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Strong responsiveness to noise interference in an anuran from the southern temperate forest

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Abstract Animals adopt different strategies to communicate by means of sound in noisy environments. Some animals increase, while others decrease, their vocal activity in the presence of interference. Anuran amphibians from diverse latitudes exhibit both kinds of responses. Recent studies have shown that males of *Batrachyla taeniata* and *Batrachyla antartandica* from the temperate austral forest do not call in response to the presentation of advertisement calls of sympatric congeneric species, but their responsiveness to other kinds of interference has not been tested. To explore the diversity in responsiveness to acoustic intrusion in a single species, we exposed males of *B. taeniata* to prolonged prerecorded natural abiotic noises of wind, creek, and rain and to a band-pass noise centered at 2,000 Hz, at 67 dB sound pressure level (SPL). The subjects drastically increased their call rate when exposed to all four sounds. Frogs also responded by augmenting their vocal activity to exposures of band-pass noise at increasing intensities (55–79 dB SPL). The increase in vocal activity in response to noise is strong relative to those of other anurans from the temperate forest studied previously under similar exposures. These results reveal a remarkable activation of vocal response to acoustic interference of continuous abiotic noise, which would allow compensating for limitations in the active communication space under background sounds. This strategy contrasts with the decrease in vocal output amid interference from heterospecific signals reported formerly for this frog, a tactic that would restrict energy expenditure to relevant acoustic competition with conspecifics.

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Introduction

Animals communicating by sound have developed different mechanisms to overcome interference. Increases in amplitude, rate, and duration of vocalizations during exposure to noises from various sources, both natural and anthropogenic, occur in various vertebrates (e.g., Pytte et al. 2003; Brumm 2004). In addition, animals communicating in natural and urban environments have been reported to produce vocalizations having spectra detuned from the range of the background noise (e.g., Slabbekoorn and Peet 2003; Feng et al. 2006; Cunnington and Fahrig 2010). Decrements of vocal activity in the presence of noise have also been reported to occur in various instances, in which animals restrict their vocal output to intervals when the interfering noise is attenuated or absent (e.g., Zelick and Narins 1983; Greenfield 1988; Römer et al. 1989; Brumm 2006).

Acoustic environments where animals communicate can be fairly complex, in particular in tropical latitudes, where a diversity of organisms creates intricate sound landscapes (Narins and Zelick 1988; Owings and Morton 1998). However, anurans from temperate environments also confront high levels of noise interference: they typically breed in dense aggregations in which high noise levels are built up (e.g., Swanson et al. 2007). Frogs from diverse latitudes have been reported to use different tactics to confront interference from biotic, abiotic, and anthropogenic origins. Some species increase, while other decrease, their vocal output when exposed to noises of different kinds (Penna et al. 2005; Sun and Narins 2005; Penna and Hamilton-West 2007; Lengagne 2008; Kaiser and Hammers 2009; Kaiser et al. 2011; Love and Bee 2010; Cunnington and Fahrig 2010). In particular, in the temperate austral forest, we found that two frogs of the genus *Eupsophus*, inhabiting the same microenvironment, respond differently to noise exposures of moderate to high levels, under similar test conditions. The vocal activity of *Eupsophus calcaratus* is enhanced (Penna et al. 2005), whereas *Eupsophus emiliopugini* does not alter or decreases its vocal responses during exposure to the same battery of noises (Penna and Hamilton-West 2007). The increase in vocal

output during noise exposures would contribute to maintaining the active distance over which animals communicate (e.g., Parris et al. 2009). On the other hand, a reduction in vocal activity would imply savings of energy expenditure for time intervals having lower background levels (e.g., Brumm 2006).

Batrachyla taeniata is a frog from the temperate austral forest, where it breeds from the end of the summer through the beginning of autumn. Its advertisement call is composed of short, 5-ms pulses repeated at a rate of 40–80 pulses/s, having a total duration of about 500 ms and a dominant frequency of about 2,000 Hz (Penna and Veloso 1990). Playback studies have shown that this frog gives vocal responses preferentially to temporal patterns approximating the typical parameters of the conspecific advertisement call (Penna 1997; Solís and Penna 1997).

In a recent study on interference of calls of the sympatric congeneric species on the vocal activity of *B. taeniata*, we found that the signals of *Batrachyla antartandica* and *Batrachyla leptopus* did not evoke vocal responses at intensities at which males of the focal species typically interact with conspecific neighbors (Penna and Velásquez 2011). Males of *B. antartandica* react similarly to interference of biotic origin, decreasing their vocal output when presented with synthetic calls of the other two species (Penna and Meier 2011).

In the current study, we evaluate the effects of continuous noises of abiotic origin and a band-pass noise centered at the typical frequency of the calls of *Batrachyla* on the vocal responses of *B. taeniata*, allowing comparisons with the responses to stereotyped biotic signals studied previously in this species (Penna and Velásquez 2011) and with responses of other frogs from the temperate forest to continuous abiotic noise (Penna et al. 2005; Penna and Hamilton-West 2007). By extending the exploration of the responses of a single species to a broad range of interferences found in nature, we sought to contribute an assessment of the adaptive values of different strategies for confronting acoustic intrusion. Recently, the relevance of biotic components of sound environments for the evolution of anuran sound communication systems has been highlighted (Amézquita et al. 2011), but the importance of abiotic sound sources has been subjected to limited explorations, which we expand in the present study.

Methods

Study site

The experiments were conducted on the southwestern shore of Tinquilco Lake (39°07′ S, 71°46′ W) located near Huerquehue National Park in southern Chile, from March 4 to 17, 2009 and from March 10 to 16, 2011. Males of *B. taeniata* breed in this locality in a forest where *Drimys winteri* and *Nothofagus*

dombeyi were the predominant trees. Frogs called from the ground, hidden underneath fallen branches and leaves, and among herbs (*Prunella vulgaris*) and grasses (*Bromus* sp.).

Synthetic stimuli and noises

A synthetic call emulating the advertisement call of *B. taeniata*, as characterized in previous studies (Penna 1997; Solís and Penna 1997), was made using the Soundmaker 1.0.4. software (Ovolab, Torino, Italy). The stimulus consisted of 25 short pulses repeated at a rate of 50 pulses/s, having a total duration of 500 ms. Pulse duration was 5 ms, having 1- and 4-ms linear rise and fall times, respectively, and a carrier frequency of 2,000 Hz. The first pulse had an amplitude of 25 %, the second and last pulses an amplitude of 50 %, and the third and penultimate pulses an amplitude of 75 % relative to the pulses in the midportion of the call.

