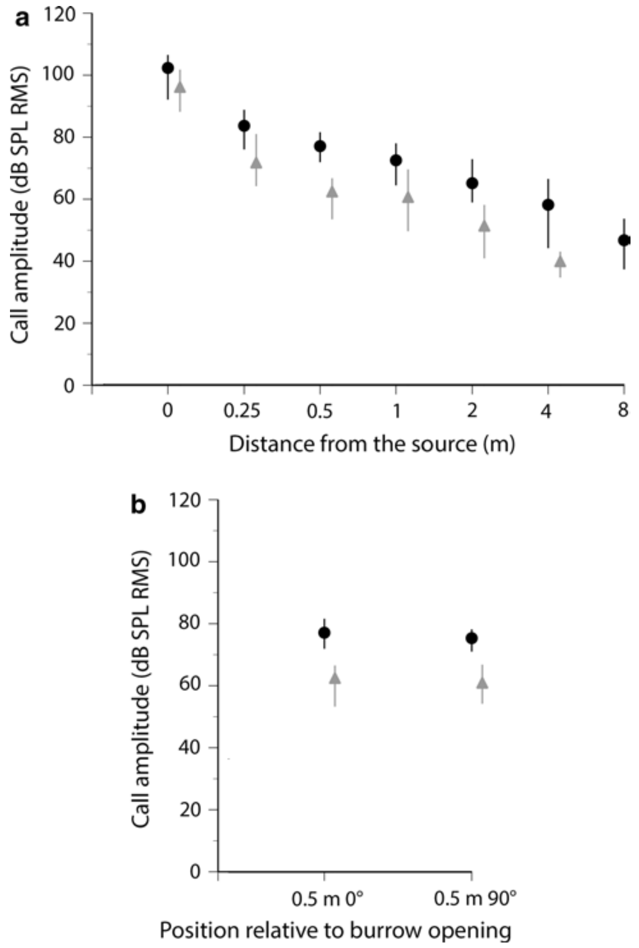

Fig. 3 **a** Sound pressure levels of advertisement calls recorded at different distances from males of *Eupsophus emiliopugini*. *Black circles* and *bars* indicate averages and ranges, respectively. Numbers of males of *E. emiliopugini* recorded at each distance are listed in Table 1. For comparison, values obtained in a former study with *E. calcaratus* (Penna et al. 2013) are represented by *grey triangles* and *bars*. **b** Sound pressure levels of calls of males of *E. emiliopugini* ($N = 9$) recorded at a distance of 0.5 m and at 0° and 90° relative to burrow openings. Values obtained in a former study with 7 *E. calcaratus* (Penna et al. 2013) are represented by *grey triangles* and *bars*

Table 2 Multiple comparisons among sound pressure levels of advertisement calls measured at five distances (0.25–4.0 m) from males of *Eupsophus emiliopugini* Friedman ANOVA: $\chi^2_4 = 38.56$, $P < 0.00001$, Wilcoxon signed-ranks tests, with Bonferroni correction

Distances (m)	Z	P
0.25–0.5	2.80	0.00506*
0.25–1.0	2.80	0.00506*
0.25–2.0	2.80	0.00506*
0.25–4.0	2.80	0.00506*
0.5–1.0	2.70	0.00691
0.5–2.0	2.80	0.00506*
0.5–4.0	2.80	0.00506*
1.0–2.0	2.80	0.00506*
1.0–4.0	2.80	0.00506*
2.0–4.0	2.60	0.00935

* Indicate values having marginal significance (significance level: $P < 0.005$)

the actual transmission loss, i.e., the decrease in average SPL from 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 call spectra are broad due to the pulsed amplitude-modulated structure of the signal. The dominant frequency showed a tendency to decrease with distance from the sound source, however, this variation did not reach the significance level (Friedman ANOVA: $\chi^2_4 = 7.34$, $P = 0.11890$; Fig. 4).

