Vocal Responsiveness to Interfering Sounds By a Frog From The Southern Temperate Forest, _Batrachyla leptopus_

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Abstract

Interfering sounds from biotic and abiotic origins are likely to shape the responsiveness of sound communicating animals. Among these sources of interference, interactions among acoustically active species have been studied to quite a limited extent. The vocal responses of 20 male frogs _Batrachyla leptopus_ from the temperate austral forest in Chile were tested with conspecific calls and with the calls of two sympatric species: _Batrachyla taeniata_ and _Batrachyla antartandica_, broadcast at amplitudes of 73, 79, 85, 91 and 97 dB SPL peak. Also, the vocal activity of the subjects during exposure to a 3-min continuous broadband noise presented at 67 dB SPL RMS was monitored. The subjects gave higher responses on average to the conspecific relative to the heterospecific calls, but in most comparisons, these differences did not reach levels of significance. In addition, the vocal activity of males of _B. leptopus_ did not increase in the presence of the continuous broadband noise. The lack of clear preferential responses for conspecific signals contrasts with the sharp selectivities that _B. taeniata_ and _B. antartandica_ have shown for their own calls in previous studies. Such different vocal behavior could be related to the extensive geographic overlap of _B. leptopus_ with the two other species in the temperate austral forest, where mixed choruses of this species with each of the two congeneric taxa have been reported to occur occasionally. The lack of vocal activation in the presence of continuous noise also contrasts with the increased vocal output with which the other two taxa respond to this intrusion and is likely to result from a relatively high spontaneous vocal activity in _B. leptopus_.

Introduction

Acoustic communication occurs amid interference from biotic and abiotic sources, and animals employ different strategies to overcome these difficulties. These tactics comprise increases in vocalization amplitude, emission rate and duration (e.g. Cynx et al. 1998; Lengagne et al. 1999; Pytte et al. 2003; Brumm 2004; Brumm & Slabbekoorn 2005), and shifts in the spectra of vocalizations to frequencies out of the range of background noise (e.g. Slabbekoorn & Peet 2003; Feng et al. 2006; Both & Grant 2012).

Interference from biotic origins is particularly significant in multispecies assemblages composed of different sound-producing vertebrates and invertebrates occurring in different landscapes, particularly in saturated tropical environments. Sound communicating insects, frogs and birds living in syntopy typically produce signals stratified in the spectral domain and exhibit different temporal patterns, which contribute to interference avoidance (e.g. García-Rutledge & Narins 2001; Luther 2009; Schmidt et al. 2013). Acoustic active insects alter their signal emission in the presence of heterospecific interference mainly by reducing their sound output (Latimer & Broughton 1984; Greenfield 1988; Römer et al. 1989; Schatral & Yeoh 1990). Similar effects have been reported in anurans (Littlejohn & Martin 1969; Wong

et al. 2009; Penna & Meier 2011; Penna & Velásquez 2011). Birds also adjust the timing of their vocalizations in the presence of heterospecific songs (Popp et al. 1985; Brumm & Slabbekoorn 2005; Brumm 2006; Luther 2008, 2009). Such strategies have been regarded as means to avoid costly energy expenditure in unfavorable acoustic environments (e.g. Wells 2001; Ophir et al. 2010).

Sensory correlates of the segregation of the acoustic space among coexisting taxa have been reported for crickets: species living in tropical environments saturated with biotic noise are endowed with auditory neurons responding to a restricted frequency range relative to species dwelling in temperate, simpler environments (Schmidt et al. 2011). In addition, studies with frogs subjected to natural biotic interference have shown a lower sensitivity in the frequency range overlapping the spectra of calls of sympatric related species (Amézquita et al. 2005).

In contrast with the use of separate channels for interference avoidance among sympatric taxa outlined above, recent studies have reported that the coexistence among singing bird species producing acoustic signals with structural similarities is likely to facilitate communal cooperative displays (Planqué & Slabbekoorn 2008; Malavasi & Farina 2012; Tobias et al. 2014a,b). Such convergence has been related to habitat similarity (Cardoso & Price 2010) or interspecific agonistic interactions (Grether et al. 2009). Clustering trends among acoustic signals of closely related sympatric species in anuran communities have also been reported recently (Chek et al. 2003; Amézquita et al. 2011).

A sensory capability that likely contributes to communication amid biotic signal interference resides in auditory perceptual scopes broader than the range of variation of signals. This relative tolerance of sensory systems would favor signal detection and processing amid degradation and noise interference occurring in natural environments (Luther & Wiley 2009; Amézquita et al. 2011; Erdtmann et al. 2011; Vélez et al. 2012).

Anurans generally communicate in dense chorusing assemblages. In lowland tropical latitudes, up to 20 different species typically build up acoustic communities (e.g. Hödl 1977; Drewry & Rand 1983; Duellman & Pyles 1983; Zimmerman 1983; García-Rutledge & Narins 2001; Chek et al. 2003; Amézquita et al. 2011). In temperate latitudes, just a small number of species are normally present, conforming simpler biotic sound environments (e.g. Penna & Veloso 1990). However, high sound levels are also built up in breeding aggregations in temperate regions (e.g. Swanson et al. 2007), interference being a widespread condition for communicating anurans.