Natural prerecorded and edited noises of wind, rain, and creek used in this study were the same recorded and used in previous field playback studies. The recording and editing procedures are described by Penna et al. (2005). Briefly, recordings of these sounds were carried out at localities in the region where the study was conducted. Recordings were done in the absence of other interfering sounds with the microphone of a sound level meter (Brüel & Kjaer 2230; Brüel & Kjaer Instruments, Boston, MA, USA), the output of which was connected to a digital tape recorder (Sony TC D10 PROII; Sony Electronics Inc., San José, CA, USA). Segments of 10-s duration judged to have spectra representative of each noise type were selected and pasted to create noise durations of 150 s. These recorded noises were digitized using the same instrumentation and settings as for the recordings of vocal responses of the experimental subjects in this study (see the "Acoustic analysis of evoked vocal responses" subsection). In addition to the natural recorded sounds, a band-pass noise was synthesized from a white noise filtered between 1,000 and 3,000 Hz and centered at 2,000 Hz. This spectrum was chosen to encompass the dominant frequency range of the advertisement call of *B. taeniata*. This noise was generated using a wave generator (WG1; Tucker-Davis Technologies, Alachua, FL, USA) and a programmable filter (PF1; Tucker-Davis Technologies, Alachua, FL, USA). The total duration of each noise was 150 s. Oscillograms and power spectra of the synthetic call and of the noises used are shown in Fig. 1.

Experimental protocols

Synthetic stimuli and noises were recorded on separate channels of an audio player (iPod nano; Apple Computer Inc., Cupertino, CA, USA), in successive tracks. Track 1 lasted 120 s, the left channel containing no stimuli. On the right channel, the first 30 s contained 20 repetitions of the synthetic advertisement call with a repetition period of 1.5 s, followed

Fig. 1 Oscillograms and power spectra of the synthetic advertisement call of *B. taeniata*, natural noises, and a band-pass noise used to create the sound tracks presented to the experimental subjects. Power spectra of the synthetic call are averaged over 500 ms and noise spectra are averaged over 10 s (bandwidth, 20 Hz)
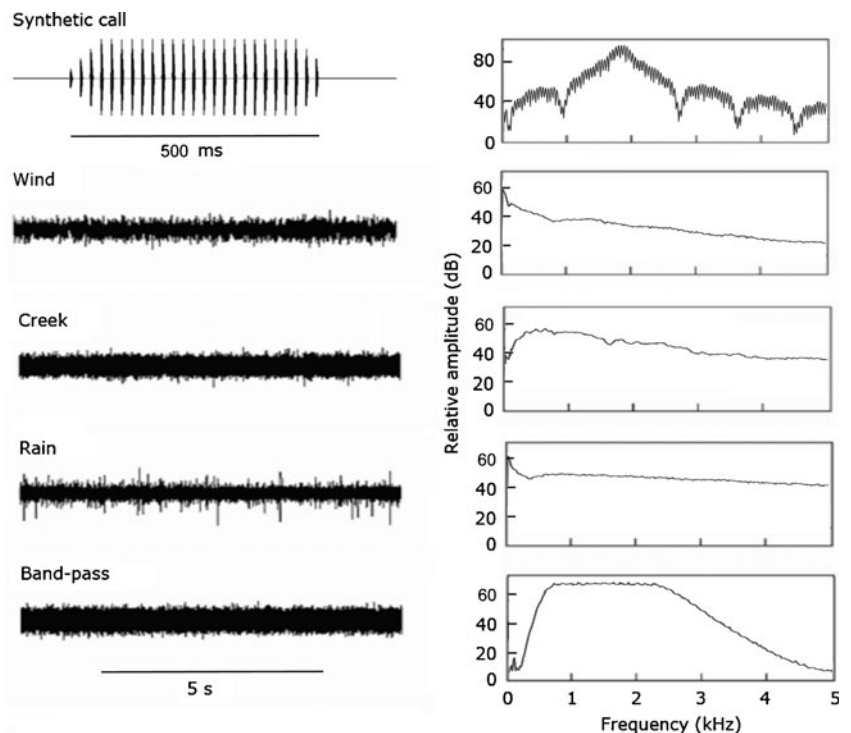
by 90 s of silence, as shown in the schematic diagram in Fig. 2. Four subsequent tracks, each of which lasted 240 s, shared a similar structure, as schematized for track 2 in Fig. 2. In this track, the left channel started with 150 s of noise followed by 90 s of silence. On the right channel, a bout of 20 synthetic calls lasting 30 s started 60 s after the noise onset on the left channel and was followed by 150 s of silence.

In 2009, two experiments were conducted: a "noise–structure experiment," in which four tracks containing the noises of different structures were played in two different orders; for sequence A, the order was wind, creek, rain, and band-pass noise, and for sequence B, the reverse order was used. Each sequence was broadcast to half of the experimental subjects. These two different orders were used as a control for the influence of the order of presentation of noises of different structures on the evoked vocal responses (EVRs). After the presentation of the fourth noise track (or fifth track of the sequence), a sixth track identical to the first track, containing a bout of 20 synthetic calls followed by 90 s of silence on the right channel and comprising 120 s of silence on the left channel, was presented to control for changes in vocal activity throughout the experiment. Upon completing this first experiment, the basal vocal activity was recorded in absence of any stimulation for 2 min. Following this recording period, the second, "noise–level experiment" proceeded, starting with a track identical to the first track of the first experiment, and subsequently, a band-pass noise track identical to the one used in the first experiment was presented five times at increasing amplitudes (see the "Instrumentation and experimental setting" subsection). The order of increasing noise levels was chosen to

minimize the effect of presentations of loud noise on vocal activity during subsequent exposures.

Because the two sequences of noises of different structures used in the noise–structure experiment during 2009 yielded different results between the responses to some of the noises employed (see the "Results" section), this experiment was replicated during 2011 using a random presentation order of these sounds.

