Female frog auditory sensitivity, male calls, and background noise: potential influences on the evolution of a peculiar matched filter

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The matched filter hypothesis proposes that the tuning of females' auditory sensitivity matches the spectral energy distribution of males' signals. Such correspondence is expected to arise over evolutionary time, as it promotes conspecific information transfer and reduces interference from other sound sources. Our main objective was to determine the correspondence between the acoustic sensitivity of female frogs of *Eupsophus roseus* and the spectral characteristics of advertisement vocalizations produced by conspecific males. We also aimed to determine how auditory sensitivity is related to the characteristics of background noise. We analysed data on the auditory sensitivity of *E. roseus* females, and recordings of conspecific male vocalizations and of the acoustic environment during the breeding period of this species. Our results indicate a concordance between the auditory sensitivity of females and call spectra that would provide an appropriate detection of these signals. In addition, this matching is large relative to the correspondence between auditory sensitivity with the spectra of the abiotic and biotic background noise, with the last component being associated with calls of the related species *Eupsophus vertebralis*. This may be an adaptation of receivers confronting sound interference, which improves the capability of *E. roseus* to communicate sexually by means of acoustic signals. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **110**, 814–827.

ADDITIONAL KEYWORDS: acoustic communication – acoustic signals – auditory tuning – automated recording systems – *Eupsophus roseus* – matched filter hypothesis.

INTRODUCTION

Animal communication systems involve the interaction of signallers and receivers by means of transmitted signals. This information transfer requires a minimum concordance between the signal design and the sensitivity characteristics of the receivers (Endler, 1993). In sexual communication, factors promoting the evolution of this matching improve individual recognition and mate acquisition. In addition,

this concordance decreases the probability of heterospecific interactions that can affect the fitness of reproductive individuals negatively, such as non-viable hybrid progeny formation and competition for a communication channel (Endler, 1993; Ryan & Rand, 1993; Endler & Basolo, 1998; Higgie, Chenoweth & Blows, 2000; Ritchie, 2007; Pfennig & Pfennig, 2009).

In sexual acoustic communication, the matched filter hypothesis proposes that the tuning of females' auditory sensitivity matches the spectral energy distribution of male acoustic signals (Capranica, 1965; Capranica & Moffat, 1983). The evolution of this matching is expected to occur as it promotes

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communication amid attenuation and interference occurring ubiquitously in natural environments (Wiley & Richards, 1982). Animals engaging in acoustic communication are exposed to background interferences from abiotic and biotic sources, which can vary in space and time (Wiley & Richards, 1982). Natural abiotic sources, such as wind and flowing water, generally contribute low frequencies to the acoustic environment. Sound-producing animals living in environments with high levels of such noises have been reported to use signals containing frequencies detuned from the noise spectra (reviewed in Brumm & Slabbekoorn, 2005).

The biotic components of the acoustic environment depend on the biodiversity level of a given area. Several species producing sound at the same location and at the same time may interfere with each other, resulting in competition for the acoustic channel (Ryan & Kime, 2003). This situation is likely to result in acoustic niche partitioning, i.e. different co-occurring species tend to have calls with non-overlapping frequencies (e.g. Sueur, 2002; Luther, 2009), and may also constrain the auditory sensitivity of females to match conspecific signals, thereby enhancing the signal-to-noise ratio (e.g. Gerhardt & Huber, 2002; Feng & Schul, 2006). Correspondences of this kind have been observed in different vertebrate and invertebrate taxa (e.g. Brzoska, Walkowiak & Schneider, 1977; Witte *et al.*, 2005; Amézquita *et al.*, 2006; Sueur, Windmill & Robert, 2010; Schmidt, Riede & Römer, 2011; Gall, Brierley & Lucas, 2012), especially in environments characterized by high levels of biodiversity and acoustic complexity. In these cases an enhanced relationship between acoustic signal and sensitivity is present (e.g. Witte *et al.*, 2005; Amézquita *et al.*, 2006, 2011; Schmidt *et al.*, 2011).

Since its original formulation, the matched filter hypothesis has received support from further studies (Gerhardt & Schwartz, 2001; Simmons, 2013); nevertheless, mismatches between signals and sensitivity have also been reported to occur, in which the lack of strict correspondence is related to interactions between natural and sexual selection, morphological and physiological constraints, and to the evolutionary history of the species (Ryan & Brenowitz, 1985; Ryan *et al.*, 1990, 2003; Ryan & Rand, 1990, 1999; Ryan, Perril & Wilczynski, 1992; Gerhardt, 1994; Endler & Basolo, 1998; Ryan, 1998; Wilczynski, Rand & Ryan, 2001). In a recent review, Simmons (2013) has pointed out that additional studies incorporating ecological data are needed to gain a deeper understanding of the factors causing variation between auditory sensitivity and vocalization characteristics. In this context, the use of automated recording systems has provided the possibility to record the vocal activity of animals over extensive time periods (e.g. Obrist *et al.*, 2010; Llusia,

Márquez & Bowker, 2011, Sueur *et al.*, 2012; Llusia *et al.*, 2013), allowing to detect and to quantify the co-occurrence of potentially interfering vocalizing species.

The anuran species inhabiting the South American temperate forest communicate sexually in relatively simple acoustic environments when compared with species found in tropical forests that generate more complex soundscapes. Most anurans in this region produce vocalizations with dominant frequencies between 1 and 4 kHz, and heterospecific choruses usually include very few taxa, ranging from two to four species (Penna & Veloso, 1990). *Eupsophus* frogs (Cycloramphidae) are endemic anurans inhabiting the South American temperate forest, where they breed mainly during the spring and early summer of the Southern hemisphere. Males typically congregate in flooded areas, attracting females with advertisement vocalizations emitted from inside burrows, where amplexus takes place. Females leave burrows after pairing and males provide parental care to the endotrophic tadpoles (Formas & Vera, 1980; Penna & Veloso, 1990; Úbeda & Nuñez, 2006; Penna & Márquez, 2007). This genus is divided into two main groups (Formas, 1992), differing in their vocalizations: species of the Roseus group emit calls with a complex harmonic structure and with frequency modulations, and species of the Vertebralis group produce vocalizations containing amplitude modulations and sidebands (Penna & Veloso, 1990; Formas, 1991, 1992; Penna & Solís, 1998; Márquez *et al.*, 2005; Opazo *et al.*, 2009). The dominant frequency is higher in the Roseus group, although some frequency overlap occurs between the two groups (e.g. Penna & Veloso, 1990). Species belonging to these two groups have been reported to respond with different behavioural strategies to interference from natural abiotic noises occurring in their native environment (Penna, Pottstock & Velásquez, 2005; Penna & Hamilton-West, 2007).

