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Signal Recognition by Green Treefrogs (*Hyla cinerea*) and Cope's Gray Treefrogs (*Hyla chrysoscelis*) in Naturally Fluctuating Noise

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Abstract

This study tested three hypotheses about the ability of female frogs to exploit temporal fluctuations in the level of background noise to overcome the problem of recognizing male advertisement calls in noisy breeding choruses. Phonotaxis tests with green treefrogs (*Hyla cinerea*) and Cope's gray treefrogs (*Hyla chrysoscelis*) were used to measure thresholds for recognizing calls in the presence of noise maskers with (i) no level fluctuations, (ii) random fluctuations, or level fluctuations characteristic of (iii) conspecific choruses and (iv) heterospecific choruses. The *dip-listening hypothesis* predicted lower signal recognition thresholds in the presence of fluctuating maskers compared with non-fluctuating maskers. Support for the dip listening hypothesis was weak; only Cope's gray treefrogs experienced dip listening and only in the presence of randomly fluctuating maskers. The *natural soundscapes advantage hypothesis* predicted lower recognition thresholds when level fluctuations resembled those of natural soundscapes compared with artificial fluctuations. This hypothesis was rejected. In noise backgrounds with natural fluctuations, the *species-specific advantage hypothesis* predicted lower recognition thresholds when fluctuations resembled species-specific patterns of conspecific soundscapes. No evidence was found to support this hypothesis. These results corroborate previous findings showing that Cope's gray treefrogs, but not green treefrogs, experience dip listening under some noise conditions. Together, the results suggest level fluctuations in the soundscape of natural breeding choruses may present few dip-listening opportunities. The findings of this study provide little support for the hypothesis that receivers are adapted to exploit level fluctuations of natural soundscapes in recognizing communication signals.

Keywords

acoustic communication; auditory masking; cocktail party problem; Cope's gray treefrog; dip listening; green treefrog; *Hyla chrysoscelis*; *Hyla cinerea*; masking release

Animals often communicate acoustically in social aggregations that include several signalers and receivers (Schwartz & Freeberg, 2008). In such environments, the background noise generated by the mixture of unattended signals poses a number of problems for communication, including impaired signal detection, recognition, and discrimination (Brumm & Slabbekoorn, 2005; Klump, 1996). How the auditory systems of nonhuman animals may be adapted to overcome or ameliorate these problems has received little attention (Bee & Micheyl, 2008; Brumm & Slabbekoorn, 2005; Hulse, 2002; Klump, 1996; Miller & Bee, 2012). Importantly, the level of background noise present in natural acoustic scenes is not constant, but instead fluctuates over time (Richards & Wiley, 1980; Nelken,

Rotnam, & Yosef, 1999; Vélez & Bee, 2010). Among the potential mechanisms animals use to communicate in noise is an ability to exploit these level fluctuations to improve signal perception (Bee & Micheyl, 2008; Klump, 1996; Langemann & Klump, 2005). We investigated this possibility in frogs, for which the noise generated in breeding choruses impairs the ability of receivers to recognize and discriminate among individual calls (reviewed in Bee, 2012; Vélez, Schwartz, & Bee, in press).

In this comparative study, we examined the extent to which female green treefrogs (*Hyla cinerea*) and Cope's gray treefrogs (*Hyla chrysoscelis*) exploit level fluctuations present in the noise of breeding choruses in recognizing conspecific advertisement calls. In both species, reproduction takes place in dense choruses in which males produce species-specific calls that mediate species recognition and female mate choice (Gerhardt, 2001). The fluctuations that occur in the level of noise in pure choruses of green treefrogs and Cope's gray treefrogs differ and exhibit species-specific patterns (Vélez & Bee, 2010, 2011; Vélez, Höbel, Gordon, & Bee, in review). In this study, we used phonotaxis experiments to measure 'signal recognition thresholds' (Bee & Schwartz, 2009) in the presence of maskers that had (i) no level fluctuations, (ii) random fluctuations, or level fluctuations characteristic of (iii) conspecific choruses and (iv) heterospecific choruses. Our objective was to test three hypotheses about signal recognition in fluctuating noise backgrounds.

According to the *dip-listening hypothesis*, listeners are able to catch brief 'acoustic glimpses' of target signals when the level of fluctuating background noise momentarily decreases. This hypothesis, therefore, predicts that receivers should recognize signals at lower thresholds in the presence of fluctuating maskers compared with non-fluctuating maskers. Dip listening is well known in the contexts of human hearing and speech perception (Bacon, Opie, & Montoya, 1998; Cooke, 2006; Füllgrabe, Berthommier, & Lorenzi, 2006; Gustafsson & Arlinger, 1994; Vestegaard, Fyson, & Patterson, 2011). Behavioral and neurophysiological studies of animals from diverse taxa show that simple tonal and narrowband noise signals can be detected at relatively lower thresholds in the presence of maskers with sinusoidal or random level fluctuations, compared with non-fluctuating maskers (birds: Bee, Buschermöhle, & Klump, 2007; Hofer & Klump, 2003; Jensen, 2007; Klump & Langemann, 1995; Langemann & Klump, 2001, 2007; Nieder & Klump, 2001; cats: Nelken et al., 2001; Rotman, & Yosef, 1999; dolphins: Branstetter & Finneran, 2008; fish: Fay, 2011; frogs: Goense & Feng, 2012). The hypothesis that dip listening contributes to the ability of nonhuman animals to recognize communication signals in noisy social aggregations (Langemann & Klump, 2005) has so far received limited attention (but see Ronacher & Hoffmann, 2003). Here, we tested the hypothesis that female treefrogs experience dip listening in the behavioral context of communication by comparing masked thresholds for recognizing conspecific advertisement calls in the presence of a non-fluctuating control noise to those measured in fluctuating noises.