Instrumentation and experimental setting

Stimuli and noises were played back with an iPod nano audio player connected to a two-channel impedance-matched operational amplifier and two attenuator sets (Hewlett-Packard 355 C and D for synthetic calls and Hewlett-Packard 350 D for noises; Hewlett-Packard, Loveland, CO, USA). The synthetic call and noises were mixed up with an electronic adder and fed into a power amplifier (Alpine 3540; Alpine Electronics of America, Torrance, CA, USA) and a two-way loudspeaker (Dynaudio BM6; frequency response, 38–20,000 Hz; Dynaudio Acoustics, Risskov, Denmark). The loudspeaker was positioned at an average distance of 0.73 m (range, 0.56–1.00 m) in front of each experimental subject. EVRs were recorded with a directional microphone (Sennheiser ME 66; Sennheiser Electronic GmbH & Co., KG, Wedemark, Germany) connected to the left channel of a digital recorder (Sony TC D10 PROII; Sony Electronics Inc., San José, CA, USA). During the experiments, the synthetic advertisement calls were recorded on the right channel of the digital recorder from the iPod audio player through a
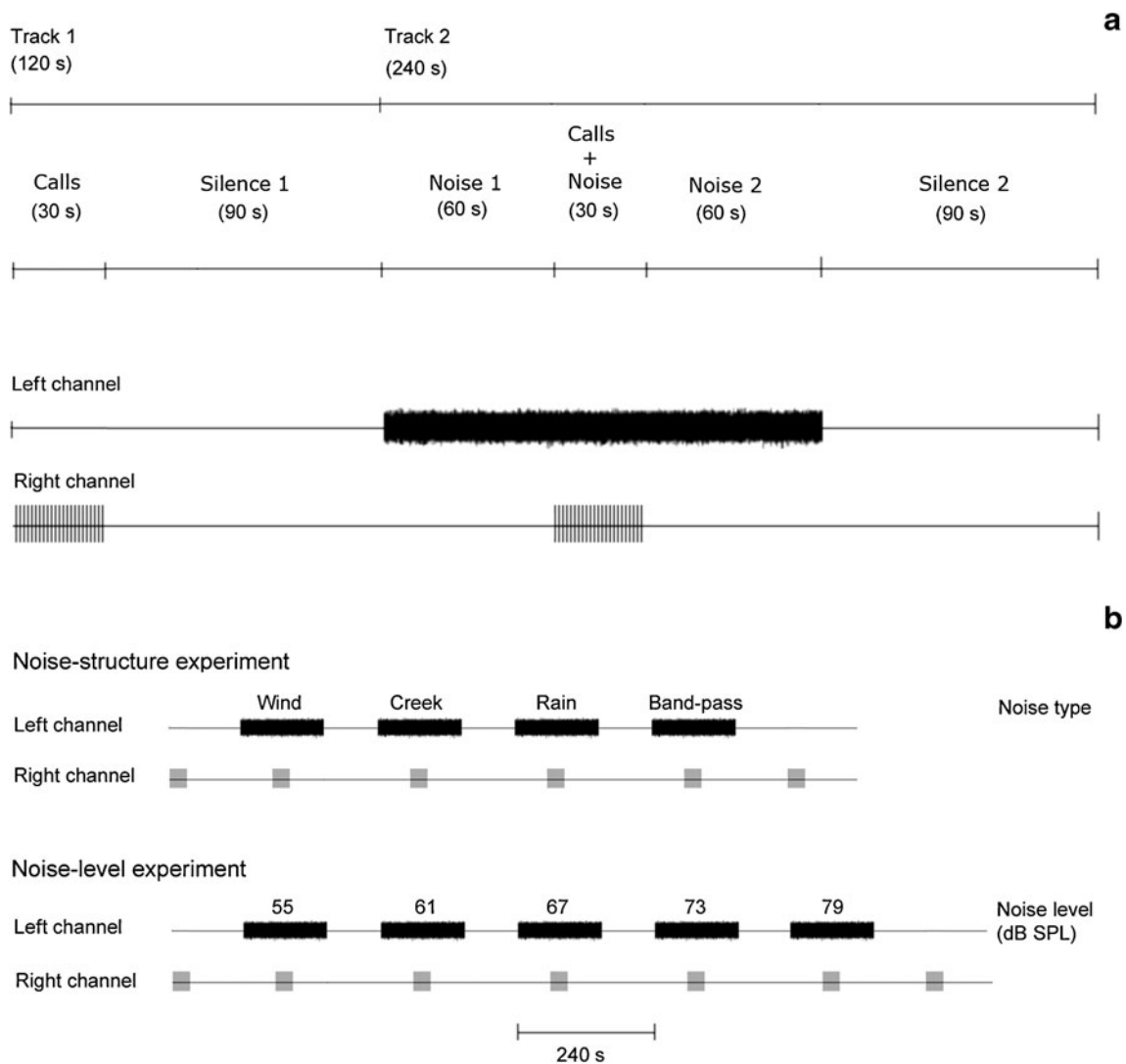
Fig. 2 **a** Schematic diagram of the time intervals for which measures of EVRs were compared during exposures to different kinds of noise and to band-pass noise at different levels. Track 1 contains silence during the 120 s on the left channel and a bout of 20 calls lasting 30 s followed by 90 s of silence on the right channel. Track 2 contains noise during 150 s followed by 90 s of silence on the left channel and 60 s of silence followed by a bout of 20 calls lasting 30 s and 150 s of silence. Silence 1, noise 1, calls+noise, noise 2, and silence 2 are the five intervals for which vocal activity in terms of call rate, call duration, and call amplitude were compared in the exposures to noise. **b** Schematic diagrams of the entire sequences of presentation of noises and synthetic calls in the noise–structure experiment and in the noise–level experiment. The order of noises in the noise–structure experiment corresponds to one of the sequences used during 2009 (see text)

connection cable to have a temporal relationship between the stimuli and the EVRs. During playbacks, special care was taken to suppress the vocal activity of neighboring frogs by gently tapping the substrate near their positions so that the EVR of the focal frog was recorded without this interference.

The synthetic calls were presented at 70 dB sound pressure level root–mean–square (SPL RMS, fast weighting scale, linear frequency weighting) at the position of the experimental subject in the first and second experiments. The noises were presented at 67 dB SPL RMS in the noise–structure experiment and at five different levels in the noise–level experiment. The value of the synthetic calls was close to the average level of the calls of the nearest neighbors in natural aggregations of

this species (MP, unpublished data), and the noise level was well above the background noise, which at the study site was typically around 40 dB SPL (see the "Results" section). Also, the 3-dB signal-to-noise ratio has been appropriate to evoke consistent vocal responses to synthetic calls in previous studies with other anurans in the temperate austral forest (Penna et al. 2005; Penna and Hamilton-West 2007). Before starting an experimental session, the SPLs of the synthetic calls and noises were measured by placing the tip of the microphone of a sound level meter (Brüel & Kjaer 2230; Brüel & Kjaer Instruments, Boston, MA, USA) as close as possible to the head of the experimental subject, without causing disturbance. These measurements allowed adjusting the attenuator settings

in order to maintain a constant SPL during playback of the synthetic call and noises for each individual. The range of SPLs used for the noise–level experiment for exposures to band-pass noise at five increasing amplitudes in 6-dB steps (55, 61, 67, 73, and 79 dB SPL) encompassed levels effective in altering frog vocal activity in previous studies (Penna et al. 2005; Penna and Hamilton-West 2007).

Experimental sessions were conducted at night, between 2100 and 0500 hours, comprising the period during which natural choruses build up and fade away. The total duration of the noise–structure experiment and noise–level experiment were 20 and 24 min, respectively. Basal vocal activity in the absence of any stimulation was recorded for 2 min before and after the noise–structure experiment and noise–level experiment were completed. At the end of the experimental session, the environmental noise level was measured by placing the microphone of the sound level meter at the position of subject. Air and substrate temperature were measured with a thermometer (Digi-Sense 8528-20) and the relative humidity with a hygrometer (Bacharach Sling Psychrometer). Whenever possible, experimental subjects were captured, and their snout–vent length and body weight measured.