In anuran species living in sympatry and therefore naturally subjected to reciprocal interference, a reduction in calling activity during exposure to heterospecific calls has been reported in some cases (Littlejohn & Martin 1969; Wong et al. 2009; Penna & Meier 2011; Penna & Velásquez 2011). In contrast with these results, males of other anurans have been shown to increase their calling activity when presented with heterospecific sympatric calls (Schwartz & Wells 1984, 1985; Phelps et al. 2006).

In addition to interference from identifiable heterospecific signals, anurans confront continuous abiotic noise of natural and anthropogenic origin with diverse strategies, that is augmenting or reducing their vocal output (Penna et al. 2005; Sun & Narins 2005; Penna & Hamilton-West 2007; Lengagne 2008; Kaiser & Hammers 2009; Love & Bee 2010; Kaiser et al. 2011; Penna & Meier 2011; Penna & Zúñiga 2014). Decreases in the vocal output would avoid costly energy expenditure in unfavorable acoustic environments (e.g. Wells 2001; Ophir et al. 2010), and increases would contribute to maintain the distance over which animals communicate (e.g. Parris et al. 2009).

Frogs *Batrachyla* in southern Chile produce advertisement calls composed of short pulses repeated in species-specific patterns (Barrio 1967; Penna & Veloso 1990; Penna 1997). *Batrachyla antartandica*, *B. leptopus* and *B. taeniata* have extensive distributions, overlapping their geographic ranges in areas of the austral temperate forest region. *B. taeniata* has the northernmost distribution, extending its range well beyond the temperate austral forest into the Mediterranean region up to latitude 32°S (Díaz et al. 1987; Brieva & Formas 2001). *B. antartandica* has the southernmost distribution, reaching latitude 51°S (Atalah & Sielfeld 1976; Díaz-Páez et al. 2002; Asencio et al. 2009). *Batrachyla leptopus* has an intermediate distribution, between about latitudes 37°S and 49°S, where it overlaps extensively with the other two species (Formas & Brieva 2000; Díaz-Páez & Ortiz 2003; Rabanal & Núñez 2008). In areas of sympatry, monospecific chorusing aggregations are the most common condition. However, instances of syntopic breeding and calling activity for this frog with the other two species have been reported (Penna & Veloso 1990; Penna 2005). No hybridization between these species has been reported and phonotactic responses of females to acoustic signals have not been explored.

In previous studies, we examined the responsiveness of males of *B. taeniata* (Penna & Velásquez 2011)

and *B. antartandica* (Penna & Meier 2011) to calls of the three species and found that male frogs give strong responses to conspecific relative to heterospecific signals. These preferences are related to stronger responses of these frogs for temporal patterns characteristic of conspecific calls (Penna 1997; Penna et al. 1997; Solís & Penna 1997). The preferences of males of *B. leptopus* for conspecific relative to heterospecific signals are of particular interest to explore as males of this species are exposed in nature to heterospecific interactions to a larger extent than the two congeneric taxa along their geographic distribution. We hypothesize that males of *B. leptopus* show a manifest responsiveness to the calls of the other species, which would indicate a relatively ample perceptual scope allowing interactive coexistence (Luther & Wiley 2009; Amézquita et al. 2011; Erdtmann et al. 2011; Vélez et al. 2012), as probably also occurs in anuran communities in which acoustic partitioning is weak or absent (Chek et al. 2003; Amézquita et al. 2011).

However, according to studies showing auditory perceptual restrictions in taxa exposed in nature to heterospecific interference (Amézquita et al. 2005; Schmidt et al. 2011), a species exposed to signals of related taxa would give stronger responses to stimuli having structure similar to their own vocalizations relative to taxa living in isolated conditions. As such, an alternative hypothesis is that *B. leptopus* responses to conspecific signals are stronger relative to those of the two species studied previously (Penna & Meier 2011; Penna & Velásquez 2011), which are less exposed to heterospecific acoustic interactions along their distribution range.

In addition to simulated biotic interference, males of the other two species have been exposed to prolonged low-pass continuous noise lacking a fine temporal segmentation, and it was found that they respond increasing their vocal output (Penna & Meier 2011; Penna & Zúñiga 2014), in contrast with their low vocal activity in the presence of heterospecific calls. In this study, we expose males of *B. leptopus* to this kind of noise to determine whether they confront this intrusion with further increases in their vocal activity or whether they use a strategy different from the one employed in response to heterospecific acoustic intrusion.

Methods

Study Site

The study was conducted from Jan. 27 through 2 Feb. 2009 and from 4–14 Mar. 2011, at the locality of Huerquehue (39°08′S, 71°42′W) in Southern Chile. The study site was located in a forest of *Podocarpus nubigenus* where males of *B. leptopus* called from inside crevices among mosses (*Rachomytrium sp.*) and ferns (*Hymenophyllum secundum* and *H. tortuosum*). Climbing plants (*Asteranthera ovata* and *Luzuriaga radicans*) were also abundant. At this site, a monospecific *B. leptopus* assemblage congregates typically every year, and a single male of *B. taeniata* called during two nights in 2011.

Experimental Protocol and Stimuli

Playback experiments were conducted nightly between 21:00 and 04:00 h. Spontaneous vocal activity of each subject was recorded for 2 min approximately, prior and after playback presentations. Air and substrate temperature, measured with a thermometer (Digi-Sense 8528-20) to the nearest 0.1°C after each recording, averaged 11.9°C (range 7.2–15.6°C) and 13.0°C (range 7.2–16.0°C), respectively, and the relative humidity averaged 91.4% (range 78–100%). Twelve experimental subjects captured after completing the playbacks had an average weight of 2.5 g (range 2.2–2.9 g, Acculab Pocket Pro Balance) and a snout-vent length of 34 mm (range 32–37 mm, Traceable Digital Caliper). The nearest neighbors were spaced at an average distance of 2.89 m (range 0.2–8.5 m) of the 20 experimental subjects.