Two representative *Eupsophus* species that can be found breeding at the same time and location are *Eupsophus roseus* (Duméril & Bibron, 1841) (geographic range from 38°S to 40°S) and *Eupsophus vertebralis* (Grandison, 1961) (geographic range from 40°S to 44°S), from the Roseus and Vertebralis group, respectively (Formas & Vera, 1980; Veloso *et al.*, 2010). The fundamental frequency (346–1019 Hz) of the call of *E. roseus* has lower energy than the second (875–1429 Hz) and third (1194–2167 Hz) harmonics, and the dominant frequency corresponds either to the second or third harmonic (Márquez *et al.*, 2005). The dominant frequency of *E. vertebralis* ranges between 700 and 1110 Hz (Penna & Veloso, 1990), overlapping with the first harmonic of *E. roseus*, but more importantly because of its higher energy content, also

overlapping with the lower range of the second harmonic of its advertisement vocalizations. The spectral structure of the call of *E. roseus*, having two discrete peaks in the high-frequency range, the lower of which overlaps with the call spectrum of the sympatric and syntopic *E. vertebralis*, provides an excellent opportunity to explore the extent to which matching between female auditory sensitivity and male signals applies.

Our main objective was to determine the matching between the spectral characteristics of the advertisement vocalization produced by males and the acoustic sensitivity of *E. roseus* females. We also aimed to determine how auditory sensitivity is related to the characteristics of the background noise. Because abiotic and biotic noise can interfere with the communication process, we expect that auditory sensitivity and vocalizations present characteristics that improve the signal-to-noise ratio. As the main biotic background noise corresponds to *E. vertebralis* vocalizations, which contain frequencies overlapping with the lower frequency range of *E. roseus* vocalizations, we expect female sensitivity to have evolved to reduce interference from this heterospecific low-frequency source. If this is the case, females should not match entirely the conspecific male frequency distribution, but instead diverge from the interfering species, shifting to higher frequencies. To test this hypothesis, we collected and analysed data of *E. roseus* female auditory sensitivity, acoustic recordings of conspecific male advertisement vocalizations, and recordings of the acoustic environment during the breeding period of this species.

MATERIAL AND METHODS

STUDY SITE

The population studied is located in a temperate forest reserve, Bosque Experimental San Martín (39°38′S, 73°07′W), managed by the Universidad Austral de Chile. The recordings of *E. roseus* male vocalizations and of the acoustic environment were conducted during the 2010 reproductive season, from September to December, and the female auditory sensitivity tests were conducted during December 2010.

MULTIUNIT AUDITORY RESPONSES OF FEMALES

Surgical procedures

Twelve females in reproductive state were captured in December 2010 (snout–vent length = 39.88 ± 3.09 mm; body mass 7.68 ± 1.83 g). Sex and reproductive state were determined by the presence of eggs. Females were transferred to the Laboratorio de Neuroetología, Universidad de Chile, Santiago, Chile. Individuals were maintained in terraria (40 × 20 × 25 cm) with vegetation from the capture site in a temperature-regulated room at 10 ± 1 °C under an inverted 12 : 12-h photoperiod. The females were fed once a week with tenebrionid larvae. All the neurophysiological procedures were performed within 3 weeks of arrival in the laboratory.

Females were anaesthetized by immersion in a 0.4% aqueous solution of MS-222 (Sigma-Aldrich). The dorsal skin of the head was incised and the skull was drilled. Dura and pia membranes covering the brain were dissected, exposing the optic tectum. Individuals recovered from anaesthesia for 12–24 h before recordings. To perform recordings, females were immobilized by injection with D-tubocurarine chloride (10 µg g^{-1} body mass). The recordings were performed in a sound-attenuated booth with walls and ceiling covered with 10-cm foam wedges. Females were positioned on a Peltier plate (Cambion) supplied with a DC current of 2 A to maintain body temperature at 10 °C, which is similar to the mean temperature under natural conditions during the breeding period. The body of the animal was covered with light moist gauze to facilitate cutaneous respiration. Body temperature was monitored throughout recordings by using a miniature thermometer probe inserted in the cloaca (Digisense 8528-20). This protocol is in agreement with the bioethical regulations of the Universidad de Chile (protocol CBA# 061FMUCH).

Stimulus generation and recording procedures

To determine the acoustic sensitivity of females at different frequencies, pure tones were synthesized with a sound generator, System II (Tucker-Davis Technologies), driven by SigGen 3.0 software (Tucker-Davis Technologies). The duration of the tones was 170 ms, with rise and fall times of 30 ms; values similar to natural advertisement vocalizations (Márquez *et al*., 2005). The frequency of pure tones was within 100 and 5000 Hz, a range encompassing the natural spectral range of advertisement vocalizations. The frequency step of pure tones was 100 Hz between 100 and 3000 Hz, and 200 Hz between 3000 and 5000 Hz. Stimuli were broadcast using an amplifier (NAD C 320 BEE) and a loudspeaker (Dynaudio BM 6, frequency response 38–20 000 Hz) positioned 1 m in front of the individual. The amplitude of the stimulus was controlled with an attenuator (PA4; Tucker-Davis Technologies). The rate of stimulus presentation was 0.5 stimuli s^{-1}. The amplitude of tones of each frequency tested was measured at the beginning of the experimental session by positioning the microphone of a sound-level meter (Brüel and Kjaer 2238) 2–3 mm above the head of the individual. The frequency response of the system was within ±6 dB.