Acoustic analysis of evoked vocal responses

Recordings were digitized with a Macintosh computer (Macintosh G4), using the Peak 2.52 software (Bias, Inc., Petaluma, CA, USA) at a 44.1-kHz sampling rate, an antialiasing filter (FT6-2; Tucker-Davis Technologies, Alachua, FL, USA), and an analogue digital interface (Motu 828). Recordings were analyzed using the Raven Pro 1.3 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA), and three measures of the frogs' EVRs were quantified: call rate, call duration, and call amplitude.

Because EVRs recorded during the presentation of noises were embedded in the broadcast noise, we corrected the amplitude values of these vocalizations with the following procedure: In the recordings, we measured the RMS amplitude of an interval containing the noise broadcast through the loudspeaker but free of evoked calls and added it to the RMS value of a call recorded at a preceding interval free of broadcast noise to calculate the predicted amplitude resulting from adding the two sounds (Bradbury and Vehrencamp 1998, pp. 34–35). Then, we corrected the RMS amplitude measured for the recorded calls embedded in noise by subtracting the predicted amplitude increment calculated. We checked the reliability of this procedure by replacing at the study site a calling frog with a small active loudspeaker (Altec Lansing, iM 207 Orbit; Altec Lansing Technologies, Inc., Milford, PA, USA) which broadcast the synthetic call. The broadcasting and recording instrumentation was set in the same way as for the experiments. The microphone used to record the EVRs during the playback experiments was positioned 15 cm in front of the

small loudspeaker and connected to the tape recorder (Sony TC D10 PROII; Sony Electronics Inc., San José, CA, USA). The BM6 loudspeaker used to broadcast noises and synthetic calls during the experiments was placed behind the directional microphone, at 60 cm in front of the small loudspeaker. The band-pass noise was broadcast with the BM6 loudspeaker at increasing intensities in 6-dB steps, from 49 to 85 dB SPL RMS. The SPL of the synthetic call at the position of the tip of the Sennheiser ME 66 microphone (Sennheiser Electronic GmbH & Co., KG, Wedemark, Germany) was set at 80 dB SPL RMS as measured by also placing at this position the tip of the microphone of a sound level meter (Brüel & Kjaer 2230; Brüel & Kjaer Instruments, Boston, MA, USA), and the SPLs of the broadcast noise were also measured with this instrument. This procedure allowed us to measure the increments in RMS amplitude of a constant-level call in the presence of noise at predetermined amplitudes. The corrections applied to the calls embedded in noise yielded values within −0.5 to +2 % of the amplitude of the call in the absence of broadcast noise.

Statistical analysis

The effects of exposure to noises of different structures broadcast at the same SPL (noise–structure experiment) were analyzed with Friedman nonparametric analyses of variance (ANOVAs) for repeated measures (Statistica 6.0 software; Statsoft, Inc., Tulsa, OK, USA; significance level $P < 0.05$) and post hoc multiple comparisons (Siegel and Castellan 1988, pp. 180–181) for the three EVR measures. The five time intervals within a noise exposure were considered as treatments: the no-playback interval preceding the noise presentation (90 s), the noise exposure before synthetic call onset (60 s), the noise exposure during the presentation of the bout of synthetic call (30 s), the noise exposure after the presentation of the synthetic call (60 s), and the no-playback interval after noise exposure (90 s). We refer to these five time intervals as S1 ("silence 1"), N1 ("noise 1"), C ("call and noise"), N2 ("noise 2"), and S2 ("silence 2"), respectively. A no-playback interval between two noise exposures was considered as S2 for the preceding exposure and as S1 for the subsequent exposure.

To compare the EVRs to the two sequences of exposures to noise of different structures (A and B) employed for the noise–structure experiment during 2009, call rate, call duration, and call amplitude were compared between corresponding intervals (S1, N1, C, N2, and S2) with Mann–Whitney U tests (significance level $P < 0.05$).

To assess the effects of band-pass noise broadcast at increasing levels presented during 2009 (noise–level experiment), nonparametric Friedman ANOVAs and multiple comparisons tests were performed as for the noise–structure experiment.

To explore the effects of prolonged noise exposure on EVRs during the entire experimental sequence, we used Wilcoxon matched-pairs signed-ranks tests ($P < 0.05$) to compare the subject's call rate, call duration, and call amplitude in response to the bout of synthetic calls presented in the absence of noise at the beginning and end of the noise–structure experiment conducted in 2011. We also compared EVRs to synthetic calls at the beginning and end of the noise–level experiment conducted in 2011. Similarly, basal vocal activity before and after the noise–structure experiment conducted in 2011 and before and after the noise–level experiment conducted in 2009 were compared with Wilcoxon matched-pairs signed-ranks tests.

Finally, the EVRs to the corresponding intervals N1, C, and N2 in the noise–structure experiment conducted in 2011 were compared with Friedman ANOVAs and post hoc multiple comparisons (Siegel and Castellan 1988, pp. 180–181).

For all the analyses performed, the absence of calls during a given time interval was quantified as a call rate of 0; however, for call duration and call amplitude, these were considered as missing data. To compare graphically the EVRs of different subjects during the five time intervals, the three EVR measures were normalized to the maximum response for each individual. Namely, the values of call rate, call duration, and call amplitude for a given subject during a particular interval were divided by the maximum value of that measure produced by the frog across all intervals

Results

Environmental conditions and basal vocal activity

At the study site, substrate temperature was the only environmental factor that differed significantly between the 16 males recorded in 2009 and the 14 males recorded in 2011 (Student's t test, $t = 3.0$, $P = 0.006$). Averages and standard deviations for this variable for the 2 years were 12.2±2.3 and 9.3±2.7 °C, respectively. The other environmental variables were similar between the 2 years, and the averages and standard deviations for the pooled data are as follows: air temperature, 9.7±3.2 °C; air relative humidity, 91.0±7.5 %; background noise at the position of the experimental subjects, 40.2±8.0 dB SPL RMS; and the distance between the experimental subjects and their nearest neighbors, 2.0±1.38 m. The biometric characteristics for 23 males captured were as follows: size, 32.1±2.75 mm and weight, 2.6±0.47 g.

Vocal activity differed between both years in terms of call rate during the initial 2 min of recording of basal vocal activity (Student's t test, $t = 2.4$, $P = 0.022$) and in response to the initial bout of 20 repetitions of the synthetic call in the absence of broadcast noise (Student's t test, $t = 2.7$, $P = 0.013$). For the 2 years, the averages and standard deviations for basal call rate

were 11.7±9.0 and 4.9±3.7 calls/min, respectively. Averages and standard deviations for call rate in response to the initial bout of stimuli were 26.5±6.2 and 19.5±7.5 calls/min, respectively. No differences in call duration and dominant frequency occurred between the 2 years (Student's t test, $t < -1.7$, $P > 0.100$ for all comparisons), and therefore, averages and standard deviations for pooled data were calculated. Call duration during the initial basal activity was 467±157 ms, call duration in response to the initial bout of stimuli was 625±166 ms, and dominant frequency measured during the initial basal activity was 2,031±174 Hz.