Stimuli were synthetic imitations of the advertisement calls of *Batrachyla leptopus*, *B. taeniata* and *B. antartandica* made with the Soundmaker 1.0.4 software (Ovolab, Torino, Italy). The stimuli were designed after the typical calls of these frogs and were close to the average for calls of the three species at about the temperatures at the study sites, as reported in previous field studies (Penna 1997; Penna et al. 1997). The structure of these stimuli has been described in detail elsewhere (Penna & Meier 2011; Penna & Velásquez 2011). Briefly, the elementary unit for the calls of the three species was a 5-ms pulse having rise and fall times of 1 and 4 ms, respectively, and a carrier frequency of 2 kHz, which is close to the average dominant frequency of the calls of the population. The call of *B. leptopus* had a relatively complex temporal structure, namely, it consisted of 4 notes, each containing eight pulses repeated with an interpulse period of 5 ms and separated by internote intervals of 40 ms and the total duration of the call was 280 ms. The call of *B. taeniata* consisted of 25 pulses repeated with an interpulse period of 20 ms and the total duration of the call was 500 ms. The call of *B. antartandica* consisted of 30 pulses repeated with an

interpulse period of 667 ms, and the total duration of the call was 20 s. Because of its long duration, a single synthetic call of *B. antartandica* was presented per trial, and the synthetic calls of *B. taeniata* and *B. leptopus* were presented in bouts of 20 calls at an intercall period of 1.5 and 1.25 s, respectively. These call rates were within the ranges measured in natural aggregations (Penna 1997). The bouts of stimuli of the three species were repeated successively, leaving 60-s intervals in between presentations. Figure 1 shows the time wave and spectra of these stimuli.

Stimuli were broadcast using the same instrumentation described in Penna & Velásquez (2011) and Penna & Meier (2011). Recordings of evoked vocal responses (EVRs) during Jan. 2009 were conducted using a directional microphone (Sennheiser ME 66), the tip of which was placed at 0.2–0.4 m in front of the subject and a digital tape recorder (Sony TC D10 PROII). During Mar. 2011, the same microphone and a digital solid-state recorder (Tascam DR 100) were used.

Before starting recordings, the amplitude of the three stimuli at the position of the experimental subject were adjusted with the attenuator to 85 dB SPL (peak) by placing the microphone of a sound-level meter (Brüel & Kjaer 2230, linear weighting scale) just above the frog position (typically 3–5 cm), making efforts to avoid disturbing the animal. This SPL was within the range of amplitudes of the calls of nearest neighbors at the position of focal subjects (Penna & Solís 1998).

To avoid interferences during the playback experiments, nearby neighbors were silenced by capturing or disturbing them with gentle vibrations of the substrate. The basal calling activity of the experimental subject was recorded during an interval of two minutes approximately, after which the conspecific stimulus was presented, and if the frog responded, the playback experiment proceeded thereafter. The experimental sequence started with a series of synthetic calls of the three species presented at 85 dB SPL. Subsequently, four series of stimuli in each of which the heterospecific synthetic calls were presented at a constant amplitude: 73, 79, 91 and 97 dB SPL. These four series were delivered in order of increasing amplitude, using the corresponding attenuator settings. In the five series, a conspecific synthetic call at 85 dB SPL was presented before and after two consecutive bouts of heterospecific stimuli to compare the responses to these different stimuli at a proximate time. At the end
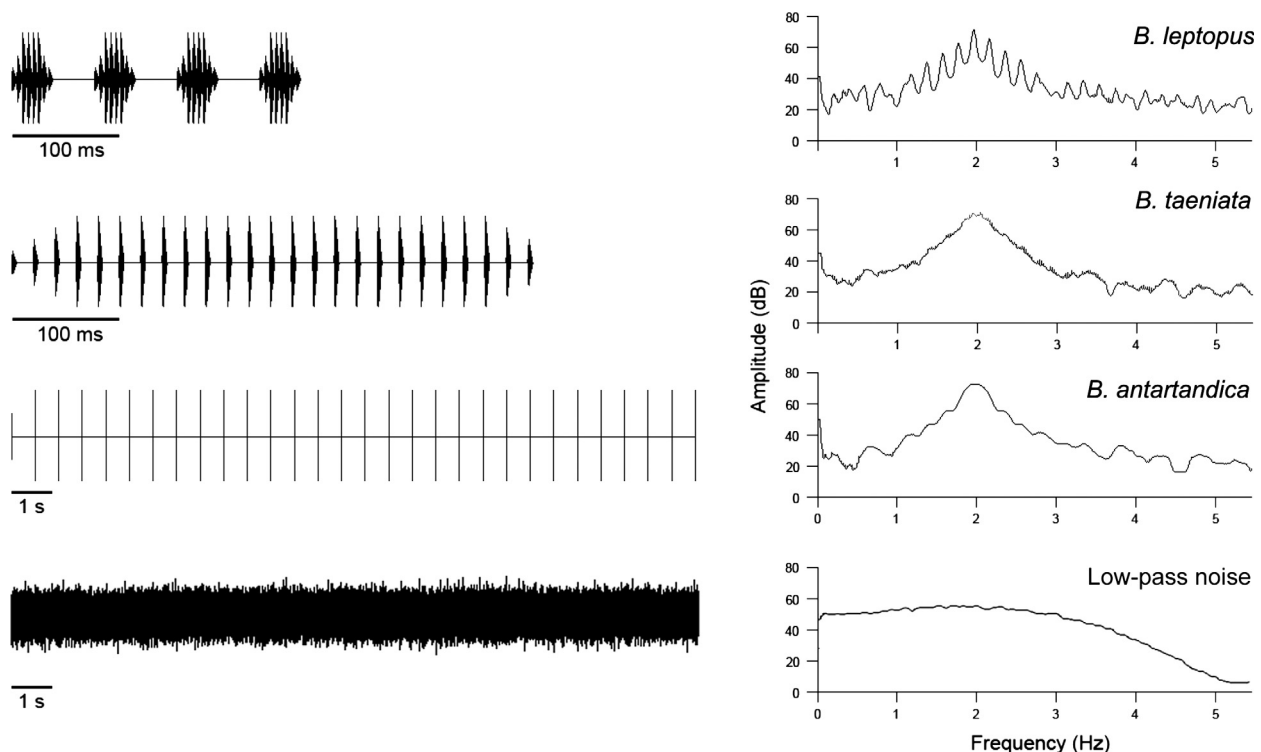
Fig. 1: Oscillograms and power spectra of synthetic stimuli imitating the calls of *Batrachyla leptopus*, *Batrachyla taeniata* and *Batrachyla antartandica*, and a low-pass continuous noise to which the experimental subjects were exposed. Sampling rate: 44.1 kHz, frequency resolution: 20 Hz.