Multiunit responses (i.e. the activity of a population of neurons) from the torus semicircularis were

recorded with a custom-made gross glass-insulated tungsten electrode (75 μm diameter). The electrode was positioned with a micromanipulator and a hydraulic microdrive (Narishige MO-8) in the intersection of the limit between the mid and the caudal third, and the limit between the medial and lateral half of the left hemisphere. The electrode was lowered into the brain while delivering white noise bursts of the same temporal structure as the pure tones, and with an amplitude of 60 dB sound pressure level (SPL), until reaching the level at which the strongest response was evoked, between 800 and 1350 μm. Thereafter, thresholds for neural responses to pure tones were determined by increasing the stimulus amplitude in 3-dB steps with the attenuator, while monitoring the neural response with an oscilloscope and a loudspeaker. Auditory responses were considered to cease when no audible neuronal discharge occurred for two out 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. The best threshold (BT) corresponded to the lowest SPL measured for a given tone frequency, and the BT frequency corresponded to that at which the BT occurred for a given audiogram. This set-up and these methods have been used in previous studies (Penna et al., 1990; Penna, Capranica & Somers, 1992; Penna, Velásquez & Solís, 2008). In addition to the BT, bandwidths for the two frequency regions of enhanced sensitivity (see Results section) were calculated, considering frequencies adjacent to the BT frequency for which thresholds were within 6 dB above the BT.

ADVERTISEMENT VOCALIZATIONS OF MALES

Acoustic recordings

Advertisement vocalizations of 50 *E. roseus* males (Fig. 1) were recorded between September and December 2010, from 22:00 to 04:00 h, with a Sennheiser ME66/K6 microphone connected to a Marantz PMD 660 digital recorder (sampling rate, 48 000 Hz; sample size, 16 bits). The microphone was placed at a distance of 100 cm from the opening of burrows from which males of this species call. The vocalization of a local male with a pulse duty cycle of 2 s was broadcast to induce a nearly constant vocalization rate of the recorded male. The loudspeaker (frequency response: 50 Hz–15 kHz) was placed by the microphone and facing the calling male. The stimulation started 30 s prior to the recording onset,
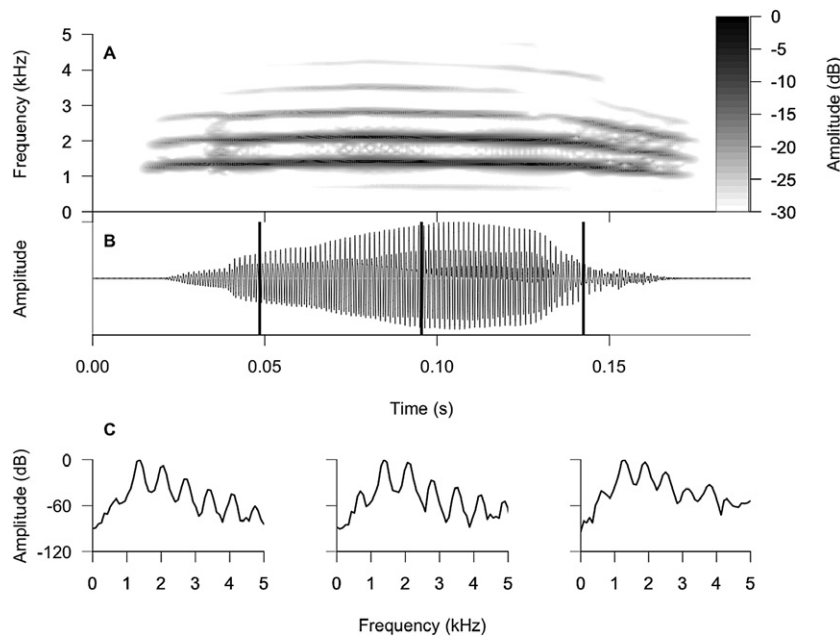

Figure 1. Example of an advertisement vocalization produced by males of *Eupsophus roseus*. A, spectrogram (Hamming window with a 95% of overlap, frequency resolution 86 Hz, and 30-dB dynamic range). B, oscillogram (time versus linear relative amplitude, with no units). C, power spectra calculated at the mid-points of the initial, mid, and final third segments of the vocalizations, as indicated by the vertical line in the oscillogram, starting from left to right (frequency resolution: 86 Hz).

which lasted 180 s. Because some burrows may have more than one opening, we recorded males that were at least 2 m apart to avoid recording individuals repetitively, and a tag was placed outside each burrow for identification.

Vocalization analysis

Because recordings of focal males contained vocalizations of surrounding males, the time position (i.e. beginning and end) of all clean vocalizations within the acoustic recording were first obtained by using the Audacity sound editor (http://www .audacity.sourceforge.net). Then, as the number of clean vocalizations within the acoustic recordings of each male was highly variable, seven vocalizations obtained at random were analysed, a number that allowed the inclusion of all recorded males. To achieve this, for each male a sequence of numbers from 1 to the total number of selected vocalizations was generated, and then seven vocalizations at random were sampled, with no replacement, using the function 'sample' of R (R Development Core Team, 2012, http:// www.R-project.org/). These selected vocalizations were then used to obtain the temporal and spectral characteristics, with a custom-automated analysis implemented with the R sound analysis package Seewave (Sueur, Aubin & Simonis, 2008).

Prior to analysis audio files were down-sampled to 22 050 Hz. This sampling rate allows the most important frequency range of advertisement vocalization to be analysed, while reducing computer memory consumption. To calculate the spectral characteristics of vocalizations, frequency parameters were obtained using a fast Fourier transform with a window length of 220 points. This procedure gives a temporal resolution of 9.98 ms, and a frequency resolution of 100 Hz, similar to the frequency resolution used for the neurophysiological measurements. Because the signals contained frequency modulations (Márquez *et al.*, 2005), power spectra were obtained at the mid points of the initial, mid and final third segments of the vocalizations. The resulting spectra were used to determine the harmonic composition by measuring the frequency and relative amplitudes of the first (f1), second (f2), and third (f3) harmonics. The dominant frequency (DF) – i.e. the frequency with the highest energy – of the mean power spectrum over the entire vocalization was obtained, which in this species corresponds either to the second (f2) or third (f3) harmonics (Márquez *et al.*, 2005).