Noise–structure experiment, 2009: a comparison between two presentation sequences

Some of the comparisons between sequences A and B presented in 2009 during the noise–structure experiment yielded significant differences, in the three EVR measures for some of the intervals considered in the analysis, as shown in Table 1. Because of these differences, and to comply with formal requirements, this noise–structure experiment was replicated in 2011, but using a randomized order of presentation of the four noises, and these results are presented in the following section. However, results from the noise–structure experiment conducted during 2009, analyzed pooling data from both orders of presentation, yielded results entirely similar to those performed in 2011. Data analyses from the later year are informed in the next subsection.

Noise–structure experiment, 2011: evoked vocal responses in randomized sequences

The analysis of the noise–structure experiment, comprising exposures to noises of different structures conducted during 2011, showed that frogs vocalized persistently during broadcast of the four noises. Call rate differed significantly among the five intervals analyzed (S1, N1, C, N2, and S2) during exposures to wind, creek, rain, and band-pass noises. To give an idea of the magnitude of noise-induced changes, call rate during band-pass noise presentation was, on average, 2.2, 20.9, 25.9, 21.6, and 1.0 calls/min for the five intervals, respectively.

Post hoc multiple comparisons showed that, for all four noises, call rate was significantly higher during N1 and C than during S1 and higher during C than during S2. In addition, call rate was higher during N1 than during S2 and higher during N2 than during S1 and S2 for creek, rain, and band-pass noise and higher during C than during N1 and N2 for wind and rain noise. Call rate was also higher during N2 as compared to N1 for creek noise. No significant differences in call rate occurred between intervals S1 and S2 for exposures to any of the four noises (Table 2 and Fig. 3a).

Table 1 Results of Mann–Whitney U test for EVR measures (call rate, duration, and amplitude) between corresponding time intervals during exposures to noises of different structure in order sequences A (wind, creek, rain, and band-pass; $N=8$) and B (reverse order; $N=8$)

EVR measure	Noise	S1	N1	C	N2	S2
Call rate	Wind	0.009	0.006		0.006	
	Creek	0.005				
	Rain					
	Band-pass			0.016		
Call duration	Wind	0.036				
	Creek	0.046				
	Rain					
	Band-pass					
Call amplitude	Wind	0.009	0.021			0.036
	Creek	0.012	0.046			
	Rain					
	Band-pass					

Significant P values (<0.05) indicate values larger for sequence A relative to B in all cases

S1 no-playback interval preceding the noise exposure, *S2* no-playback interval after the noise exposure, *N1* noise exposure preceding the bout of synthetic calls, *N2* noise exposure after the presentation of the bout of synthetic calls, *C* noise exposure during the presentation of the bout of synthetic calls

The analysis of call duration also showed differences among the five intervals analyzed for exposures to wind, creek, rain, and band-pass noise, although these differences reached lower levels of significance relative to call rate. To give an idea of the magnitude of noise-induced changes, call duration during band-pass noise presentation was, on average, 463, 609, 696, 688, and 415 ms for intervals S1, N1, C, N2, and S2, respectively.

Multiple comparisons showed that call duration was longer during C than during N1 for wind noise. In addition, call duration was longer during C than during S2 for creek noise and longer during N2 than during S2 for creek noise (Table 2 and Fig. 3b).

Call amplitude showed significant differences among the five intervals analyzed for exposures to wind, creek, rain, and band-pass noise. To give an idea of the magnitude of noise-induced changes, call amplitude during band-pass noise presentation was, on average, 650, 791, 817, 848, and 482 mV for intervals S1, N1, C, N2, and S2, respectively.

Multiple comparisons showed that call amplitude was larger during C than during S1 for wind noise. Call amplitude was also larger during N2 than during S2 for creek and band-pass noise (Table 2 and Fig. 3c).

The restricted statistical significance of multiple comparisons for call duration and call amplitude was likely due to the cessation of calling by the experimental subjects during intervals in which no noise was broadcast, which yielded missing cases for these two variables.

To compare subjects' responses in the noise–structure experiment during corresponding intervals of exposure, we made comparisons between intervals N1, C, and N2 using Friedman ANOVAs. These intervals were chosen because a high vocal activity occurred during them, thus facilitating comparisons among exposures. Comparisons yielded significant differences in call rate for intervals N1, C, and N2. Multiple comparisons showed that call rate during interval N1 was higher for creek, rain, and band-pass than for wind

Table 2 Results of Friedman ANOVA and multiple comparisons for EVR measures (call rate, duration, and amplitude) between time intervals during exposures to noises of different structure

EVR measure	Noise	χ^2	P value	S1/N1	S1/C	S1/N2	S1/S2	N1/C	N1/N2	N1/S2	C/N2	C/S2	N2/S2
Call rate	Wind	32.9	<0.001	<	<			<			>	>	
	Creek	45.9	<0.001	<	<	<			<	>		>	>	>
	Rain	41.5	<0.001	<	<	<		<		>	>	>	>
	Band-pass	37.5	<0.001	<	<	<				>		>	>
Call duration	Wind	10.2	0.037					<					
	Creek	13.4	0.010									>	>
	Rain	10.9	0.027										
	Band-pass	10.1	0.038										
Call amplitude	Wind	13.0	0.011		<								
	Creek	13.0	0.011										>
	Rain	10.9	0.027										
	Band-pass	10.9	0.027										>

Nonsignificant P values for the Friedman ANOVA (>0.05) are omitted

S1 no-playback interval preceding a noise exposure, *S2* no-playback interval after a noise exposure, *N1* noise exposure preceding synthetic call onset, *N2* noise exposure after the presentation of the bout of synthetic call, *C* noise exposure during the presentation of the bout synthetic calls, < and > relative magnitudes for significant multiple comparisons ($P<0.05$)