of the experimental sequence, the basal calling activity of the experimental subject was recorded as at the beginning of the experiment, during an interval of approximately two minutes. The total duration of the experimental sequence from the onset of the initial conspecific bout of synthetic calls to the end of the final conspecific bout of synthetic calls was 23 min and 15 s.

The heterospecific synthetic calls were broadcast following two presentation orders: for 12 frogs the sequence was *B. taeniata* first, *B. antartandica* second, and for the other eight subjects, the presentation followed the reverse order. An order of increasing intensity was chosen for the presentation of heterospecific stimuli to minimize eventual inhibitory effects of high sound levels on the ongoing vocal activity or positioning of the experimental subjects during subsequent stimuli presentations. Previous experimental work with other species has shown that males reduce their calling rate when exposed to synthetic calls at high amplitudes (e.g. Penna et al. 2008). Additional randomization of stimuli presentation al different amplitude levels would have implied larger availability of subjects and experimental time.

After completing the conspecific and heterospecific stimulus presentation schedule, and after an interval of at least 3 min during which basal vocal activity was recorded, the experimental subjects were exposed to a continuous low-pass noise (cutoff frequency: 3000 Hz) of 3-min duration. Figure 1 (bottom) shows the time wave and power spectrum of this sound. The amplitude of the noise was 67 dB RMS SPL at the position of the experimental subject. Such level is well above the background noise at the study site during the experiments, which averaged 45 dB SPL RMS (range 39–57 dB SPL RMS). Furthermore, the noise level used for exposures has been shown to produce increments in vocal activity in previous studies on the effect of noise on the vocal behavior of other anuran species (Penna et al. 2005; Penna & Meier 2011).

Acoustic Analysis

The measures of the evoked vocal responses used were call rate (calls/min) and call duration (ms). These call attributes are significant for calling competition in different anurans (Gerhardt & Huber 2002) and in one congeneric species (Penna & Velásquez 2011). The two measures were computed for calls produced by the experimental subjects in response to bouts of 20 synthetic calls of *B. leptopus* and *B. taeniata* and to a single synthetic call of *B. antartandica,* and to the no-stimulus intervals preceding the presentation

of stimuli of the three species, which were labeled silent intervals S1, S2 and S3, respectively.

Recordings of frog vocalizations and stimuli conducted during 2009 were digitized with a analog to digital interface (Motu 828) and a computer (Macintosh G4) using Peak 4.0 software. Recordings conducted during 2011 were transferred directly from a secure digital (SD) card to the hard disk of a computer (Macintosh G4 Power PC). Onset and end times of evoked calls and stimuli were measured with Raven 1.3 software. The call's dominant frequency was measured during the initial period of basal activity for 19 subjects (0–22 000 Hz, frequency resolution: 20 Hz, sampling rate: 44 100 Hz, Raven 1.3 software).