Laboratory midbrain recordings

The stimuli used to evoke neural responses are displayed in Fig. 1. The 6 males used for recordings had an average

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

Distance (m)	Number of males	Call amplitude (dB SPL RMS)	Excess attenuation (dB)	Dominant frequency (Hz)
0	9	102.3 (92.1–102.3)		1,059 (758–1,344)
0.25	10	83.7 (76.0–88.8)		1,001 (711–1,206)
0.5	10	77.1 (71.9–81.6)	0.6	1,040 (732–1,314)
1	10	72.5 (64.5–78.0)	−0.8	1,054 (775–1,378)
2	10	65.1 (58.9–72.9)	0.6	958 (689–1,309)
4	10	58.2 (44.2–66.5)	1.5	931 (670–1,206)
8	7	46.7 (37.4–53.7)	7.0	858 (689–1,212)

Measurements at 0 m correspond to recordings obtained with the microphone placed at burrow openings. Excess attenuations were calculated from the average SPLs at 0.25–8 m (see text)

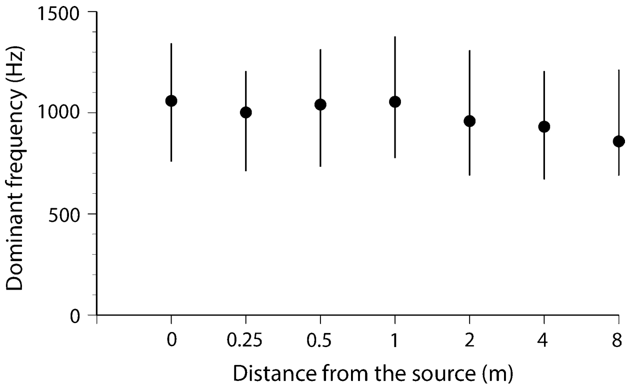

Fig. 4 Dominant frequencies of calls of *E. emiliopugini* recorded at different distances from burrow openings. *Symbols* as in Fig. 3

weight of 14.8 g (range 12.4–17.9 g) and a snout-vent length of 50.7 mm (range 48.8–55.2 mm). The cloacal temperatures at which recordings were conducted averaged 10.3 °C (range 9.7–10.9 °C).

Audiograms showed regions of enhanced sensitivity at a low-frequency range, between ca. 0.1 and 0.4 kHz and at a high-frequency range, between ca. 0.8 and 1.8 kHz. Audiograms measured in both hemispheres of a given individual were very similar, differences in BTL, BTH and CFH averaged 2.3 dB SPL (range 0–6 dB SPL), 1.2 dB SPL (range 0–6 dB SPL) and 78 Hz (range 0–213 Hz), respectively. Audiograms obtained from both hemispheres of an individual and thresholds to synthetic calls are shown in Fig. 5. Comparisons for BTL, BTH and CFH of audiograms and of thresholds to the synthetic calls between both hemispheres performed for the six individuals showed no significant differences (Wilcoxon signed-rank test: $Z < 1.83$, $P > 0.068$ for all comparisons). For each individual, averages of BTL, BTH and CFH of audiograms and thresholds to the synthetic calls obtained from values measured in both hemispheres were calculated for further analysis.

The BTL reached minima values averaging 48.3 dB SPL (range 45.0–51.5 dB SPL), and occurred at the lower frequencies tested (0.1 or 0.2 kHz), and the BTH averaged 43.3 dB SPL (range 37.0–49.5 dB SPL). The frequencies at which BTH occurred ranged from 1.0 to 1.4 kHz. The center frequency for the high-frequency region (CFH) averaged 1,168 Hz (range 1,082–1,306 Hz) and was not significantly correlated with body weight or body length (Spearman correlation: $R_s = -0.086$, $P < 0.05$ and $R_s = -0.600$, $P > 0.05$, respectively). Average values of BTL and BTH are listed in Table 3 and an average audiogram is shown in Fig. 6a.

Thresholds of responses to the synthetic call averaged about 44 dB SPL, and were similar to the thresholds to the 1-kHz pure tone (Table 3; Fig. 6b).

