Sound level discrimination by gray treefrogs in the presence and absence of chorus-shaped noise

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An important aspect of hearing and acoustic communication is the ability to discriminate differences in sound level. Little is known about level discrimination in anuran amphibians (frogs and toads), for which vocal communication in noisy social environments is often critical for reproduction. This study used two-choice phonotaxis tests to investigate the ability of females of Cope's gray treefrog (*Hyla chrysoscelis*) to discriminate between two advertisement calls differing only in sound pressure level by 2, 4, or 6 dB. Tests were conducted in the presence and absence of chorus-shaped noise (73 dB) and using two different ranges of signal levels (73–79 dB and 79–85 dB). Females discriminated between two signals differing by as little as 2–4 dB. In contrast to expectations based on the "near miss to Weber's law" in birds and mammals, level discrimination was slightly better at the lower range of signal amplitudes, a finding consistent with earlier studies of frogs and insects. Realistic levels of background noise simulating a breeding chorus had no discernable effect on discrimination at the sound level differences tested in this study. These results have important implications for studies of auditory masking and signaling behavior in the contexts of anuran hearing and sound communication. © *2012 Acoustical Society of America*. [http://dx.doi.org/10.1121/1.3699271]

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I. INTRODUCTION

Anurans are important animal models for understanding hearing and sound communication in noisy environments (Gerhardt and Huber, 2002; Narins *et al.*, 2007; Bee, 2012; Schwartz and Bee, 2012; Vélez *et al.*, 2012). Male frogs commonly aggregate in large numbers during a breeding season and produce advertisement calls to attract gravid females as mates (reviewed in Gerhardt and Huber, 2002). Advertisement calls are loud (Gerhardt, 1975; Penna and Solís, 1998), and the levels of noise generated in breeding choruses are often intense (Narins, 1982) and may be heard from distances of up to 2 km (Arak, 1983). In many species, reproduction requires that females successfully detect a calling male, recognize it as a conspecific based on species-specific call properties, and localize it in the structurally and acoustically complex habitat of a breeding chorus (reviewed in Gerhardt and Huber, 2002). In addition, female frogs often exhibit directional mate preferences based on perceiving individual differences among the calls produced by different males (reviewed in Ryan and Keddy-Hector, 1992; Gerhardt and Huber, 2002). Auditory masking by the high levels of background noise in breeding choruses can constrain these functions of vocal communication in frogs (reviewed in Vélez *et al.*, 2012).

Numerous behavioral (e.g., Ehret and Gerhardt, 1980; Gerhardt and Klump, 1988; Simmons, 1988; Schwartz and Gerhardt, 1989, 1998; Bee and Schwartz, 2009) and physiological (e.g., Freedman *et al.*, 1988; Ratnam and Feng, 1998; Lin and Feng, 2001, 2003; Bibikov, 2002) studies have investigated the influence of noise on how anuran auditory systems process vocal signals and other sounds. Recent studies of auditory masking in Cope's gray treefrog (*Hyla chrysoscelis*) have used "chorus-shaped noise" (i.e., noise with the long-term spectrum of conspecific breeding choruses) to investigate masking release in the context of call recognition and discrimination. In phonotaxis tests, females of this species experience on the order of 2–6 dB release from masking when sources of conspecific calls are spatially separated from sources of noise (Bee, 2007, 2008a; Nityananda and Bee, 2012) or when background noise fluctuates in amplitude (Bee and Vélez, 2008; Vélez and Bee, 2011). Interestingly, males appear not to increase the amplitude of their calls as a function of increasing background noise levels (Love and Bee, 2010). While previous work in other species indicates that frogs can discriminate level differences on the order of 2–6 dB (reviewed in Fay and Simmons, 1999), we currently lack such data for Cope's gray treefrogs. More importantly, we lack estimates of signal level discrimination abilities measured at multiple signal levels under controlled conditions in both the absence and presence of masking noise in any frog species.

Here, we investigated level discrimination by females of Cope's gray treefrog using two-choice phonotaxis experiments