Statistical Analysis

We carried out six kinds of statistical analyses. First, the dependence of call rate and call duration on air and substrate temperature and of dominant frequency on body weight and size was explored with GLM multiple regressions ($p < 0.05$). Second, GLM repeated measures ANOVAs ($p < 0.05$) were used to compare the vocal activity between the initial and final interval of recording of basal activity. Third, vocal activity to the presentation of the six conspecific synthetic calls presented along the experimental sequence were compared with GLM repeated measures ANOVAs ($p < 0.05$), to check for changes in responsiveness during the experimental sequence. Fourth, GLM one-way ANOVAs ($p < 0.05$) were used to compare the responses to heterospecific stimuli in the two sequences in which these sounds were presented. For these comparisons, the differences in call rate and call duration in response to a stimulus bout and the preceding silence were computed and compared between the two sequences of stimuli presentation. Fifth, GLM repeated measures ANOVAs ($p < 0.05$) were used to compare responses to conspecific and heterospecific calls. For this analysis, responses among six time intervals: the times of presentation of a bout of *B. leptopus* call and the two following heterospecific stimuli and the silent intervals preceding the conspecific, the B. *taeniata* and the *B. antartandica* stimuli, defined as silent intervals 1, 2 and 3, respectively. Five of such ANOVAs were performed for call rate and call duration, one for each series of heterospecific stimuli presented at intensities of 85, 73, 79, 91 and 97 dB SPL. *Post hoc* comparisons among the six time intervals were performed using Tukey tests ($p < 0.05$). In the sixth statistical analysis, vocal activity during the presentation of a 3-min noise and the preceding and following 2-min intervals of silence were compared with

Table 1: Results of GLM repeated measures ANOVAs and *post hoc* multiple comparisons (Tukey test) for EVR measures between stimuli presentation and interstimuli intervals

EVR measure	Heterospecific stimuli amplitude (dB SPL)	F	df	p	S1/BL	S1/S2	S1/S3	S1/BT	S1/BA	S2/BL	S2/BT	S2/S3	S2/BA	S3/BL	S3/BT	S3/BA	BL/BT	BL/BA	BT/BA
Call rate	85	10.85	5,95	<0.00010	0.00012			0.02464		0.00013				0.00015			0.00450	0.00118	
	73	3.65	5,95	0.00462										0.00322	0.03570				
	79	4.36	5,95	0.00128	0.03098					0.00378				0.00149					
	91	5.32	5,95	0.00024	0.00244				0.03044	0.00200			0.02566	0.04786				0.01184	
	97	4.00	5,95	0.00246										0.00100					
Call duration	85	2.28	5,55	0.05888															
	73	2.83	5,55	0.02391															
	79	5.83	5,30	0.00071	0.01364					0.00050				0.01023			0.00367	0.00923	
	91	2.88	5,60	0.02141	0.02275					0.02425							0.00436		
	97	5.93	5,35	0.00045	0.00156					0.00056				0.00701			0.03192		

BL, *B. leptopus* stimulus; BT, *Batrachyla taeniata* stimulus; BA, *B. antartandica* stimulus; S1, silence 1; S2, silence 2; S3, silence 3.
Stimuli amplitudes indicate levels at which heterospecific stimuli were presented, the conspecific stimulus was presented at 85 dB SPL in the five experimental series.

GLM repeated measures ANOVA ($p < 0.05$) and Tukey tests ($p < 0.05$).

Results

Basal Vocal Activity

The basal vocal activity was recorded previous to the presentation of the series of stimuli, during an average interval of 141.2 s (range 104–184 s). Nineteen of 20 frogs called during this interval, producing advertisement calls at an average call rate of 12.0 calls/min (range 2.0–36.9 calls/min). The average call duration was 227 ms (range 105 - 571 ms). The dominant frequency was measured for the 19 experimental subjects in the calls emitted during the initial period of basal recording yielded an average of 1968 Hz (range 1731–2230 Hz). Upon completion of the experimental series, the basal vocal activity was recorded again during a similar interval as previous to the presentation of the series of stimuli. Eighteen frogs called during this interval, producing advertisement calls at an average call rate of 11.7 calls/min (range 0.7–28.0 calls/min). The average call duration was 247 ms (range 122–529 ms). Call rate and call duration did not differ between the initial and final periods of recording of basal activity (GLM repeated measures ANOVA: $F_{1,19} = 0.54$, $p = 0.47238$ and $F_{1,16} = 0.44$, $p = 0.51617$, respectively).

The dependence of call rate and call duration on air or substrate temperature for the 19 males that called during the initial period of basal activity recording was explored with GLM multiple regressions. Call rate and call duration were not significantly related to these environmental factors ($F_{1,18} = 0.02$, $p = 0.88575$). Dominant frequency was not dependent on weight or SVL, as examined for the 12 experimental subjects that were captured (GLM multiple regression: $F_{1,10} = 0.11$ and $p = 0.75028$).

### Evoked Vocal Responses to Synthetic Calls

All 20 frogs responded to the initial bout of 20 repetitions of the conspecific stimulus with an average call rate of 41.1 calls/min (range 4.8–108.0 calls/min) and an average call duration of 290 ms (range 122–577 ms). The experimental subjects responded to most of the 6 presentations of bouts of the conspecific stimulus throughout the experimental sequence (109 of 120 stimuli presentations analyzed). Call rate and call duration evoked during the presentation of six bouts of the conspecific stimulus at 85 dB SPL did not differ significantly (GLM repeated measures ANOVA:

$F_{5,95} = 1.69$, p = 0.14485 and $F_{5,50} = 0.71$, p = 0.62079, respectively).

No differences in call rate and call duration occurred between the corresponding heterospecific stimuli presented in the two experimental sequences in which, the order was reversed (GLM one-way ANOVA: $F_{1,18} < 2.12$, p > 0.16235 and $F_{5,50} < 3.42$, p > 0.08198 for all comparisons, respectively). For this analysis, the correspondence of the intervals preceding the synthetic heterospecific calls presented in two different orders was maintained, so that the silent interval preceding the bout of synthetic calls of *B. taeniata* and the synthetic call of *B. antartandica* were labeled silent intervals S2 and S3, correspondingly, irrespective of the actual presentation order of the stimuli of each species.