Our second hypothesis stems from growing evidence that auditory systems are adapted to process physical properties of natural sounds (Rieke, Bodnar, & Bialek, 1995; Lewicki, 2002; Woolley, Fremouw, Hsu, Theunissen 2005; Smith & Lewicki, 2006). For example, auditory neurons transmit information more efficiently when stimuli have properties of natural sounds, compared to stimuli with artificial properties (Rieke et al., 1995; Woolley et al., 2005). In species that communicate in social aggregations, therefore, the auditory system may be adapted to exploit physical properties of natural soundscapes to improve signal recognition. However, the extent to which receivers exploit *natural* level fluctuations to improve signal recognition has not been tested. The few available studies on recognition of communication signals in fluctuating noise have used *artificial* (sinusoidal) level fluctuations (Ronacher & Hoffmann, 2003; Vélez & Bee, 2010, 2011; Vélez et al., in review). According to the *natural soundscapes advantage hypothesis*, we predicted that

masked signal recognition thresholds would be lower when level fluctuations in the noise resembled those of natural soundscapes compared to completely artificial level fluctuations.

Our third hypothesis originates from our recent findings showing that level fluctuations in the sounds of frog choruses exhibit species-specific patterns that result from species differences in the temporal properties of signals and signaling behaviors (Vélez & Bee, 2010, 2011; Vélez et al., in review). Langemann and Klump (2005) have suggested that animals may be adapted to exploit level fluctuations typical of the natural soundscape in recognizing communication signals. Thus we might expect animals to be relatively less impaired by noise typical of *conspecific* aggregations. The extent to which an ability to recognize communication signals in fluctuating backgrounds is ‘tuned’ to species-specific fluctuation patterns of the soundscape remains an open question. According to this *species-specific advantage hypothesis*, then, we predicted lower signal recognition thresholds in the presence of noise with fluctuations typical of conspecific choruses compared with heterospecific choruses.

General Methods

Subjects and Study Sites

Procedures used to collect, handle, and test animals were approved by the University of Minnesota’s Institutional Animal Care and Use Committee (IACUC No. 0809A46721). We collected female green treefrogs (*H. cinerea*) in amplexus between 2200 and 0200 hours in April and May 2011 from artificial ponds at the Jasper State Fish Hatchery in Jasper, Texas (30° 57' 4.01" N, 94° 7' 39.53" W; Jasper County, Texas, U.S.A.). Female Cope’s gray treefrogs (*H. chrysoscelis*) were collected in amplexus between 2100 and 0100 hours in May and June 2011 from wetlands at the Carver Park Reserve (44° 52' 49.29" N, 93° 43' 3.10" W; Carver County, Minnesota, U.S.A.) and the Crow Hassan Park Reserve (45° 11' 18.71" N, 93° 39' 9.05" W; Hennepin County, Minnesota, U.S.A.). Females of both species were kept in coolers at approximately 2–4°C to delay oviposition until tested (usually within 24–48 hours) in the laboratory on the St. Paul campus of the University of Minnesota. Female green treefrogs were shipped overnight between Jasper, TX, and St. Paul, MN, in plastic containers in coolers with frozen gel packs. Just prior to testing, we placed females in an incubator for at least 45 minutes to allow their body temperature to reach $24 \pm 1^\circ\text{C}$ (green treefrogs) or $20 \pm 1^\circ\text{C}$ (Cope’s gray treefrogs). After completing all tests, females were released at the pond from which they were collected (usually within three days).