Fig. 3 Normalized measures of EVRs: **a** call rate, **b** call duration, and **c** call amplitude of 14 males of *B. taeniata* during the sequence of exposures to noises of different structure. *Numbers* in **a** indicate the number of individuals calling during time intervals. *Empty rectangles on the horizontal axis* indicate the presentation of bouts of synthetic calls, having a duration of 30 s. *Filled rectangles* indicate the presentation of different noises, having a duration of 150 s. Intervals of silence lasting 90 s, preceding and following presentation of noises, are indicated by *ticks between horizontal rectangles* (see the "Methods" section). The three measures of evoked call response were normalized to the maximum response for each individual. Namely, the values of call rate, call duration, and call amplitude for a given subject during a particular interval were divided by the maximum value of that measure produced by the frog across all intervals. *Filled circles* and *vertical bars* represent averages and standard errors, respectively
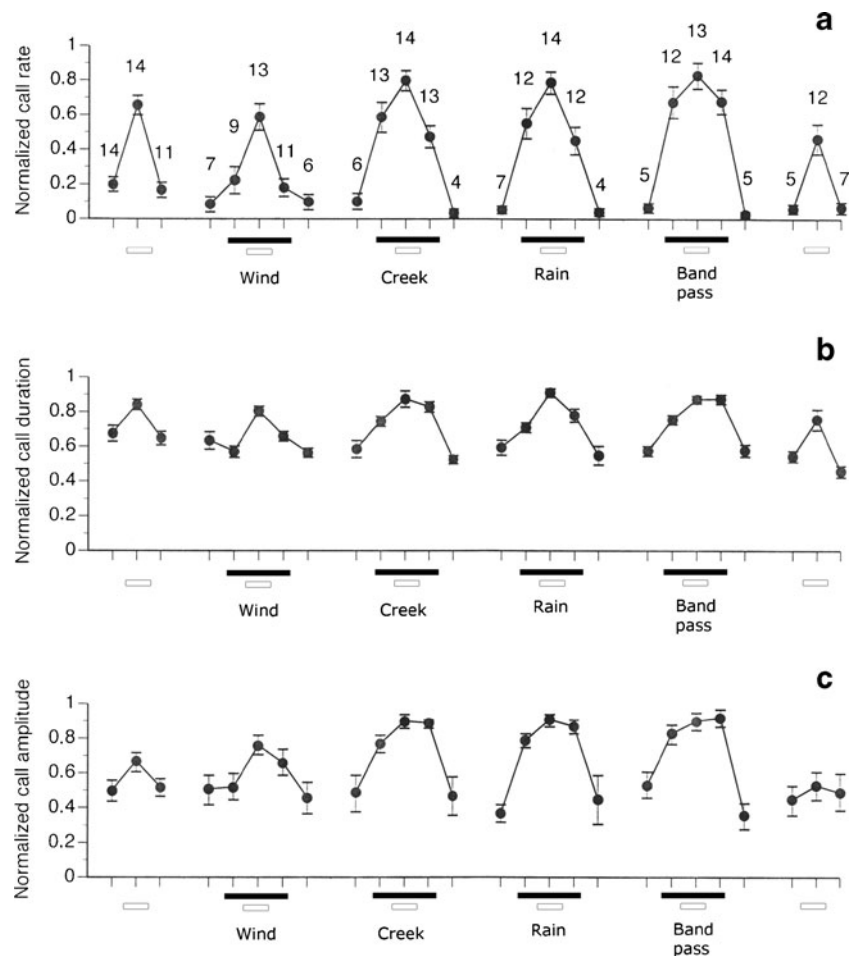
noise, call rate during interval C was higher for band-pass than for wind noise, and call rate during interval N2 was higher for creek, rain, and band-pass than for wind noise and also higher for band-pass than for creek and rain noise (Table 3).

Call duration differed significantly for intervals N1, C, and N2. Multiple comparisons showed that call duration during interval N1 was longer for band-pass and creek than for wind noise, call duration during interval C was longer for creek and rain than for wind noise, and call duration during interval N2 was longer for creek, rain, and band-pass noise than for wind noise (Table 3).

Call amplitude differed significantly for intervals N1, C, and N2. Multiple comparisons showed that call amplitude during interval N1 was larger for rain and band-pass than for wind noise and call amplitude during interval C and N2 was larger for creek rain and band-pass than for wind noise (Table 3).

Comparisons between vocal basal activity in the absence of generated noises and stimuli at the beginning and at the end of the noise–structure experiment showed that vocal activity decreased at the end of the experiment: 14 and 7 experimental subjects vocalized during the initial and final periods, respectively (Fig. 3a). Also, call rate was significantly lower at the

end of the experiment (Wilcoxon matched-pairs signed-ranks test: $Z=3.47$, $P=0.001$), but call duration and amplitude did not differ between both time intervals ($Z=0.76$, $P=0.450$ and $Z=0.89$, $P=0.374$, respectively). EVRs to the bout of synthetic calls presented in the absence of noise at the beginning and at the end of this experimental series were similar: 14 and 12 experimental subjects responded, respectively (Fig. 3a). No differences in the response to these stimuli occurred for call rate, call duration, and call amplitude (Wilcoxon matched-pairs signed-ranks test: $Z=0.80$, $P=0.423$; $Z=0.29$, $P=0.773$; and $Z=0.89$, $P=0.374$, respectively).

Noise–level experiment: evoked vocal responses to band-pass noise of increasing intensities

The experimental subjects vocalized persistently during broadcast of band-pass noise at different intensities. Call rate differed significantly among the five intervals analyzed (S1, N1, C, N2, and S2) during exposures at 55, 61, 67, 73, and 79 dB SPL. Post hoc multiple comparisons analysis showed that, for all five noise levels, call rate was significantly higher during N1 and C than during S1. Call rate was also higher during C than during S2 for all five noise levels. In addition,

Table 3 Results of Friedman ANOVA and multiple comparisons for EVR measures (call rate, duration, and amplitude) among noises of different structure for intervals N1, C, and N2

EVR measure	Interval	χ^2	P value	W/C	W/R	W/B	C/R	C/B	R/B
Call rate	N1	13.6	0.003	<	<	<			
	C	8.8	0.033			<			
	N2	28.2	<0.001	<	<	<		<	<
Call duration	N1	13.8	0.003	<		<			
	C	11.7	0.008	<	<				
	N2	15.3	0.002	<	<	<			
Call amplitude	N1	12.4	0.006		<	<			
	C	16.6	0.001	<	<	<			
	N2	15.6	0.001	<	<	<			

W wind noise, *C* creek noise, *R* rain noise, *B* band-pass noise, *N1* noise exposure preceding synthetic call onset, *C* noise exposure during the presentation of the bout synthetic calls, *N2* noise exposure after the presentation of the bout of synthetic calls, < and > relative magnitudes for significant multiple comparisons ($P<0.05$)

call rate was higher during N1 than during S2 for the exposures from 61 to 79 dB SPL and higher during N2 than S2 for exposures from 67 to 79 dB SPL. Finally, call rate was higher during N2 than during S1 for exposures at 55, 67, and 79 db SPL (Table 4 and Fig. 4a).

The analysis of call duration showed differences among the five intervals analyzed for the exposures at 55, 61, and 67 dB SPL. No significant differences occurred for the exposure at 73 dB SPL, and the absence of individuals producing calls during all the five time intervals during the 79-dB SPL exposure precluded applying the ANOVA to these data. Multiple comparisons showed that call duration was significantly longer during C than during S2 for the three exposure levels and during C than during S1 for the exposure at 55 dB SPL. In addition, call duration was longer during N1 than during S2 for the exposure at 61 dB SPL, longer during N2 than during S1, and longer during N2 than during S2 for the exposure at 67 dB SPL (Table 4 and Fig. 4b).