The analysis of the EVR measures showed significant differences in call rate and call duration among the six time intervals compared (synthetic calls of *B. leptopus, B. taeniata* and *B. antartandica*, and the corresponding silent intervals preceding these stimuli: S1, S2 and S3). Significant differences in both variables occurred for most of the five series in which heterospecific stimuli were presented at 85, 73, 79, 91 and 97 dB SPL. The only comparison on the border of statistical significance was call duration for the series in which heterospecific stimuli were presented at 85 dB SPL (GLM repeated measures ANOVA, Table 1).

Post hoc comparisons (Tukey tests, p < 0.05) showed a significant higher call rate in response to the conspecific stimulus relative to most of the silent intervals (11 of 15 comparisons). The four comparisons in
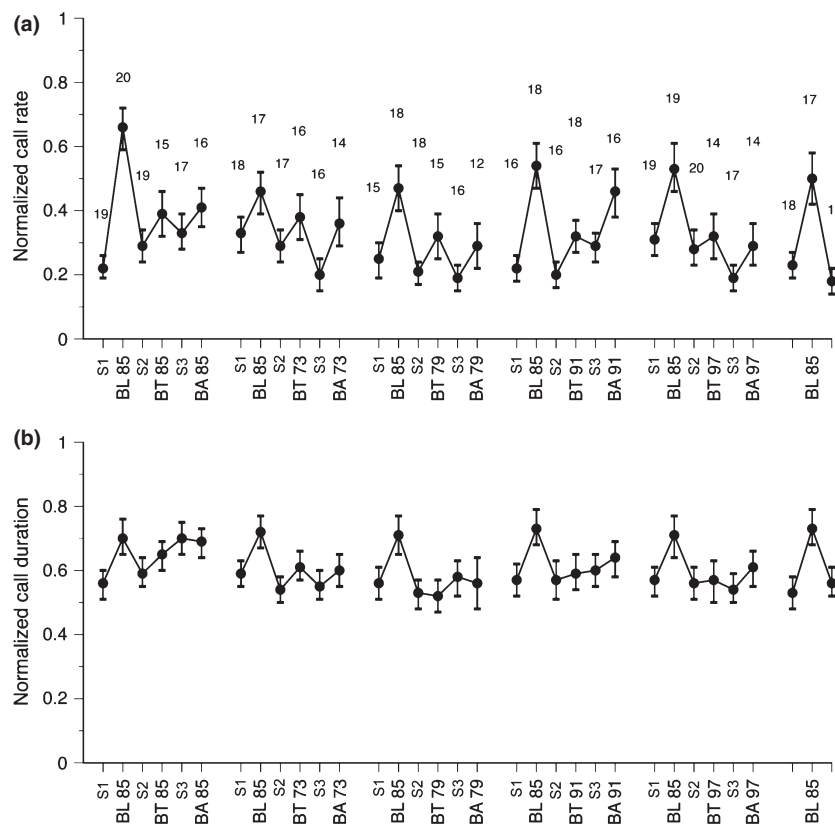
Fig. 2: Normalized EVRs given by 20 males of *Batrachyla leptopus* during the intervals of presentation of a conspecific and two heterospecific stimuli. Call rate (a) and call duration (b) were computed during the interval of presentation of the stimuli plus the 1.25 s following the cessation of the stimuli. To compare graphically, the EVRs of different individuals to a series of stimuli, the EVR measures were normalized to the maximum response for each individual. Namely, the value of an EVR measure for a given subject in response to a particular bout of stimuli or silent interval was divided by the maximum value of that EVR measure produced by the frog to any bout of stimuli. Filled circles correspond to averages and bars to standard errors. BL: *B. leptopus* stimulus; BT: *Batrachyla taeniata* stimulus; BA: *Batrachyla antartandica* stimulus; S1, S2 and S3: silent intervals preceding BL, BT and BA, respectively. Numbers: 73, 79, 85, 91 and 97 indicate the SPLs at which the conspecific and heterospecific stimuli were broadcast. Numbers above symbols in graph (a) indicate frogs responding in each condition. Series of stimuli follow the order of presentation during the experimental sequence. Presentation of the heterospecific stimui follows the order in which the Batrachyla taeniata stimulus precedes the Batrachyla antartandica stimulus (see Methods).

which call rate to the conspecific stimulus was similar to silent intervals corresponded to S1 and S2 in the series in which, the heterospecific stimuli were presented at 73 and 97 dB SPL. In contrast, call rate differed only in 3 of 10 comparisons between the conspecific and the two heterospecific stimuli. Call rate in response to the conspecific stimulus was higher than to the *B. taeniata* stimulus in the series in which the heterospecific stimuli were presented at 73 dB SPL and higher than to the *B. antartandica* stimulus in the series in which the heterospecific stimuli were presented at 73 and 97 dB SPL (Table 1, Fig. 2a).