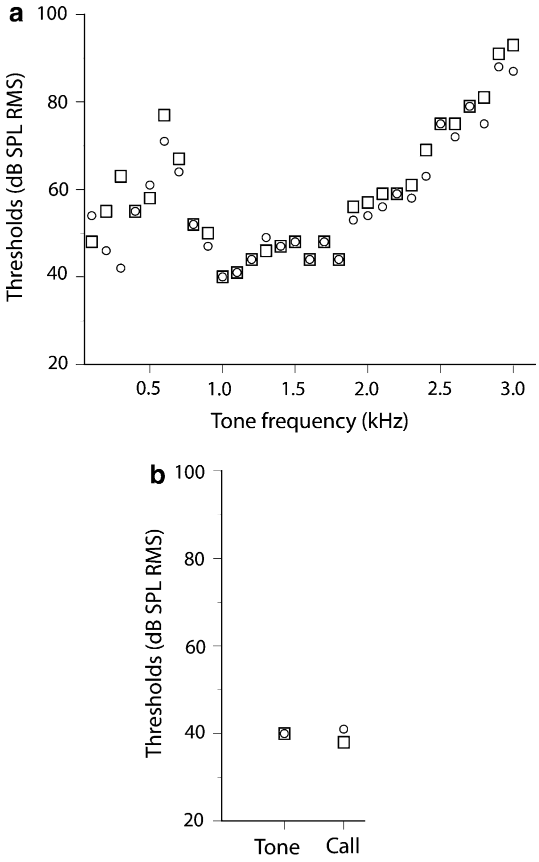

Fig. 5 Thresholds of multi-unit midbrain auditory responses in both hemispheres of a male of *E. emiliopugini*: **a** audiograms obtained with pure tones. **b** Thresholds to a 1-kHz tone and the synthetic advertisement call. *Circles* and *squares* correspond to thresholds in the left and right hemisphere, respectively

Comparisons between *E. emiliopugini* and *E. calcaratus*

The variables measured in the calls and audiograms of *E. emiliopugini* were compared with similar measurements carried out in a previous study with the related species *E. calcaratus* which calls and breeds at the study site earlier in the year (Penna et al. 2013). The call SPLs at 0.25 and 4 m were significantly higher for *E. emiliopugini* relative to *E. calcaratus* (Mann–Whitney U test, $P < 0.05$). For the audiograms, the BTLs did not differ between both species, but the BTH, thresholds for conspecific calls and CFH were higher for *E. calcaratus* as compared to *E. emiliopugini* (Table 3).

Discussion

Results of this study show that SPLs of the advertisement calls of *E. emiliopugini* decrease progressively with distance from the source. A drop of about 20 dB occurs

Table 3 Comparisons of call and audiogram parameters between *E. emiliopugini* and *E. calcaratus* (Mann–Whitney *U* test, $P < 0.05$)

Variable	Species	*N*	Average (range)	*Z*	*P*
Call SPL at 0.25 m	EE	10	83.7 (76.0–88.8)	3.40	0.00067
(dB SPL)	EC	10	71.7 (64.1–80.9)		
Call SPL at 4 m	EE	10	58.2 (44.2–66.5)	3.42	0.00064
(dB SPL)	EC	10	39.9 (34.6–42.9)		
Audiogram BTLs	EE	6	48.3 (45.0–51.5)	0.53	0.59873
(dB SPL)	EC	13	48.9 (25.1–57.9)		
Audiogram BTHs	EE	6	43.3 (37.0–49.5)	−2.98	0.00285
(dB SPL)	EC	13	52.1 (43.2–57.8)		
Thresholds for calls*	EE	6	44.3 (36.6–50.5)	−3.07	0.00214
(dB SPL)	EC	13	57.6 (46.8–62.1)		
Audiogram CFHs	EE	6	1.2 (1.1–1.3)	3.42	0.00063
(kHz)	EC	13	1.8 (1.3–2.5)		

Data for *E. calcaratus* from Penna et al. (2013)

* Stimuli used to measure thresholds for calls were the synthetic call of *E. emiliopugini* and a natural call of *E. calcaratus* having about mid values for its frequency components (mid-frequency advertisement call, see Fig. 6b and Penna et al. 2013)

from the burrow opening to 0.25 m, the minimum external distance measured. At 8 m from the source, the signal amplitude is clearly above background noise in animals for which measurements were carried out at this distance; measurements for 3 out of 10 individuals could not be performed because of interference from biotic background noise corresponding mainly to chorusing by conspecific males calling nearby this microphone 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 power at the sound source. Simultaneous recordings of calls with at least two 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 yield values of excess attenuation well below measurements of a recent study on propagation of vocalizations of the congeneric *E. calcaratus* (Penna et al. 2013) in which excess attenuations of about 8 dB were measured at 4 m from the source, in contrast with the average 1.5 dB measured at this distance in the current study. The lower attenuation rates of the vocalizations of *E. emiliopugini* are likely due to the lower frequency contents of these signals relative to the calls of *E. calcaratus*, a spectral feature inversely related to body size of these species. Dominant frequency of anuran calls in general and in particular in species of the southern temperate forest is inversely related to body size (Penna and Veloso 1990). Our results of transmission losses rather show general similarity with measurements on propagation of vocalizations of Iberian toads *Alytes* (Penna et al. 2012), which yielded excess attenuations of about 5 dB at 8 m from the sound source. These calls have a pure tone structure with a dominant frequency of about 1,400 Hz, devoid of frequency components at higher frequencies as is characteristic of the call of *E. calcaratus*.