In addition, to make vocalization spectra statistically comparable with audiograms, in terms of frequency resolution, we obtained the mean power spectra using a fast Fourier transform with a window length of 880 points, which yields a frequency resolution of 25 Hz. Using these data we calculated the

average amplitude between the two bins encompassing the frequency of the tones tested in the audiograms. For instance, in the spectrum of a vocalization the amplitude at 100 Hz was obtained by averaging the 25-Hz bins starting at 75 and 100 Hz, the amplitude at 200 Hz was obtained from the 25-Hz bins starting at 175 and 200 Hz, and so on. The resulting amplitude values were assigned to frequency values in 100-Hz steps, yielding a vocalization spectrum comparable with audiograms.

ACOUSTIC ENVIRONMENT

Acoustic recordings

An automated recording device was used (Song Meter SM2, Wildlife Acoustics) to record the background noise during the breeding season. The recorder was installed at sites where reproductive males were known to vocalize actively. Recordings were conducted between September and December 2010. The automated recorder was programmed to record (sampling rate, 48 000 Hz; sample size, 16 bits) for seven consecutive days each month, except for December, when 4 days were sampled. The equipment recorded 10 min every hour, starting 1 hour after sunset for 6 hours.

Background noise analysis

Background noise recordings were analysed using the same settings as for the analysis of individual vocalizations (sampling frequency, 22 050 Hz; window length, 220 points; frequency resolution, 100 Hz). The DF of the entire acoustic environment was obtained from the mean power spectrum of each 10-min recording. In addition, to obtain the spectral characteristics of the background noise, for each recording a 200-ms interval devoid of *E. roseus* vocalizations was selected and the DFs of the mean power spectra of these selections were obtained. These 200-ms samples were obtained by generating a random number sequence from 1 to the total number of seconds in the recording (i.e. 600 s). The random number indicated the beginning of the 200-ms sample interval. If a generated interval included *E. roseus* vocalizations, we moved forward until a segment devoid of these sounds was found. Mean spectra of these intervals statistically comparable with audiograms were obtained by using the same procedures as for vocalizations.

STATISTICAL ANALYSIS

Neurophysiological and acoustic data were summarized by classical descriptive statistics, i.e. means and standard deviations for symmetric distributions, and medians and ranges for skewed distributions (Quinn & Keough, 2002). Decibel (dB) values were converted to a linear scale (Pa) prior to arithmetic calculations, and then converted back to logarithmic dB values.

Cross-correlation analyses were performed: (1) to compare female auditory sensitivity spectra and the spectra of vocalizations; and (2) to compare the female auditory sensitivity spectra and the background noise spectra. Female audiograms were previously inverted to obtain the same type of profiles as vocalizations and background noise spectra. Because audiograms present two regions of enhanced sensitivity (see Results section), two cross-correlation coefficients were obtained, one for each region. The cross-correlations were performed under a bootstrap procedure with 10 000 repetitions to obtain the distribution of the corresponding coefficients (Quinn & Keough, 2002). Because the number of females was lower than the number of males, and of the biotic background noise spectra, sampling with replacement was set to the low value of 12 individuals (the total number of females) for each of the 10 000 repetitions. In each repetition, the 12 samples were averaged and the resulting average audiogram and average spectra were subjected to cross-correlation analysis. The confidence interval of the statistic at 95% was estimated by obtaining the lower (0.025) and upper (0.975) quantiles of the statistic distribution (Crawley, 2007).

All analyses were performed using R (R Development Core Team, 2012, http://www.R-project.org/).

RESULTS

FEMALE AUDITORY SENSITIVITY

Eupsophus roseus female multi-unit audiograms were characterized by two regions of enhanced sensitivity: a low-frequency region (< 700 Hz, LFR) and a high-frequency region (> 700 Hz, HFR), with a decreasing sensitivity above 3500 Hz (Fig. 2A). The BT of the LFR had a median of 37 dB SPL (range: 34–54 dB SPL) at 200 Hz (range: 200–500 Hz). The BT of the HFR had a median of 37 dB SPL (range: 35–50 dB SPL) at 1900 Hz (range: 1700–2000 Hz) (Fig. 2A). The BT frequencies of both LFR and HFR were not significantly correlated with body size (LFR, Spearman correlation, $R_S = -0.037$, $P = 0.910$; HFR, Spearman correlation, $R_S = -0.121$, $P = 0.708$, $N = 12$). The bandwidths calculated for the two frequency regions of enhanced sensitivity, i.e. the frequency range presenting thresholds within 6 dB above the BT, in the LFR had a median for the low and high limit
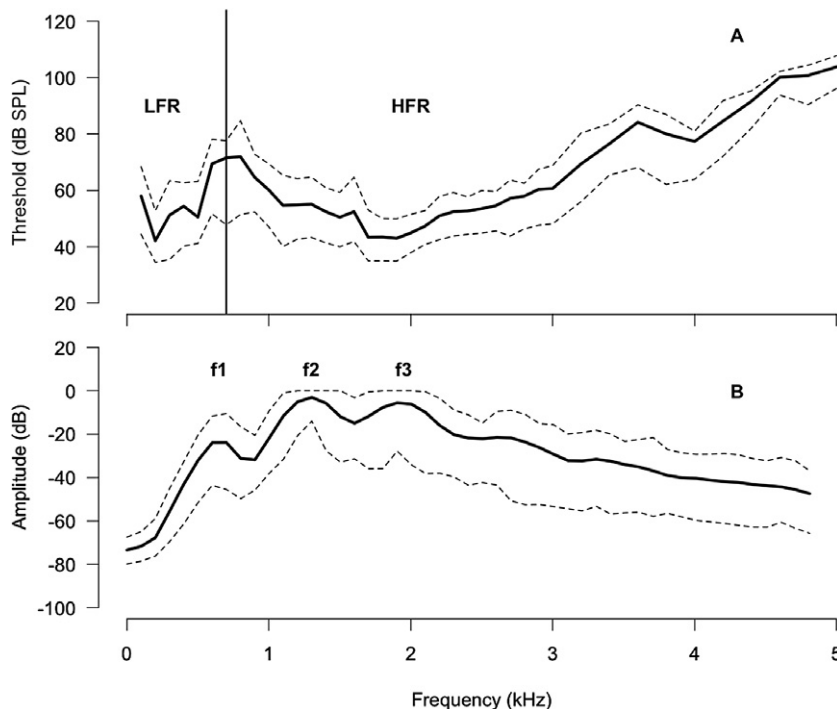