Call duration in response to the conspecific stimulus showed a more restricted tendency to differ from call duration during the silent intervals than call rate: significantly longer call durations occurred in 8 of 15 comparisons between responses to the conspecific stimulus than during the silent intervals. Call duration in response to conspecific versus heterospecific stimuli showed a similar tendency as for call rate: longer call durations to conspecific stimuli than to heterospecific stimuli occurred in 4 of 10 comparisons (Table 1, Fig. 2b),

Vocal Activity During Exposure to Prolonged Noise

Eighteen subjects were exposed to 3-min low-pass noise. The exposure to low-pass noise did not produce a significant change in call rate (GLM repeated measures ANOVA: $F_{2,38} = 1.71$, p = 0.19377) but significantly increased call duration (GLM repeated measures ANOVA: $F_{2,22} = 4.28$, p = 0.02701). *Post hoc* comparisons showed that call duration was longer during the 3-min noise exposure than during the following 2-min silent interval (Tukey test, p = 0.02275, Fig. 3).

Discussion

Results of this study show that in most cases, responses to conspecific calls were not significantly stronger than for heterospecific calls. Responses to the conspecific calls in terms of call rate were stronger relative to the calls of *B. taeniata* only in the series in which the heterospecific calls were presented at 85 dB SPL and stronger relative to the calls of *B. - antartandica* when the heterospecific calls were presented at 85 and 97 dB SPL. In contrast with these results, a previous study using the same stimuli showed that males of *B. taeniata* responded with higher call rate to the conspecific stimulus relative to the *B. leptopus* and *B. antartandica* stimulus in all the five series of stimuli (Penna & Velásquez 2011; see
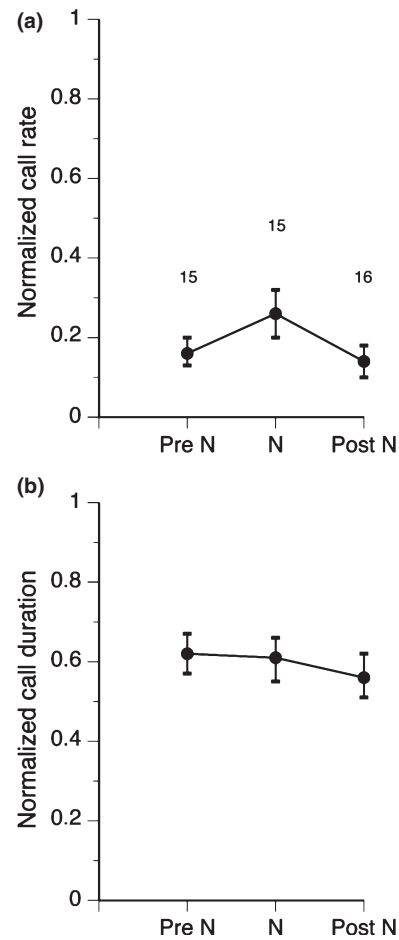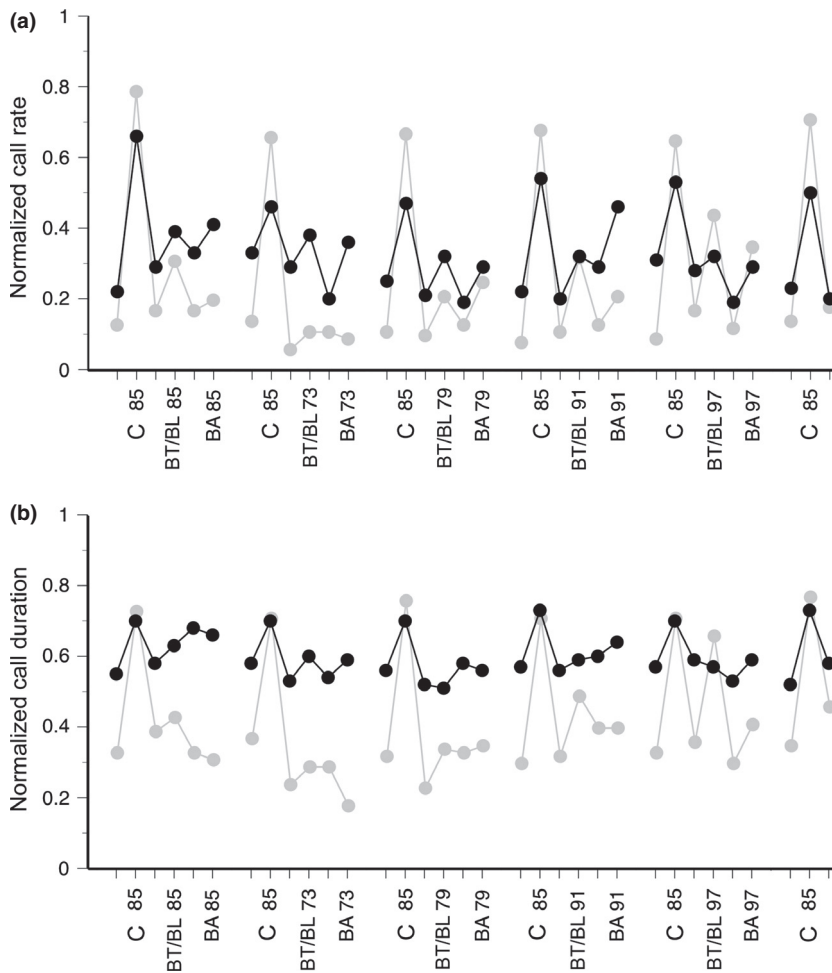

Fig. 3: Normalized call rate (a) and call duration (b) during exposure to a 3-min continuous low-pass noise (cutoff: 3 kHz, 67 dB RMS SPL) and to preceding and following 2-min intervals. Symbols and normalization procedure as in Fig. 3.