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(Gerhardt, 1995). The study had three objectives. First, we investigated the ability of subjects to discriminate between two calls differing only in sound pressure level (SPL) by magnitudes of 2, 4, or 6 dB. Our tests were designed to estimate the "just meaningful difference" (JMD; Nelson and Marler, 1990) in SPL that elicits differential phonotaxis by females. We consider these estimates to be JMDs, and not "just noticeable differences" (JNDs), because in phonotaxis tests, it is always possible that females can perceive smaller differences than those that elicit behavioral discrimination. Based on previous work in other species (reviewed in Ryan and Keddy-Hector, 1992; Gerhardt and Huber, 2002), our *a priori* hypothesis was that females would preferentially choose the alternative presented at the higher SPL. A second objective was to determine the extent to which level discrimination may depend on ambient noise. Therefore, all choice tests were conducted under both quiet conditions in the absence of noise and in the presence of an artificial chorus-shaped noise similar to those used in previous studies of auditory masking in this species. The chorus-shaped noise was broadcast at 73 dB SPL to reflect the natural background noise levels recorded in gray treefrog choruses (Schwartz *et al.*, 2001; Swanson *et al.*, 2007). Following the work of Zelick and Narins (1983) on the Puerto Rican coquí frog (*Eleutherodactylus coqui*), we predicted that JMDs would be relatively smaller in the absence of noise. Finally, our third objective was to assess the possibility that JMDs for level discrimination ability might depend on overall signal level. To this end, we replicated all of our choice tests using two different ranges of SPLs in which the alternative with the higher relative amplitude was presented at either 85 or 79 dB SPL at a distance of 1 m (hereafter referred to as the "nominal signal level" of the test). The alternative was attenuated by 2, 4, or 6 dB relative to the nominal signal level. A signal level of 85 dB corresponds to the lower end of the range of natural variation in the amplitude of gray treefrog calls recorded at 1 m (Gerhardt, 1975). Based on a previous study of green treefrogs (*Hyla cinerea*), we predicted that JMDs would be relatively smaller at lower nominal signal levels (Gerhardt, 1987).

II. METHODS

A. Subjects and study sites

All experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (#0809A46721) and were carried out in strict accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals of the National Institutes of Health*. Between June 5 and June 15, 2011, we collected and tested 49 gravid females of the western mtDNA lineage of Cope's gray treefrog (Ptacek *et al.*, 1994). Females were collected in amplexus with a male (i.e., as a breeding pair) between 2130 and 0200 h from wetlands located in the Carver Park Reserve (Carver Co., MN) and the Crow-Hassan Park Reserve (Hennepin Co., MN). Upon return to the laboratory, we maintained subjects at approximately 2 °C until testing to delay egg deposition. All testing took place within three days of collection. At least 30 min prior to testing, subjects were placed in an incubator set to 20 °C to allow their body temperatures to reach 20 ± 1 °C. After testing was completed, subjects were returned to their location of capture.

B. Apparatus

We conducted our experiments inside a temperature controlled (20 ± 1 °C), hemi-anechoic sound chamber (L × W × H = 220 cm × 280 cm × 216 cm; Industrial Acoustics Company, Bronx, NY). With its temperature control unit running, measurements of ambient noise in the chamber ranged between 2 and 12 dB SPL (re. 20 μPa, LZF) in the 1/3-octave bands between 0.5 and 4 kHz, which spans the frequency range of interest in this study. Additional details on the sound chamber have been described elsewhere (Bee and Schwartz, 2009). Phonotaxis tests were performed in a circular test arena (2-m diameter) with walls (60-cm height) that were constructed from hardware cloth covered in black fabric so that they were acoustically transparent but visually opaque (Fig. 1). During a test, subjects were released from a small, acoustically transparent cage located at the center of the arena (Fig. 1). We presented target signals (Sec. II C 1) using two Orb Mod1 speakers (Orb Audio LLC, New York, NY) located on the floor of the sound chamber separated by 90° around the perimeter of the test arena and directed toward the central site where subjects were released (Fig. 1). We used a third Orb Mod1 speaker suspended from the ceiling of the sound chamber 1.9 m above the central release site to broadcast noise (Sec. II C 2). Broadcasting noise from the overhead speaker created uniform noise levels (±1.5 dB) across the entire floor of the circular test arena.

Phonotaxis tests were conducted under infrared (IR) illumination. Two observers recorded subject behavior in real time on a video monitor located outside the chamber using an IR-sensitive Panasonic WV-BP334 video camera (Panasonic Corporation of North America, Secaucus, NJ) mounted from the center of the sound chamber's ceiling. The camera's output was also encoded in real time into digital video files and stored to hard disk for 283 of the 288 phonotaxis tests conducted in this study (5 videos were lost due to software malfunctions at the time of encoding). Had any discrepancies arisen between the two observers in

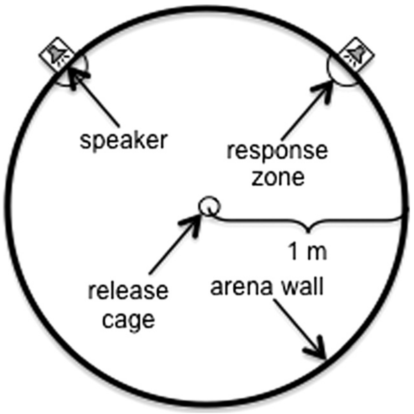

FIG. 1. The test arena. Schematic diagram of the circular test arena (2 m diameter) used for phonotaxis tests showing the positions of the central release cage, the speakers, and the response zones relative to the arena wall.