Figure 2. Mean audiogram of females and mean power spectrum of advertisement vocalization produced by males of *Eupsophus roseus* frogs. A, multi-unit audiogram of females ($N = 12$). The bold line corresponds to the average dB sound pressure level (SPL) for each frequency tested, and the dashed lines indicate the confidence interval at 95%. The vertical line indicates the separation between the low (LFR) and high (HFR) frequency regions of enhanced sensitivity. B, mean power spectrum of advertisement vocalizations produced by males ($N = 50$). The bold line corresponds to the mean spectrum for all males and the dashed lines indicate the confidence interval at 95%. The first three harmonics are depicted (f1, f2, and f3). Frequency resolution: 100 Hz.

of 200 Hz (range: 100–200 Hz) and 400 Hz (range: 300–500 Hz), respectively. In the HFR the low and high limits had a median of 1700 Hz (range: 1100–1700 Hz) and 2100 Hz (range: 1700–2700 Hz), respectively.

MALE ADVERTISEMENT VOCALIZATIONS

The advertisement vocalizations had a duration of 163 ± 17 ms. When considering the mean spectrum of the entire vocalization, the DF presented a bimodal distribution with a median of 1482 Hz and a range of 1131–1976 Hz, indicating that it corresponds either to the second (f2) or third (f3) harmonics (Fig. 2B). A detailed analysis of the harmonic composition performed at the initial, mid and final third segments of the vocalizations indicates the existence of frequency modulations in an ascending–descending mode, with the highest frequency occurring in the mid third segment of the vocalizations. The harmonic amplitude ratio indicates that f2 was well above f1, but similar to the amplitude of f3 during the three segments (Table 1).

ACOUSTIC ENVIRONMENT

The spectral characteristics of the acoustic environment indicated that *E. roseus* was the most conspicuous species vocalizing at night-time throughout the months analysed. During September the DF was mostly related to abiotic noise. In October the DF corresponded to f2 of *E. roseus* vocalizations. During November another sound corresponding to the calls of *E. vertebralis*, a species related to *E. roseus* (Penna & Veloso, 1990), contributed to the acoustic environment, lowering the DF to values between the abiotic noise and the advertisement vocalizations of *E. roseus*. Finally, in December the spectral characteristics are similar to those in October, with *E. roseus* being the most important sound source (Fig. 3; Table 2). The spectra of the 200-ms background noise samples indicate that during September, October, and December the DF was associated with abiotic background noise; however, in November the DF was higher as a result of *E. vertebralis* vocalizations (Table 2).

COMPARISON BETWEEN AUDITORY SENSITIVITY AND SOUND SPECTRA

The comparison between *E. roseus* female audiograms and the spectral characteristics of advertisement vocalizations was only performed for the HFR because the LFR was out of the frequency range of vocalizations. The BT of the HFR of *E. roseus* females' audiograms matches the frequency modulation range of f3 in males' advertisement vocalizations. The HFR bandwidth encompasses mainly the frequency range of f3 (see Female auditory sensitivity; Fig. 4A; Table 1). The bootstrapped cross-correlation analysis between the audiograms and the conspecific vocalization spectra yielded a cross-correlation coefficient with a median of 0.4 (95% CI: 0.3–0.5) at zero lag (Table 3).

The comparison between the audiograms of *E. roseus* females and the 200-ms background noise samples indicates that females were less sensitive to the abiotic and biotic sound sources as compared with conspecific vocalizations. The BT of the LFR was located at frequencies higher than abiotic noise. In November, when *E. vertebralis* was vocally active, the DF was in between the BTs of the LFR and HFR. The cross-correlation coefficients calculated for noise are lower than those obtained for *E. roseus* vocalizations, reaching negative values in November when *E. vertebralis* contributes to the acoustic environment (Fig. 4B; Table 3).

DISCUSSION

The analysis of *E. roseus* sound production and reception shows a general concordance between female auditory sensitivity and the acoustic signals produced by males. The multi-unit audiograms indicated the existence of two regions of enhanced sensitivity, i.e. LFR and HFR, the limit between which is around

Table 1. Spectral characteristics of the advertisement vocalizations of *Eupsophus roseus* males (*N* = 50) obtained at the initial, middle, and final third segments of calls

Vocalization segment	f1 (kHz)	f2 (kHz)	f3 (kHz)	f2/f1	f2/f3
Initial	0.6 (0.5–0.8)	1.3 (1.0–1.6)	1.9 (1.5–2.3)	25.2 (2.3–334.6)	1.9 (0.3–11.5)
Middle	0.7 (0.6–0.8)	1.3 (1.1–1.5)	2.0 (1.7–2.3)	26.3 (1.7–254.3)	1.1 (0.2–8.1)
Final	0.6 (0.5–0.8)	1.2 (1.0–1.5)	1.7 (1.4–2.3)	14.3 (1.3–104.4)	1.1 (0.1–54.1)