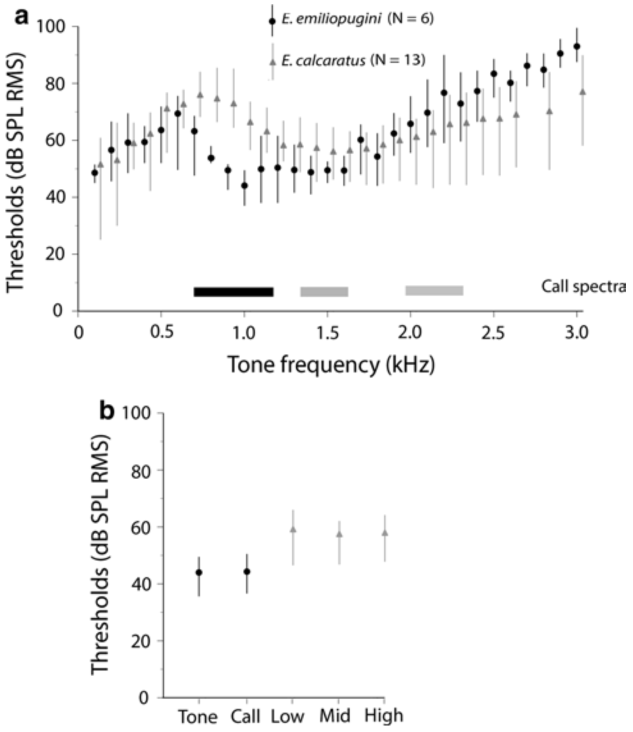

Fig. 6 Thresholds of multi-unit midbrain auditory responses in six males of *E. emiliopugini*: *Black circles* and *bars* indicate averages and ranges, respectively. For comparison, values obtained in a former study with *E. calcaratus* ($N = 13$, Penna et al. 2013) are represented by *grey triangles* and *bars*. **a** Audiograms obtained with pure tones. The *horizontal black bar* shows the dominant frequency range for the call of *E. emiliopugini*. The *two horizontal grey bars* indicate the frequency range of the two harmonics of higher energy content in the call of *E. calcaratus*. **b** Thresholds for the a 1-kHz tone and for the synthetic advertisement call of *E. emiliopugini*. For comparison, values obtained in the study *E. calcaratus* (Penna et al. 2013) in response to natural conspecific calls having spectral components within the conspecific low-, mid- and high-frequency range of variation of the second harmonic are presented as *grey triangles* and *bars*

The average amplitude of the calls of E. *emiliopugini* at 8 m (46.7 dB SPL) is still above the average of about 44 dB SPL obtained for auditory thresholds to conspecific advertisement calls measured with multi-unit recordings in the auditory midbrain. According to this combined evidence, male frogs would be able to communicate beyond 8 m, in contrast with *E. calcaratus*, which is constrained to distances below 2 m (Penna et al. 2013). However, playback experiments of evoked vocal responses conducted formerly for *E. emiliopugini* (Penna et al. 2005a) have shown that these behavioral thresholds are on average 60 dB SPL peak (corresponding to about 50 dB SPL RMS) which is 6 dB above the auditory thresholds of 44 dB SPL RMS for synthetic calls measured in the current study. Dissimilarities between auditory thresholds and thresholds for evoked vocal responses have been reported in other anurans, prompting for caution in assessments of active space in acoustic interacting animals (e.g., Wilczynski and Brenowitz 1988; Brenowitz 1989; Gerhardt et al. 1989; Dyson and Passmore 1992).

It would be of particular interest to record auditory thresholds from females to estimate the distance range over which they orient to calling males and the potential consequences of this detection for sexual selection processes. Unfortunately, females are very seldom seen at the study site: only four or five specimens have been spotted during more than 20 years of fieldwork in this locality.

The radiation pattern of vocalizations by males of *E. emiliopugini* is not directional, a result concordant with measurements reported for other anurans (Narins and Hurley 1982; Wells and Schwartz 1982; Penna et al. 2012, 2013), indicating that this is a general trend for the group, in contrast with the directional patterns observed in insects (e. g. Michelsen and Elsner 1999; Michelsen and Fonseca 2000) and birds (e. g. Brumm 2002; Patricelli et al. 2007).