scoring responses (none did), they could have been resolved immediately after the trial by watching the recorded video. Acoustic playbacks were controlled from a PC (Dell Optiplex 980; Dell Computer Corp., Round Rock, TX) located outside the sound chamber. We used Adobe Audition v1.5 (Adobe Systems Inc., San Jose, CA) to broadcast digital stimuli (11.025 kHz, 16-bit) through an M-Audio FireWire 410 soundcard (M-Audio USA, Irwindale, CA). The output of the soundcard was amplified using a DMA-1275 multichannel amplifier (Home Theater Direct Inc., Plano, TX). The C-weighted, root-mean-square SPLs of target signals (LCF_{max}) and noise (LC_{eq}) were calibrated by placing the microphone of a Brüel and Kjær Type 2250 sound level meter (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) at the approximate position of a subject's head while located at the central release site. All sound levels and level differences for this study refer to measurements taken at the subject's release site.

C. Acoustic stimuli

1. Target signals

We tested subjects in a series of six, two-choice discrimination tests in which they were required to choose between two synthetic advertisement calls that differed in amplitude by 2, 4, or 6 dB in the presence and absence of noise. Aside from the level difference, which we implemented in software, the two alternative target signals in a test were identical. Target signals were created to reflect average temporal and spectral properties for calls recorded in local populations and adjusted to 20 °C. Each call comprised 32 pulses that were 11 ms in duration and separated by 11-ms interpulse intervals. Hence, the pulse period was 22 ms, the pulse duty cycle was 50%, the pulse rate was 45.5 pulses s^{-1}, and the call duration was 693 ms. Each pulse was created by adding two phased-locked sinusoids (starting phase 0°) with frequencies (and relative amplitudes) of 1.25 kHz (−9 dB) and 2.5 kHz (0 dB). The amplitude envelope of each pulse was shaped with a 4-ms exponential rise time and a 7-ms inverse exponential fall time. Each call was shaped with a 50-ms linear onset. During a phonotaxis test, each call was repeated in sequence with a period of 5 s (simulating a natural call rate), and the two calls were broadcast from different speakers in an alternating fashion such that the two alternatives were 180° out of phase (i.e., each was preceded and followed by equivalent intervals). Different groups of subjects ($N = 24$ per group) were always tested with one signal presented at the designated nominal signal level (either 79 or 85 dB; the un-attenuated signal); on different tests, the alternative signal was attenuated by 2, 4, or 6 dB relative to that presented at the nominal signal level. For half of the subjects, the un-attenuated signal initiated the sequence of alternating calls; the other half of the subjects heard sequences initiated by the attenuated signal. The two speakers broadcasting signals had similar frequency responses (±2.5 dB) over the range of 0.5–4 kHz. For each speaker, the measured difference in its frequency response at the two frequencies contained in signals (1.25 and 2.5 kHz) was less than 1.0 dB (0.5 and 0.7 dB). Thus, when both speakers were calibrated at the nominal signal level, which was largely determined by the level of the 2.5 kHz component (recall that the 1.25 kHz component had a relative amplitude of −9 dB), any level differences between the same components broadcast by each speaker were small compared to the magnitude of level differences manipulated as part of this study. Each speaker was used in each relative physical position in the chamber (i.e., left or right; Fig. 1) for half of the subjects tested, and physical position was counter-balanced across both nominal signal levels (79 and 85 dB) and initial signals (un-attenuated or attenuated).

2. Chorus-shaped noise

All of our two-choice tests were conducted in both the absence of noise and in the presence of artificial, chorus-shaped noise broadcast at 73 dB SPL (LC_{eq}) from the overhead speaker. We used 24 different exemplars of chorus noise. Two subjects were tested with each exemplar; one subject was tested at the nominal signal level of 79 dB and the other was tested at 85 dB. The frequency spectra of the noises were designed to reflect the long-term spectrum of a natural breeding chorus of gray treefrogs. Between May and July, 2007–2010, we recorded 24 different gray treefrog choruses (1.5-min recording durations) at our study sites using Marantz PMD670 digital recorders (D&M Holdings US Inc., Mahwah, NJ) and omnidirectional Sennheiser ME62 microphones (Sennheiser Electronic Corporation, Old Lyme, CT). We made recordings near the nightly peaks of calling activity (2200–0000 h). We placed recording microphones at heights of 5 cm above ground or water level and at distances between 4 and 10 m from the nearest calling male present in the chorus. We selected specific locations, and times of year and night, for making chorus recordings so that gray treefrogs were the only frog species heard calling. We created 24 different exemplars of chorus-shaped noise by shaping the spectra of 24 different white noises so that each had the long-term average spectrum of one of the breeding choruses we recorded. The spectra of these chorus-shaped noises were subsequently bandpass filtered between 0.85 and 3.3 kHz and converted to the time domain using an inverse fast Fourier transform.