Fig. 4). In terms of call duration, responses to the conspecific calls were significantly longer relative to the calls of *B. taeniata* and *B. antartandica* in the series in which the heterospecific calls were presented at 79 and 97 dB SPL. In contrast with these results, the previous study with *B. taeniata* showed that males of this species responded with longer calls to the *B. leptopus* stimulus in three of five experimental series and gave longer calls relative to the *B. antartandica* stimulus in all the five series of stimuli (Penna & Velásquez 2011; see Fig. 4).

The results of the present study also contrast with a previous study conducted with *B. antartandica*, in which the same series of stimuli were used (Penna & Meier 2011), although the results are not strictly comparable in quantitative terms as the results for *B. taeniata* (Penna & Velásquez 2011), because the temporal structure of *B. antartandica* call, composed of

Fig. 4: Comparison of average normalized call rate (a) and call duration (b) of 20 males of *Batrachyla leptopus* in this study (black circles) and of 20 males of *Batrachyla taeniata* in a former study (grey circles, Penna & Velásquez 2011) to conspecific and heterospecific stimuli. Standard errors are omitted to simplify the graphical comparison between the two species. BA: *Batrachyla antartandica* stimulus, BT/BL: *B. taeniata* stimulus presented to males of *B. leptopus* (black circles) or *B. leptopus* stimulus presented to males of *B. taeniata*, C: conspecific stimulus (corresponding to BL and BT for black and grey circles, respectively). Numbers: 73, 79, 85, 91 and 97 indicate the SPLs at which the conspecific and heterospecific stimuli were broadcast. Abbreviations for silent intervals preceding stimuli are omitted for clarity.

short pulses repeated at a slow rate is quite different, and therefore, the EVR variables measured were not the same. In that study for instance, latencies of response to the conspecific stimulus were shorter relative to the *B. leptopus* and *B. taeniata* stimulus in all the five series of stimuli.

Overall, results of the current study show that males of *B. leptopus* respond consistently to conspecific calls, but the vocal activity shows a tendency to persist in the presence of heterospecific calls, in contrast with the two congeneric species which show a clear tendency to give much stronger responses to conspecific relative to heterospecific calls over most conditions of stimulation (Penna & Meier 2011; Penna & Velásquez 2011). As stated above, the contrast with *B. taeniata* is more straightforward than with *B. antartandica*, because the call of the former species has a general timing similar to *B. leptopus*, emitting pulsed calls of a few hundred milliseconds separated by approximately 1-s intervals. Focusing on this comparison, it is

apparent that the vocal activity of *B. leptopus* is not as dependent from external input as that of *B. taeniata*, showing a higher spontaneous call rate in absence of stimulation. For instance, in the current study during the initial period of basal vocal activity, only one of 20 experimental subjects remained silent, whereas in the study with *B. taeniata* (Penna & Velásquez 2011) 10 of 20 males remained silent during the corresponding period.

The sustained vocal activity of males of *B. leptopus* in the presence of heterospecific sounds suggests a tolerance to interference allowing signal detection and processing amid sounds of different origins occurring in natural environments. Extended response ranges beyond the characteristics of conspecific signals have been reported for anurans and birds (Luther & Wiley 2009; Amézquita et al. 2011; Erdtmann et al. 2011; Vélez et al. 2012). An ample responsiveness of that kind would allow the occasional mixed ensembles of syntopic breeding and calling activity of *B. leptopus*

with the other two species reported by Penna & Veloso (1990) and Penna (2005).

Results of the current study contrast with studies on signal recognition and auditory processing showing that species coexisting with related taxa respond restrictively to the properties of their own vocalizations as compared to species living in isolated conditions (Amézquita et al. 2005; Schmidt et al. 2011). Recent studies reporting clustering trends in vocalizations among coexisting anuran (Chek et al. 2003; Amézquita et al. 2011) and bird species (Planqué & Slabbekoorn 2008; Malavasi & Farina 2012; Tobias et al. 2014a,b) also suggest that perceptual permissiveness rather than segregation supports communication in these communities. The communication networks arising in these circumstances have been proposed to facilitate communal cooperative displays in bird assemblages (Planqué & Slabbekoorn 2008; Malavasi & Farina 2012; Tobias et al. 2014a,b). Conceivably, concurrent calling of anurans in multispecies aggregations may have similar synergic consequences.

No adaptive explanations in terms of breeding activity can be offered to account for the different dependence of calling activity on conspecific signals among the species of *Batrachyla*. The time course of breeding activity of the three species is similarly extended, from late summer to early fall and nothing is known about female selectivity for male advertisement calls.

The relatively unaltered vocal activity of males of *B. leptopus* in the presence of continuous low-pass noise contrasts with the marked increase in call rate exhibited by males of the other two congeneric species during exposures to similar sounds, which yield highly significant increases relative to the pre-exposure intervals (Penna & Velásquez 2011; Penna & Zúñiga 2014). Such increase in vocal activity may be adaptive to communicate amid interfering noises of different origin, which occur ubiquitously in the temperate forest. The relatively unaltered vocal activity of *B. leptopus* males in the presence of continuous background noise could result from its relatively high basal vocal rate. Further sustained increases would imply an excessive energy expenditure in this highly demanding metabolic activity (Ophir et al. 2010).

Acknowledgements

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