Table 4 Results of Friedman ANOVA and multiple comparisons for EVR measures (call rate, duration, and amplitude) among time intervals during exposures to band-pass noise at different levels

EVR measure	Noise level (dB SPL)	χ^2	P value	S1/N1	S1/C	S1/N2	S1/S2	N1/C	N1/N2	N1/S2	C/N2	C/S2	N2/S2
Call rate	55	38.6	<0.001	<	<	<						>	
	61	37.8	<0.001	<	<					>		>	
	67	36.0	<0.001	<	<	<				>		>	>
	73	38.4	<0.001	<	<					>		>	>
	79	37.9	<0.001	<	<	<				>		>	>
Call duration	55	13.0	0.011		<							>	
	61	13.8	0.008							>		>	
	67	13.6	0.009			<						>	>
	73	6.4											
	79	NA											
Call amplitude	55	15.8	0.003		<								
	61	17.1	0.002	<		<						>	>
	67	13.0	0.011							>		>	
	73	7.2											
	79	NA											

Nonsignificant *P* values for the Friedman ANOVA (>0.05) are omitted

S1 no-playback interval preceding a noise exposure, *S2* no-playback interval after a noise exposure, *N1* noise exposure preceding synthetic call onset, *N2* noise exposure after the presentation of the bout of synthetic calls, *C* noise exposure during the presentation of the bout synthetic calls, < and > relative magnitudes for significant multiple comparisons ($P<0.05$), *NA* Friedman ANOVA was not applied since no individuals produced calls during all the five time intervals of the 79-dB SPL exposure
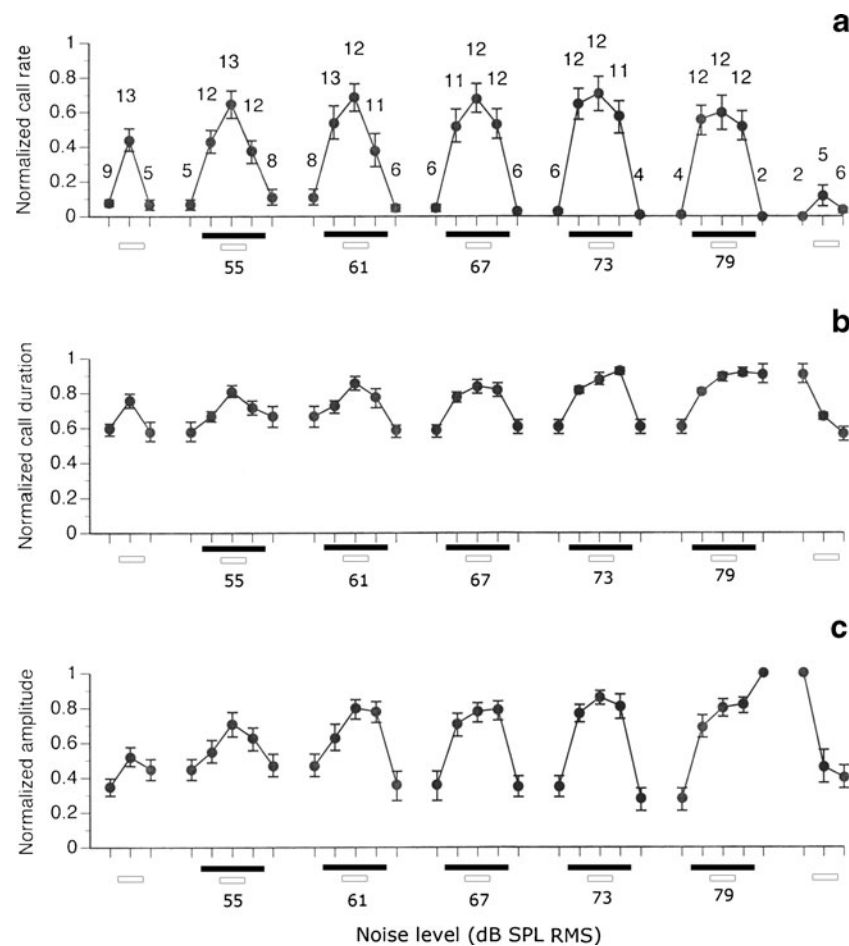
Fig. 4 Normalized measures of EVRs: **a** call rate, **b** call duration, and **c** call amplitude of 14 males of *B. taeniata* during the sequence of exposures to band-pass noise (center frequency, 2,000 Hz; cutoff frequencies, 1,000 and 3,000 Hz). *Numbers* in **a** indicate the number of individuals calling during time intervals. *Empty rectangles on the horizontal axis* indicate the presentation of bouts of synthetic calls, having a duration of 30 s. *Filled rectangles* indicate the presentation of different noises, having a duration of 150 s. Intervals of silence lasting 90 s are indicated by *ticks between horizontal rectangles* (see the "Methods" section). *Numbers below rectangles* indicate levels of noise exposures. The three measures of evoked call response were normalized to the maximum response for each individual. Namely, the values of call rate, call duration, and call amplitude for a given subject during a particular interval were divided by the maximum value of that measure produced by the frog across all intervals. *Filled circles* and *vertical bars* represent averages and standard errors, respectively

Call amplitude also showed significant differences among the five intervals analyzed during exposures at 55, 61, and 67 dB SPL. No significant differences occurred for the exposure at 73 dB SPL. No individuals produced calls during all the five time intervals during the 79-dB SPL exposure so the ANOVA was precluded for these data. Multiple comparisons showed that call amplitude was significantly larger during C than during S1 for the exposures at 55 and 61 dB SPL. In addition, call amplitude was larger during C than during S2 for the exposures at 61 and 67 dB SPL, larger during C than during S2 for the exposures at 61 and 67 dB SPL, larger during N2 than during S1 for the exposure at 61 dB SPL, and larger during N1 than during S2 for the exposure at 67 dB SPL (Table 3 and Fig. 4c).

In this experiment, as in the noise–structure experiment, the restricted statistical significance of multiple comparisons for call duration and call amplitude was due to the missing cases for these two variables.

Comparisons between vocal basal activity in the absence of generated noises or stimuli at the beginning and at the end of the noise–level experiment showed a moderate decrease: nine and six experimental subjects vocalized during the initial and final periods, respectively (Fig. 4a). Call rate, call duration, and call amplitude did not show significant differences between both intervals (Wilcoxon matched-pairs signed-ranks test: $Z=1.89$, $P=0.059$; $Z=0.94$, $P=0.345$; and $Z=0.94$, $P=0.345$, respectively). However, EVRs to the bout of synthetic calls presented in the absence of noise at the beginning and at the end of this experiment showed a decrease: 13 and 5 experimental subjects gave EVRs to the initial and final bouts of stimuli, respectively (Fig. 4a). Call rate was significantly lower at the end of the experiment (Wilcoxon matched-pairs

signed-ranks test: $Z=2.97$, $P=0.003$) but call duration and amplitude did not show significant differences ($Z=0.67$, $P=0.500$ and $Z=1.75$, $P=0.080$, respectively).