D. Experimental protocol

Each subject was tested in six randomly ordered tests that included all possible combinations of level difference between the un-attenuated and attenuated alternatives (3 levels) and the presence/absence of noise (2 levels). Prior to beginning the experiment, we had decided to exclude any female that failed to make a choice in all six tests. Ultimately, only one potential subject was excluded from analyses because it failed to complete all six tests, yielding our final sample size of $N = 48$ frogs (24 per nominal signal level). Between consecutive tests, subjects experienced a 5–15-min timeout in the incubator. Previous studies indicate gray treefrogs do not exhibit directional biases or carry-over effects in repeated tests (Gerhardt *et al.*, 2000).

To begin each test, the subject was removed from the incubator and placed in the cage at the release site at the center of the test arena. After a 1.5-min acclimation period, we began alternating broadcasts of the two alternatives. After the subject had heard three presentations of both stimuli, we remotely released it using a rope and pulley system operated from outside the sound chamber. In tests with noise, we started broadcasting the noise immediately after the first minute of the acclimation period (i.e., 30 s prior to initiation of signal broadcasts). After their release, subjects were given up to 5 min to make a choice by entering a 10-cm diameter hemi-circular response zone centered in front of a playback speaker (Fig. 1).

E. Statistical analyses

Our experiment was based on a 2 nominal signal level (79 or 85 dB SPL; between-subjects) × 3 level difference (2, 4, and 6 dB; within-subjects) × 2 noise condition (present and absent; within-subjects) fully factorial design. The response variable was binary (1 = chose the un-attenuated call; 0 = chose the attenuated call). To examine how our experimental treatments affected female preferences, we used the binary data to fit a generalized linear mixed model with a logit link function (GLMM; see review in Bolker *et al.*, 2009). We treated level difference as a continuous variable, and the other treatments (nominal signal level and noise condition) as two-level categorical variables. We included which stimulus initiated playback sequences on each trial (either un-attenuated or attenuated) as an additional two-level categorical variable. In our full model, we also included pairwise interactions between experimental treatments. To account for the repeated-measures design as well as un-modeled individual variation in response, we included "subject" as a random grouping effect. Our objective was to identify the most parsimonious model, so we sequentially removed uninformative parameters (i.e., the parameter with the lowest β/SE) from the full model until further removal resulted in an increase in the model's AIC value (Pagano and Arnold, 2009; Arnold, 2010). This analysis was conducted in R (R Development Core Team, 2011) using the glmer function within the lme4 package (Bates *et al.*, 2011).

As is customary in analyses of two-choice tests with female frogs (Gerhardt, 1995), we also analyzed the outcome of all two-choice tests using binomial tests of the null hypothesis that females chose the attenuated and un-attenuated alternatives in proportions equal to 0.5. We tested this null hypothesis against the one-tailed alternative that a proportion of subjects significantly greater than 0.5 chose the un-attenuated signal. The one-tailed nature of our alternative hypothesis is justified based on previous studies of frogs showing preferences for higher call amplitudes (reviewed in Ryan and Keddy-Hector, 1992; Fay and Simmons, 1999; Gerhardt and Huber, 2002). We also used repeated measures analysis of variance to compare response latencies across different conditions, which we measured as the time from subject's release until it entered the response zone. A significance criterion of $\alpha = 0.05$ was used for binomial tests and analysis of variance.

From time to time, the valid concern is raised in reviews of manuscripts or grant proposals that scoring phonotaxis experiments in real time lacks independence and does not meet the requirements of a double-blind experiment. Real-time scoring of phonotaxis tests is commonly accepted practice in the field, but there are few data in the literature comparing the accuracy of real-time scoring to that of double-blind scoring. Therefore, we had an independent observer, who was not involved in designing or conducting our study and who did not know the aims of the study, view all 283 of our video recordings of phonotaxis trials, which had coded file names. They were asked to simply score whether the frog they observed chose the speaker on the left or the right (Fig. 1) and to record its response latency. After scoring, this blind observer mapped left and right choices onto names of the experimental conditions (e.g., presence/absence of noise and which stimulus was broadcast from the chosen speaker). We then used Cohen's Kappa (Cohen, 1960), a statistical measure of inter-observer agreement on categorical outcomes, to determine the concordance between real-time and independent, double-blind measurements. When observers are in complete agreement, $\kappa = 1$; when agreement does not exceed that expected by chance, $\kappa = 0$.