The tendency to a decrease in dominant frequency with distance from the source does not reach a level of significance, which would indicate that spectral changes are not a reliable indicator of signal degradation as it occurs in the congeneric species *E. calcaratus* (Penna et al. 2013). The broad spectral composition of the call of *E. emiliopugini*, with side-bands originated in its pulsed amplitude-modulated structure also contributes to limit the relevance of spectral cues for identifying degraded signals. In this species, the depth of amplitude modulation is likely to decrease over distance, a degradation in the temporal structure that could be explored accurately with simultaneous recordings of vocalizations at different positions from the source.

The auditory sensitivity in the high-frequency range is tuned to a frequency 167 Hz higher than the average dominant frequency of the calls, a mistuning that may not be relevant due to the broad spectra of the calls of this species, which spreads amply into frequencies above and below the dominant frequency. The audiograms of *E. emiliopugini*, showing minimum thresholds to pure tones at about 43 dB RMS SPL are within the typical range measured in previous studies in other anurans, spanning from 30 to 50 dB RMS (e.g., Penna et al. 1990, 1992, 2008, 2013). Auditory thresholds to pure tones in the high-frequency range are similar to thresholds for synthetic imitations of the natural conspecific calls having their main spectral components in this frequency domain.

Overall, our study shows that *E. emiliopugini* (belonging to the Vertebralis group of *Eupsophus*), has considerable advantages for distant sound communication relative to *E. calcaratus* (belonging to the Roseus group of this genus) in the native environment shared by both species. The amplitude of the advertisement call is higher, the attenuation properties of the physical environment are lower and the auditory thresholds for conspecific calls are lower as compared to *E. calcaratus*. All these factors add up to extend the communication range likely of *E. emiliopugini* to a radius beyond 8 m, comparable to active spaces reported for other anurans (e. g., Boistel et al. 2011; Penna et al. 2012). The different characteristics of the vocalizations and the acoustic communication systems of these two taxa are likely related to their evolutionary history, as phylogenetic relationships indicate a strong support for the separation between the Vertebralis and the Roseus groups within the genus *Eupsophus* (Correa et al. 2006). The vocalizations of species within each group have similar structure: amplitude-modulated in Vertebralis and have a harmonic profile in Roseus (Formas 1985; Penna and Veloso 1990; Formas and Brieva 1994; Márquez et al. 2005, Opazo et al. 2009).

A possible advantage that the production of soft calls would confer to *E. calcaratus* and related species is to minimize detection by predators, a trade-off that has been proposed to occur in birds using low-amplitude vocalizations in agonistic contexts (e.g., Dabelsteen et al. 1998; Hof and Hazlett 2010).

The low environmental noise of biotic origin present at the breeding sites (Penna et al. 2005a, 2013; Penna and Hamilton-West 2007; this study) would also contribute to facilitate the detection of the calls of *E. emiliopugini*. Such relative advantageous condition may account for the lack of change in vocal activity of this frog in the presence of natural abiotic noises at moderate levels (Penna and Hamilton-West 2007). This stability contrasts with the strong vocal activation that the same interferences induce in *E. calcaratus* (Penna et al. 2005a), a responsiveness that could counteract the additional limitations that noise would impose on the restricted active space over which this frog communicates in the same microenvironment as *E. emiliopugini* (Penna et al. 2013).

The similarity of the microenvironment in which the two species compared breed and call is remarkably accurate: individuals of both taxa occupy the same burrows in successive breeding seasons, as exemplified by three of our experimental subjects which occupied burrows inhabited by males of *E. calcaratus* in the previous year (see "Results" section). There are also no detectable changes in the vegetation coverage throughout the breeding season of both species in these evergreen landscapes. Furthermore, the overlap of the breeding seasons between the two taxa, which is partial at the study site is a condition that occurs to a larger extent in other related species of the austral temperate forest region. For two species belonging to the Vertebralis and Roseus group of *Eupsophus,* the overlap in breeding seasons is complete: *E. vertebralis* calls during about 1 month during the peak of the four-month breeding period of *E. roseus* (September–December; Moreno-Gómez et al. 2013). Additional comparisons of the active spaces between these species would determine the extent to which contrasting communication strategies are employed by different taxa living in similar microenvironments within the austral temperate forest.

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