f1, fundamental frequency; f2, second harmonic; f3, third harmonic; f2/f1, amplitude ratio between f2 and f1; f2/f3, amplitude ratio between f2 and f3. Frequencies of harmonics are in kHz. Amplitude ratios are in linear scale. Because not all data were normally distributed, medians and ranges (in parenthesis) are given.
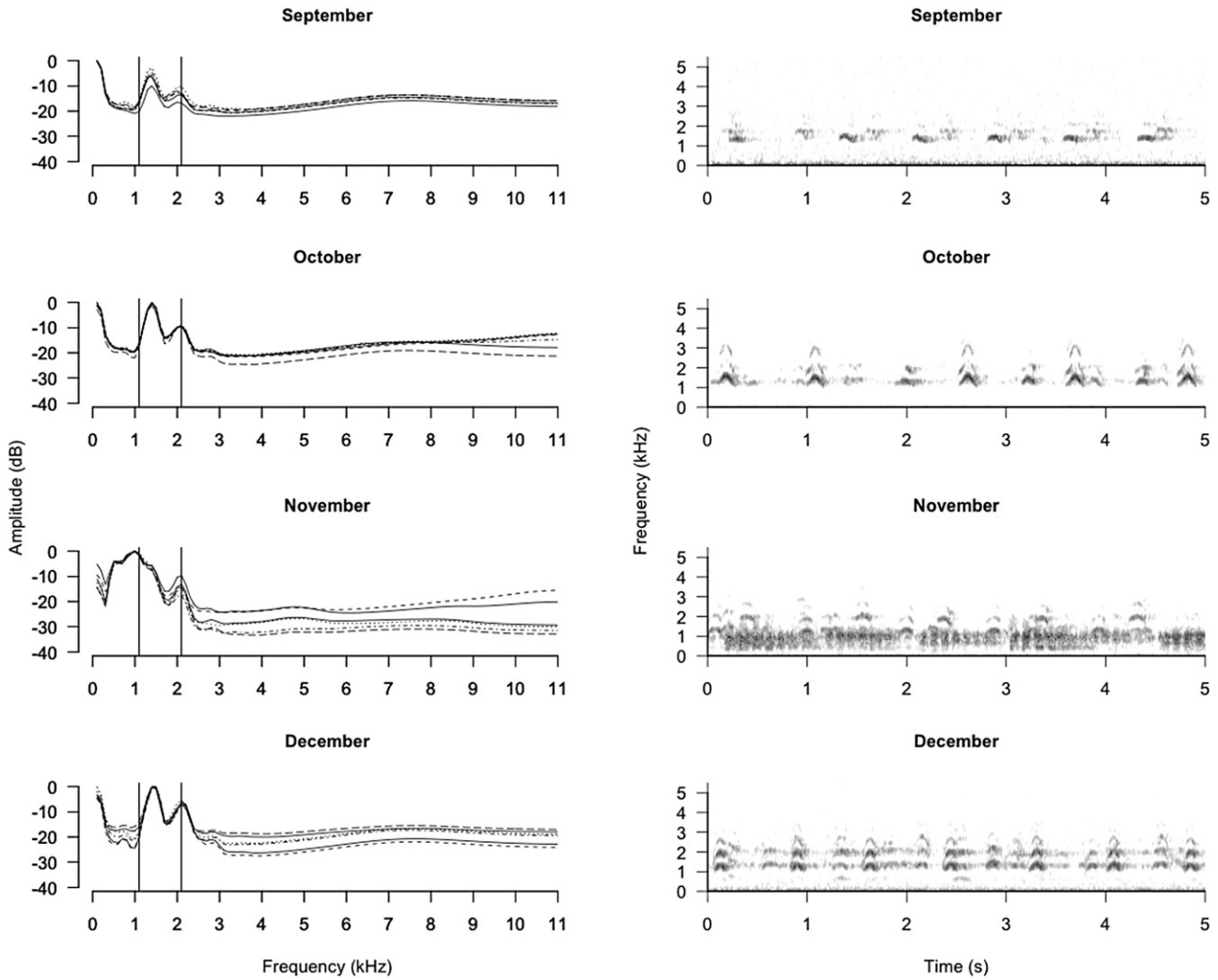
Figure 3. Ambient noise variations within the reproductive season of *Eupsophus roseus*. Left: power spectrum for each month. Each curve indicates the mean amplitude for recordings performed at the same hour (recording duration: 10 min). Note that amplitude values are in relative scale, and that for November it does not necessarily indicate that *E. roseus* is less abundant. During November, *Eupsophus vertebralis* vocalizations contribute to the acoustic environment. Vertical bars indicate the range of the dominant frequency of *E. roseus* advertisement vocalizations. Right: spectrograms of recordings (5-s extract) performed 2 hours after sunset.

Table 2. Dominant frequencies of the overall acoustic environment (10 min) and of background noise 200-ms samples during the reproductive season of *Eupsophus roseus*

Sample type	September	October	November	December
Acoustic environment	< 0.1 (< 0.1–1.3)	1.3 (< 0.1–1.4)	0.9 (0.4–1.3)	1.3 (< 0.1–1.4)
Background noise	< 0.1 (< 0.1–0.1)	< 0.1 (< 0.1–0.1)	0.9 (0.4–1.1)	< 0.1 (< 0.1–0.1)