III. RESULTS

As reported in other studies of phonotaxis behavior in treefrogs (reviewed in Rheinlaender and Klump, 1988), females typically exhibited characteristic patterns of zig-zag hopping and walking directed toward one or the other speaker. On average (±SD), subjects required 76.0 ± 44.4 s to enter the response zone. Response latencies did not differ depending on the presence or absence of noise ($F_{1,46} = 2.0$, $P = 0.16$), the nominal signal level ($F_{1,46} < 0.1$, $P = 0.89$), or the attenuation level ($F_{2,92} < 0.1$, $P = 0.92$). As might be expected for observations of an animal moving at an average velocity of 0.043 km/h (1.18 cm/s) from the release site toward the response zone, there was perfect agreement between real-time and double-blind scoring of their choices ($\kappa = 1.0$). The average latency computed by the blind observer was 76.3 ± 44.4 s, a difference of 300 ms from that determined from real-time scoring of responses.

Subjects behaviorally discriminated between two identical calls differing only in SPL and more often chose the un-attenuated (higher-amplitude) alternative. Figure 2(a) depicts the proportions (and 95% binomial confidence intervals, CI) of females choosing the un-attenuated alternative as a function of level difference, with nominal signal level and presence/absence of noise as the parameters.

The most parsimonious GLMM included only two fixed effects: level difference and nominal signal level (Table I). The presence or absence of noise, the initial stimulus (un-attenuated versus attenuated), and all interactions were found to be uninformative. There was some degree of individual variation in response [the variance for the random-effect term was 0.294, the implications of which can be seen in the dashed lines of Figs. 2(b) and 2(c)]; however, a larger sample size would be required to accurately estimate variability in the population. Subjects tended to choose the un-attenuated signal, even when the difference in level was only 2 dB; however, selection of the un-attenuated alternative at