Discussion

Results of this study show a remarkable increment in the vocal activity of males of *B. taeniata* during presentations of natural abiotic noises of different origins and a band-pass noise encompassing the dominant frequency of the advertisement call of this frog. The four noises produce by themselves significant increases in call rate in the absence of the synthetic call stimulus. Call duration and call amplitude also increase on average during noise exposures; however, due to missing cases, significant increases occurred only for a few comparisons. Comparisons particularly relevant to test the effect of noise are those between the intervals with no playback and the intervals with noise alone, i.e., S1 and S2 versus N1 and N2. In the noise–structure experiment, among the 16 possible comparisons between these time intervals, only two comparisons yielded significant differences for call duration and call amplitude (see Table 2). In the noise–level experiment, only 3 among the 16 possible comparisons yielded significant differences for call duration and call amplitude (see Table 4).

The existence of the Lombard effect in the vocal responses of anurans, i.e., the increase of call amplitude in the presence of noise, remains an unsettled issue (Love and Bee 2010). Our data on call amplitude, because of the restricted statistical significance of comparisons provide a limited support for the occurrence of the Lombard effect in the responses of *B. taeniata* to noise interference.

The increases in vocal activity produced by noises in *B. taeniata* as measured by call rate resemble those observed in a previous study with another frog of the temperate austral forest, *E. calcaratus* (Penna et al. 2005). However, the vocal output of *B. taeniata* is remarkably sensitive to these interferences, since in that former study, wind noise did not produce an increase in calling activity. This noise has a spectrum restricted to low frequencies, in contrast with rain, creek, and band-pass noise, which have components above 1,000 Hz, comprising the range of the frequencies contained in the calls of both species. In general, noises that have been shown to produce a larger increase in the vocal output of vertebrates are those having spectra that overlap the range of the vocalizations of the experimental subjects (Brumm and Slabbekoorn 2005), a pattern of response appropriate to optimize intraspecific communication. The adaptive value of the increase in vocal output of males of *B. taeniata* in the presence of wind noise, which does not represent an interference for vocal communication, is not manifest. However, in this species, the responsiveness to wind noise is limited relative to creek, rain, and band-pass noise exposures, since the three

EVR measures were significantly lower during wind noise than during exposures to the three other noises.

The results of the current study are in sharp contrast with those obtained in a previous study with another frog from the southern temperate forest, *E. emiliopugini* (Penna et al. 2005), which does not increase its calling activity during exposures to any of the noises used in the present study. Similarly, we have recently observed that *B. leptopus*, a species sympatric to *B. taeniata* in the temperate forest, does not alter its vocal activity when exposed to noises alone (Cisternas et al. 2009).

Different strategies for confronting interference have also been reported for other anurans exposed experimentally to different noises in latitudes different from the temperate austral forest. Sun and Narins (2005) and Kaiser et al. (2011) reported that, in tropical assemblages of chorusing frogs, some species increase, while other decrease their call rate during exposures to anthropogenic noise. Cunnington and Fahrig (2010) also report interspecific differences in effects of noise exposure in frogs from temperate environments. In studies focused on single species, dissimilar results have also been obtained: Kaiser and Hammers (2009) found that *Dendropsophus triangulum* increases its call rate in the presence of anthropogenic noise; however, Lengagne (2008) reported a decrease in call rate but a stable call duration and dominant frequency by *Hyla arborea* in the presence of traffic noise. Love and Bee (2010) found an increase in call duration, a decrease in call rate, and a constant amplitude in *Hyla chrysoscelis* exposed to conspecific chorus noise. The adaptive value and origins of these differences in confronting acoustic intrusion have not been addressed and remain appealing issues for future studies.

The order of presentation of the noises in the noise–structure experiment influenced the responses, as shown by the differences in EVR measures between the two sequences of presentation used in 2009, and justify the replication of this experiment in 2011 using a randomized sequence. The results of the noise–level experiment could also have been influenced by the order of presentation of the exposures. However, the extent of the effects of this schedule does not seem to have been critical, since EVRs in the presence of band-pass noise at different levels are similar to those observed in the noise–structure experiment, with increases in vocal activity for all the range of intensities used.

The results of the noise–level experiment are in partial agreement with the former study with *E. calcaratus* (Penna et al. 2005); however, increments in the vocal output of that frog do not occur at the lower levels, but just for exposures at 66 dB SPL RMS and above. The increment in vocal activity of *B. taeniata* during exposures at levels below these values again indicates that this species is highly sensitive to noise intrusion.

The differences in the effect of noise between the two *Eupsophus* species had been related to potential differences

in noise level during the breeding activity of these frogs, since *E. calcaratus*, which is stimulated to call by noise, breeds from midwinter through midspring, and *E. emiliopugini*, which does not increment its vocal output in the presence of noise, breeds from the end of spring through midsummer, when atmospheric noise sources are likely to be less active (Penna and Hamilton-West 2007). However, this interpretation does not apply to *B. taeniata* which breeds from the end of summer through the beginning of autumn, when weather conditions are milder. In fact, background noise levels during our recordings at the study site in Tinquilco were quite low, averaging 40 dB SPL RMS. Overall, the unpredictable character of weather conditions in southern Chile may preclude establishing clear relationships between seasonality of abiotic noise levels and frogs' vocal responsiveness.

The results of this study contrast with the virtual lack of vocal responses of *B. taeniata* to calls of the congeneric species *B. antartandica* and *B. leptopus* (Penna and Velásquez 2011). A similar lack of responsiveness to heterospecific calls was found for *B. antartandica* (Penna and Meier 2011). Such restricted responsiveness to heterospecific signals is likely to be mediated by the selectivity of males of *B. taeniata* for temporal parameters of sound similar to those of conspecific vocalizations (Penna 1997; Solís and Penna 1997) and is likely to contribute to the segregation in conspecific choruses typically found in areas of sympatry among species of *Batrachyla*. In tropical species in which heterospecific choruses are a common occurrence, preferential vocal responses to conspecific calls save energy expenditure for confrontations with relevant competitors for access to females (Amézquita et al. 2005; Rojas et al. 2006).

The vocal activation by prolonged continuous noises of different structures found in the current study is paralleled by the activation observed during exposure to a broadband synthetic noise in the study with *B. antartandica* (Penna and Meier 2011) and could be adaptive to compensate for the reduction in vocal space caused by masking from abiotic noises having an ubiquitous presence in the temperate forest (Penna et al. 2005).

The contemporary ubiquitous noise pollution is a subject of increasing concerns and studies (e.g., Barber et al. 2009; Francis et al. 2009). Research efforts on the diversity of adaptations of animals to natural noise interferences, which have conditioned the evolutionary history of organisms, are particularly relevant to evaluate the consequences of these present-day environmental alterations.

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