Recordings were performed in September ($N = 42$), October ($N = 42$), November ($N = 42$), and December ($N = 28$). Values are in kHz, and correspond to medians and ranges (in parentheses). Values expressed as < 0.1 indicate that the dominant frequency was between 1 and < 100 Hz, i.e. in the lowest frequency bin (see text).
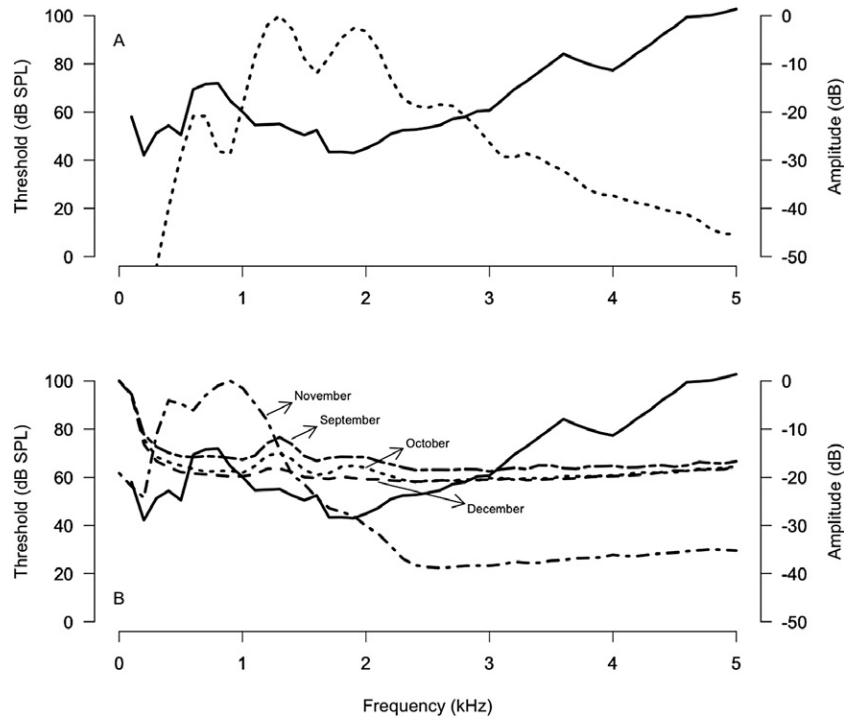
Figure 4. A, mean audiogram of *Eupsophus roseus* females (continuous line; *y*-axis on left) and mean power spectrum of advertisement vocalizations of *E. roseus* males (dotted line; *y*-axis on right). B, mean audiogram of *E. roseus* females (continuous line; *y*-axis on left) and mean power spectra of 200-ms background noise samples (September, dashed line; October, dotted line; November, dot-dashed line; December, two-dashed line; *y*-axis on right side). During September, October, and December, background noise was mainly abiotic. November was the month in which *Eupsophus vertebralis* generates biotic background noise.

Table 3. Cross-correlation coefficients between *Eupsophus roseus* female auditory regions of enhanced sensitivity and the spectra of conspecific male advertisement vocalizations ($N = 50$) recorded throughout the breeding period, and 200-ms samples of background noise devoid of conspecific vocalizations recorded over 4 months: September ($N = 42$), October ($N = 42$), November ($N = 42$), and December ($N = 28$)

Female sensitivity region	*E. roseus* calls	September 200 ms	October 200 ms	November 200 ms	December 200 ms
LFR	–	0.05 (−0.40 to 0.32)	0.08 (−0.38 to 0.34)	−0.42 (−0.75 to −0.06)	0.05 (−0.41 to 0.32)
HFR	0.40 (0.31–0.50)	0.02 (−0.22 to 0.18)	0.21 (0.06 to 0.33)	−0.15 (−0.36 to 0.04)	0.20 (0.07 to 0.32)

LFR and HFR: low and high frequency range, respectively. Values indicate median and confidence intervals at 95% (in parenthesis). The cross-correlation between LFR and *E. roseus* calls was not calculated because the frequency range of calls is above this region of enhanced sensitivity.

700 Hz. These two regions presumably correspond to the input of the amphibian papilla and the basilar papilla, the auditory organs of the inner ear, tuned to low and high frequencies, respectively (e.g. Purgue & Narins, 2000; Schoffelen, Segenhout & van Dijk, 2008). In contrast with other anuran species, such as *Physalaemus pustulosus* (Cope, 1864), in which two different spectral components of advertisement vocalization match the low- and high-frequency range of

enhanced auditory sensitivity (Wilczynski *et al.*, 2001), the most important frequencies of advertisement vocalizations of *E. roseus* are restricted to the HFR of enhanced sensitivity of females. This restricted type of spectral correspondence has been observed in other anurans, such as *Alytes cisternasii* (Boscá, 1879), *Pleurodema thaul* (Lesson, 1826), and *Bufo* spp., and would imply that the low range of enhanced sensitivity of females is not associated with

sexual communication (Walkowiak, Capranica & Schneider, 1981; Bosch & Wilczynski, 2003; Penna *et al.*, 2008).

Our results indicate a general concordance between the HFR of enhanced female auditory sensitivity and the spectral energy distribution of advertisement vocalizations produced by males. The tuning of the BT and bandwidth limits of the HFR spreads over the f3 frequency range of the calls, and in some females it extends into the high f2 range. The relatively low cross-correlation value between the female audiograms and power spectra of male vocalizations is related to the bimodal structure of the sounds. The existence of these two harmonics, either of which can concentrate the highest energy content (i.e. the DF) and present frequency modulations, is congruent with the wide auditory sensitivity observed in the HFR of the females' audiograms, encompassing a full range from 1100 to 2700 Hz, as measured by the bandwidth limits of the HFR. Such an extended range could provide an appropriate sensitivity for the overall spectral domain of these signals. A relationship between widths of the auditory sensitivity with vocalizations containing frequency modulations has been proposed for species of the neotropical frog genus *Physalaemus* (Wilczynski *et al.*, 2001). Studies in *Physalaemus* and *Hyla* indicated that signals stimulating the amphibian papilla and the basilar papilla could be more attractive to females, as predicted by the sensory exploitation hypothesis (e.g. Ryan & Rand, 1990; Gerhardt, 2005). Additional studies exploring female preferences for synthetic stimuli containing frequencies in the ranges of both auditory organs would be necessary to test this possibility in *E. roseus* (e.g. Gerhardt *et al.*, 2007).

When sensitivity and signal features are precisely matched, it is expected that the females' preferences exert stabilizing selection on the signal, whereas a mismatch would suggest the occurrence of directional selection (Ryan & Keddy-Hector, 1992; Gerhardt & Schwartz, 2001). Because the BT of the HRF of females matches f3, it is expected that intersexual selection would promote this harmonic to become the DF of advertisement vocalizations. Nevertheless, the existence of two harmonics with nearly equal energy content (i.e. f2 and f3) could be explained by restrictions derived from the morphology of the larynx and other structures involved in sound production (Gerhardt & Huber, 2002; Walkowiak, 2006), the spectral complexity of the signals associated with individual variation (Márquez *et al.*, 2005), and the degradation and attenuation of signals during transmission (Wiley & Richards, 1982).