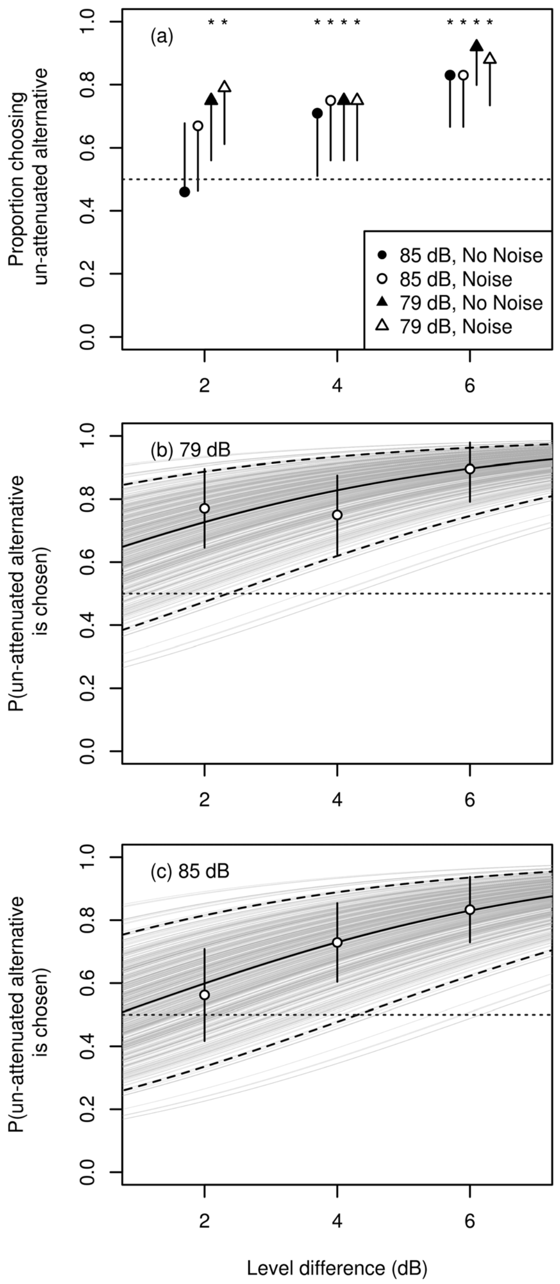

FIG. 2. Results of two-choice phonotaxis tests of level discrimination. (a) Depicted here are the proportions of females ($N = 24$ per test) choosing the un-attenuated alternative as a function of the level difference between the un-attenuated and attenuated alternatives. Error bars depict the 95% binomial confidence interval (one-tailed). Data are shown for tests conducted at nominal signal levels of 85 dB (circles) and 79 dB (triangles) in the presence (open symbols) and absence (filled symbols) of chorus-shaped noise. Asterisks indicate significance ($P < 0.05$) in one-tailed binomial tests of the hypothesis that proportions equaling 50% would choose both the attenuated and un-attenuated alternatives. (b) and (c) depict the probabilities of choosing the un-attenuated alternative for the 79 dB (b) and 85 dB (c) nominal signal levels as functions of level difference. Circles depict the actual proportions (calculated over both noise conditions) and are surrounded by 95% CIs (based on 10 000 bootstrap samples). The solid lines depict the probabilities predicted by the most parsimonious GLMM (Table I), the light-gray lines illustrate the expected response for 500 simulated individuals (i.e., for each line, the random component of the intercept was drawn from a Normal distribution with mean = 0, SD = 0.542), the dashed lines represent the 2.5% and 97.5% quantiles of expected individual responses. The horizontal dotted line in all three panels depicts the expected (null) proportion of 0.5 in a two-choice test.

small level differences (2 dB) was less frequent when the nominal signal level was 85 dB compared with 79 dB. This can also be seen in the observed proportions of subjects that chose the un-attenuated alternative [Figs. 2(b) and 2(c)]. All proportions are above 0.5, regardless of nominal signal level; however, the bootstrapped 95% CI for the 2 dB difference overlaps 0.5 when the nominal signal level was 85 dB [Fig. 2(c)] but not 79 dB [Fig. 2(b)]. Although the intercept of the GLMM is not zero (corresponding to a 0.5 probability of choosing each speaker when there is no difference in signal level), a 95% CI around this parameter includes 0 (Table I) and further, examining this model below a 2 dB difference would be extrapolating beyond the data and should therefore be avoided.

Traditional binomial analyses of the results were consistent with patterns revealed by the GLMM. At the nominal signal level of 85 dB, significantly higher proportions ($P < 0.05$) of females than expected by chance chose the un-attenuated alternative when it was paired against one attenuated by 4 and 6 dB [Fig. 2(a)]. When the nominal signal level was 79 dB, subjects chose the un-attenuated alternative in proportions significantly higher than expected by chance at level differences of 2, 4, and 6 dB [Fig. 2(a)]. These patterns of statistical significance for both the 79 and 85 dB nominal signal levels were consistent in both the presence and absence of noise [Fig. 2(a)]. There was a non-significant trend ($P < 0.08$) for females to preferentially choose the un-attenuated 85-dB call in noise when the alternative was attenuated by 2 dB.

IV. DISCUSSION

A. Level discrimination in frogs

Our results indicate that the JMD of female Cope's gray treefrogs for discriminating between two calls differing only in SPL was at least as small as 2–4 dB, depending on the nominal signal level. These results are in line with expectations from previous studies of other frog species using two-choice phonotaxis tests and conspecific calls to investigate level discrimination. Fellers (1979), for example, showed that females of the eastern gray treefrog (*Hyla versicolor*) preferentially chose a call played back at 92 dB over one presented at 90 dB. Females of the natterjack toad (*Bufo calamita*) also discriminated between two signals differing by 2 dB in SPL (82 dB versus 80 dB; Arak, 1983). Forester and Czarnowski (1985) found that female spring peepers (*Pseudacris crucifer*) discriminated level differences at least as small as 5 dB. Female painted reed frogs (*Hyperolius marmoratus*) discriminated between two identical stimuli that differed by 6 dB (Dyson and Passmore, 1988). Smaller differences on the order of 2–4 dB were not tested in these previous studies of spring peepers and painted reed frogs. Gerhardt (1987) found that female green treefrogs (*H. cinerea*) discriminated differences as small as 3 dB when the alternatives were presented at 63 dB versus 60 dB, and at 73 dB versus 70 dB, but not at 83 dB versus 80 dB. At the highest signal levels tested in that study, a 6 dB difference (i.e., 86 dB versus 80 dB) was required to elicit differential phonotaxis to the higher-

TABLE I. Generalized linear mixed-models showing model selection results and the parameters (β) and standard errors (SE) for each factor included in each model (**$P < = 0.01$, *$0.05 < P < = 0.1$).

Model	ΔAIC	AIC	Random effect variance	Parameter value	Intercept	Level difference	Nominal level	Noise	Initial stimulus	Nominal level × Noise	Level difference × Noise	Level difference × Nominal level
1	6.2	317.8	0.311	β	0.202	0.315*	−1.350*	0.738	0.248	0.456	−0.200	0.154
				SE	0.692	0.168	0.788	0.800	0.333	0.586	0.184	0.184
2	4.9	316.5	0.299	β	−0.095	0.400**	−0.818*	0.735	0.245	0.483	−0.206	
				SE	0.576	0.131	0.441	0.781	0.331	0.585	0.183	
3	4.1	315.7	0.287	β	0.259	0.298**	−0.793*	0.000	0.243	0.450		
				SE	0.487	0.091	0.433	0.437	0.328	0.582		
4	2.7	314.3	0.282	β	0.140	0.297**	−0.578*	0.252	0.242			
				SE	0.457	0.091	0.329	0.288	0.327			
5	1.3	312.9	0.299	β	0.263	0.297**	−0.581*	0.252				
				SE	0.427	0.091	0.331	0.288				
6	0	311.6	0.294	β	0.387	0.296**	−0.579*					
				SE	0.403	0.091	0.330					
7	1.0	312.6	0.382	β	0.100	0.296**						
				SE	0.367	0.091						
Null model	10.0	321.6	0.306	β	1.214							
				SE	0.161							

amplitude alternative. Together, results from these phonotaxis studies suggest that female frogs have JMDs for level discrimination on the order of 2–4 dB (and perhaps smaller), which is similar to that of 3–6 dB reported from a field study of call suppression in male *E. coqui* (Zelick and Narins, 1983). These estimates are similar to intensity difference limens reported for some species of birds and mammals (reviewed in Fay, 1988; Forrest, 1994).

Results from Gerhardt's (1987) study of green treefrogs (*H. cinerea*) indicated that level discrimination in anurans may deteriorate at higher nominal signal levels, a result also found in some insects (reviewed in Forrest, 1994; Gerhardt and Huber, 2002). Although we tested only two nominal signal levels, our results appear consistent with these earlier findings. In our study, females discriminated between signals differing by as little as 2 dB when the nominal signal level was 79 dB (i.e., 79 dB versus 77 dB) but not when it was 6 dB higher (i.e., 85 dB versus 83 dB). At the higher nominal signal level, significant preferences for the alternative with a higher SPL were only found when the difference in amplitude was 4 dB or higher. We would note, however, that Fellers (1979) showed in a field playback experiment that females of the closely related eastern gray treefrog (*H. versicolor*) discriminated between two calls broadcast simultaneously (not alternating, as in the present study) when they differed by 2 dB at higher amplitudes (92 dB versus 90 dB). It remains unclear whether this difference between two closely related frogs represents a species difference or a methodological difference (e.g., simultaneous versus alternating playbacks; field site versus laboratory sound chamber). Forrest (1994) reviews results from studies of insects showing deterioration in level discrimination at higher intensities similar to that reported here and by Gerhardt (1987) for frogs. Together, these studies suggest that level discrimination in frogs (and insects) could differ in some important respects from that in birds and mammals, in which level discrimination actually improves slightly at higher amplitudes (the so-called "near-miss to Weber's Law") (reviewed in Forrest, 1994; Gerhardt and Huber, 2002). These potential taxonomic differences in level discrimination deserve further and more rigorous investigation.

B. Implications for studies of auditory masking

The intensity difference limen (between 3 and 6 dB) estimated by Zelick and Narins (1983) in their field study of *E. coqui* was obtained under noisy conditions in the animal's natural breeding habitat. They predicted even better performance (i.e., smaller difference limens) in a quiet environment. Results from our study do not seem to support this prediction. Indeed, one of the most important results from our study is that the JMD for level discrimination in female gray treefrogs did not differ between quiet and noisy conditions. To our knowledge, this study is the first to investigate level discrimination in frogs in both the presence and absence of noise. The chorus-shaped noise used in our study was broadcast at 73 dB SPL. Previous studies in our lab have shown that this and similar noise levels, which reflect naturalistic sound amplitudes (Schwartz *et al.*, 2001; Swanson *et al.*, 2007), are adequate to interfere with a female's perception of conspecific calls (e.g., Bee, 2007; Bee and Swanson, 2007; Bee and Schwartz, 2009; Vélez and Bee, 2010; Nityananda and Bee, 2012). Under the conditions tested in the present study, however, we were unable to demonstrate that performance in a level discrimination task deteriorated in the presence of chorus-like noise relative to quiet conditions. Because we did not test level differences smaller than 2 dB, however, we cannot rule out the possibility that a female's ability to discriminate very small differences in level (i.e., <2 dB) deteriorates in the presence of noise. Nevertheless, our results suggest that level discrimination is robust in noise across multiple signal levels and level differences.