Regarding signal transmission, *E. roseus* males produce advertisement calls from inside burrows where amplexus takes place. Inside burrows of *E. roseus* and the closely related *E. calcaratus* the amplitude of f2 relative to f3 of incoming vocalizations is further enhanced, and this relationship can vary according to fluctuations in the water level inside burrows (Penna, 2004; Penna & Márquez, 2007). It is possible that this resonance also affects signals broadcast from inside burrows, which could add variation to signal structure, by enhancing the amplitude of f2 relative to f3. Furthermore, the signals can be modified during transmission from emitters to receivers, as a general trend in acoustic signals is for higher frequencies to attenuate more than lower frequencies as a function of the propagation distance (Wiley & Richards, 1982), a tendency that also occurs in the calls of anurans of the temperate forest (Penna & Solís, 1998). The effects of an enhanced female sensitivity for f3 and the degradation patterns preserving the amplitude of f2 may counterbalance each other: i.e. there is potential for an interaction between sexual and natural selection, resulting in the extant spectral characteristics of the advertisement call, with two main harmonics of similar amplitudes.

Other factors that can affect the auditory sensitivity and its tuning relative to conspecific signals include the hormone levels associated with reproductive state and environmental temperature (e.g. Hubl & Schneider, 1979; Mohneke & Schneider, 1979; Penna *et al.*, 1992; Narins, 2001; Arch & Narins, 2009). Furthermore, the harmonic structure of advertisement vocalizations could generate additional temporal cues for signal recognition, encoded in rapid amplitude modulation patterns inherent to this spectral configuration (see Fig. 1B), as has been shown to occur for females of other anuran species (Klump *et al.*, 2004). The importance of these factors in the context of the matching between auditory sensitivity and signal frequency remain to be addressed, and should be considered for future studies.

The acoustic environment analysis showed that *E. roseus* vocalizations are the principal biotic component of the nocturnal acoustic environment during September, October, and December; however, *E. vertebralis* also contributes significantly to the sound environment during November. The finding of this co-occurring species was facilitated by the use of automated recording systems that, for the first time, provided samples of the acoustic environment throughout an extensive period of the *E. roseus* breeding season, and contributed a means to estimate how the spectral characteristics of the abiotic and biotic background noise affect the matching between the auditory sensitivity of females and the spectral energy distribution of advertisement vocalizations.

The abiotic low-frequency noise was prominent in the acoustic environment during September, October, and December. The DF of abiotic noise was lower than

the BT of the LFR; however, there was some overlap with the bandwidth limits of this region of enhanced sensitivity. The main harmonics of the advertisement vocalizations of *E. roseus* are well above the range of the abiotic noise recorded, however, and are therefore not subject to this source of masking interference (e.g. Brumm & Slabbekoorn, 2005). A previous study has shown that males of *Eupsophus calcaratus* (Günther, 1881), a species closely related to *E. roseus*, do not alter their vocal activity when exposed to abiotic noises of low frequencies (Penna *et al*., 2005). In addition, the vibration of the tympanic membrane in response to high-frequency tones is not altered with noise exposure (Penna, Gormaz & Narins, 2009).

Our data indicate a spectral overlap between the calls of *E. roseus* and the biotic background noise of November, mainly composed by *E. vertebralis* vocalizations. This overlap comprises primarily the fundamental frequency and also partially the f2 of *E. roseus* calls. The frequencies containing higher energy in the recordings when this biotic noise source was present lie between the LFR and the HFR of enhanced sensitivity of females of *E. roseus*. Nevertheless, there is a partial spectral overlap of f2 with the upper bandwidth limit of the LFR, which in some females also extends into the lower limit of the HFR.

A main finding of this study is the considerable matching between HFR of female audiograms and f3 of vocalizations of conspecific males. In addition, the overlap between the auditory sensitivity of *E. roseus* females and the abiotic and biotic background noise is considerably lower. These results support the prediction that females should not match entirely the conspecific male frequency distribution, but instead diverge from the frequency range of the background noise towards the HFR. The mismatch between the auditory sensitivity of *E. roseus* females with the background abiotic noise and with the biotic noise associated with *E. vertebralis* would enhance the signal-to-noise ratio, helping to separate the communication channels used by these syntopic species, with each occupying a distinct acoustic niche. The influence of the signals of coexisting species on patterns in preferences of behavioural responses and auditory selectivity has been observed in other amphibians (Brzoska *et al*., 1977; Witte *et al*., 2005; Amézquita *et al*., 2006; Klimus, Humfeld & Gerhardt, 2012), as well as in insects (Oberdörster & Grant, 2007; Sueur *et al*., 2010; Schmidt *et al*., 2011), birds (Gall *et al*., 2012), and mammals (Schuchmann & Siemers, 2010).

As mentioned in the introduction, the concordance between conspecific signal design and sensitivity decreases the probability of heterospecific interactions, and their negative consequences, on the reproductive fitness of individuals (Endler, 1993; Ryan & Rand, 1993; Endler & Basolo, 1998; Higgie *et al*.,

2000; Ritchie, 2007; Pfennig & Pfennig, 2009). *Eupsophus* frogs of the Roseus group have 28 chromosomes and species of the Vertebralis group have 30 chromosomes (Formas, 1991, 1992), and phylogenetic data identifies these as sister taxa (Correa *et al*., 2006). Interestingly, it is possible to find species of each of these two groups breeding in sympatric and allopatric conditions (Formas & Vera, 1980; Rabanal & Nuñez, 2009). We hope that these results encourage future work to elucidate whether the acoustic separation between these species could have arisen from an ecological and/or reproductive character displacement process (Pfennig & Pfennig, 2009), and to determine the importance of other factors, such as selection for body size (e.g. Bertelli & Tubaro, 2002; Amézquita *et al*., 2006).

Overall, sexual acoustic communication in *E. roseus* apparently represents a model of matched filter operating under environmental determinants. Nevertheless, further work comparing multiple populations needs to be conducted to determine whether the presence of *E. vertebralis* and other sources of background noise affect the evolution of both the auditory sensitivity of females and the frequency components of advertisement vocalizations. It is possible that the extant matching between both domains is an adaptation of the auditory system confronting a relatively simple acoustic environment, with interference in the lower frequency range from both biotic and abiotic sources, allowing *E. roseus* to communicate sexually by means of acoustic signals amid reduced interference.

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