Our results have important implications for studies of auditory masking in frogs. For example, a neurophysiological study of spatial release from masking in northern leopard frogs, *Rana pipiens*, indicated improvements in signal detection thresholds of up to about 4 dB in auditory nerve fibers and 9 dB in midbrain neurons (Lin and Feng, 2001). Behavioral studies of spatial unmasking in gray treefrogs (*H. chrysoscelis*) and green treefrogs (*H. cinerea*) have reported improvements in signal recognition thresholds (Bee and Schwartz, 2009) on the order of 3–6 dB when signals and noise are spatially separated by 90° compared with co-localized conditions (Schwartz and Gerhardt, 1989; Bee, 2007, 2008a; Nityananda and Bee, 2012). Signal recognition thresholds are also about 2–4 dB lower in the presence of a chorus-shaped noise masker that slowly fluctuates with sinusoidal amplitude modulation (e.g., 0.625–2.5 Hz) compared to masking conditions with steady-state noise having the same frequency spectrum (Vélez and Bee, 2011). Females also appear capable of exploiting spectro-temporal correlations in amplitude across the frequency spectrum to achieve a comodulation masking release on the order of 3–5 dB (Bee and Vélez, 2008).

Across studies of auditory masking in frogs, the magnitudes of masking release measured in various experiments commonly fall in the range of about 2–6 dB. Here, we found that female gray treefrogs discriminated in favor of alternatives with higher SPLs between two signals differing only in level by as little as 2–4 dB in the presence of chorus-shaped noise. This finding suggests that masking release on the order of 2–6 dB is biologically important and has potential to influence the behavioral decisions female frogs make in choosing a mate in a chorus. Such a conclusion is consistent with the findings of Wollerman (1999), who showed that females of the hourglass treefrog (*Dendropsophus ebraccatus*, formerly *Hyla ebraccata*) preferred a stimulus combining a signal with chorus noise over one composed of the same chorus noise alone when the signal-to-noise ratio (SNR) was +3 dB and +6 dB, but not 0 dB or +1.5 dB. Gerhardt and Klump (1988) found preferences in a similar test with green treefrogs (*H. cinerea*) at a SNR of 0 dB, but not −6 dB; smaller differences in SNR were not tested. In other studies that have measured the strength of female preferences for certain call attributes by attenuating preferred alternatives, differences in sound level on the order of 2–6 dB can eliminate or even reverse some preferences (Gerhardt *et al*., 2000; Bee, 2008b). Hence, based on results from this and previous studies, we would suggest that estimates of masking release on the order of 2–6 dB are biologically important in the context of frog vocal communication in noisy chorus environments.

C. Implications for the evolution of signaling behavior

Over the years, numerous studies have investigated the signals and signaling behavior of male frogs in an effort to understand how they may be adapted to ameliorate the problem of auditory masking for receivers (reviewed in Schwartz and Bee, 2012). For example, some species may partition the time of day, the breeding area, or the frequency spectrum to avoid calling at times, places, and frequencies overlapping with other species. Male frogs can also precisely time their calls to avoid call overlap from nearby neighbors. A recent study of Cope's gray treefrogs investigating the Lombard effect failed to find any evidence that males of this species increase the amplitude of their calls as a function of the level of background chorus noise (Love and Bee, 2010). One hypothesis for the absence of a Lombard effect proposed in that study was that males were currently under intense selection (or had been in the past) to always call as loudly as possible. The data presented here, showing that females of this species are quite selective for calls produced at higher amplitudes both in the presence and absence of noise, provide robust support for this hypothesis. Male gray treefrogs that produced calls at lower amplitude relative to other nearby males in the chorus would be at a mating disadvantage.

V. CONCLUSIONS

This study yielded three main results in relation to its stated objectives (Sec. I). First, we showed that the JMD in Cope's gray treefrogs for discriminating between two signals differing only in SPL was at least as small as 2 dB; smaller differences were not tested. Second, we found that JMDs for level discrimination were similar in the presence and absence of noise over the range of level differences tested. Finally, we found a difference in estimated JMDs based on nominal signal level; JMDs for level discrimination were smaller at a lower sound level (i.e., ≤ 2 dB at 79 dB SPL; >2 dB but ≤ 4 dB at 85 dB SPL). Based on these results, we conclude that Cope's gray treefrogs exhibit robust abilities to discriminate between signals differing in level. By extension, our results indicate that small improvements in SNR as a result of masking release on the order of 2–6 dB are biologically relevant for frogs making mate choice decisions in noisy environments.

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