Phonotaxis Experiments

We used the same general protocol and testing equipment as described in recent studies of green treefrogs and Cope’s gray treefrogs (Bee & Schwartz, 2009; Vélez & Bee, 2011; Vélez et al., in review). Readers are referred to those studies for additional details not reported here. Briefly, single-speaker phonotaxis tests (Gerhardt, 1995) were conducted under infrared illumination (IR) inside a walk-in, temperature-controlled, hemi-anechoic sound chamber (details in Bee & Schwartz, 2009). The temperature inside the chamber was set to $24 \pm 1^\circ\text{C}$ for tests with green treefrogs and to $20 \pm 1^\circ\text{C}$ for tests with Cope’s gray treefrogs; these temperatures are close to the average temperatures at which each species breeds in local populations. We tested females in a circular test arena (2 m diameter) with walls made of 60-cm high hardware cloth covered in black fabric so that it was acoustically transparent but visually opaque. The floor of the arena consisted of low-pile carpet covering the vibration isolation floor of the sound chamber (Bee & Schwartz 2009). The perimeter of the arena was divided into 24 15° arcs. An IR-sensitive video camera mounted from the ceiling of the chamber above the center of the test arena was used to score behavioral responses in real time by two observers viewing a monitor outside the chamber. The video

feed was simultaneously encoded to digital video files (Supplementary Material 1). Any discrepancies or uncertainties about an animal's response, which were exceedingly rare, were immediately resolved by watching the video before proceeding to the next trial. This procedure was necessary for accurately determining signal recognition thresholds (see below). We have recently shown our real-time scoring methods to be error-free and as accurate as post-test scoring from offline analyses of videos (Cohen's Kappa = 1.0; Bee, Vélez, & Forester, 2012) and we refer readers to that paper for further discussion.

Digital acoustic stimuli (11,025-Hz sampling rate, 16-bit resolution) were generated using Matlab v7.6 (Mathworks, MA, USA) and broadcast through Orb Mod 1 speakers. The speaker used to broadcast target signals was placed on the floor, just outside the arena wall, centered in one of the 15° arcs, 1 m away from a subject release point at the center of the arena. The position of the target speaker was randomly varied around the arena's perimeter between tests of two to four subjects to eliminate any possibility of directional response bias. We have not observed such biases in our experimental set-up. Masking noises were broadcast from an overhead speaker mounted from the ceiling of the chamber 190 cm above the central release point. The frequency response of our playback system was flat (± 2.5 dB) between 500 Hz and 5,000 Hz, and the overhead speaker created uniform noise levels (± 2 dB) across the entire floor of the test arena. We calibrated sound levels by placing the microphone of a Brüel & Kjær Type 2250 sound level meter at the approximate position of a subject's head at the central release point.

We initiated a phonotaxis test by placing a subject in a small (9 cm diameter, 3.5 cm height) acoustically transparent holding cage made of plastic mesh (0.3 cm mesh size) and located at the central release point of the arena. Subjects could freely reorient inside the holding cage. Broadcast of a masking noise initiated after a 1-min silent acclimation period and continued throughout the entire test. In tests with target signals in experiment 1, broadcast of the target signal initiated 30 s after the onset of the masker. Using a rope and pulley system operated from outside of the chamber, subjects were released after 15 s of signal presentation.

Experiment 1: Signal Recognition in Fluctuating Backgrounds

Methods

Acoustic stimuli

Target signals: For each species, the target signal was a synthetic advertisement call with values of temporal and spectral properties close to the averages of calls recorded in local populations of that species (Figure 1). For green treefrogs, the target signal was a 147-ms long synthetic call that repeated at a rate of approximately 107 calls/min (562 ms call period). The call comprised three phase-locked sinusoids with equal relative amplitudes and frequencies of 850 Hz, 2,550 Hz, and 2,833 Hz. The amplitude-envelope of the call was shaped with a 25-ms inverse exponential rise time and a 50-ms inverse exponential fall time. For Cope's gray treefrogs, the target signal comprised 32 pulses (11 ms pulse duration) delivered at a rate of 45.5 pulses/s (22 ms pulse period). Each pulse consisted of two harmonically related, phase-locked sinusoids with frequencies (and relative amplitudes) of 1,250 Hz (−9 dB) and 2,500 Hz (0 dB). The amplitude-envelope of each pulse was shaped with a 4-ms inverse exponential rise time and a 7-ms exponential fall time. The first 50 ms of the call was shaped with a linear onset. The Cope's gray treefrog target signal repeated at a rate of 12 calls/min (5 s call period).

Masking noises: We measured signal recognition thresholds (see below) in four different masking treatments. Across treatments, the masking noises had the same long-term frequency spectrum (500 Hz – 4,500 Hz; Figure 1) but different temporal envelopes (Figure

2). The masking noise in the control treatment lacked level fluctuations; we refer to this masker as the ‘non-fluctuating’ treatment (Figure 2A). In a ‘randomly fluctuating’ noise treatment, the masker had a random temporal envelope (Figure 2B). The third and fourth treatments were both ‘naturally fluctuating’ treatments in which the maskers had envelopes extracted from recordings of green treefrog choruses (Figure 2C) or Cope’s gray treefrog choruses (Figure 2D). We refer to these maskers as the conspecific treatment and the heterospecific treatment, depending on the species identity of the subject. In the conspecific treatment, green treefrogs experienced maskers with envelopes from green treefrog choruses, and Cope’s gray treefrogs experienced maskers with envelopes from Cope’s gray treefrog choruses. In the heterospecific treatment, each species experienced maskers with envelopes from choruses of the other species.

Figure 3 summarizes the methods used to generate each of our four types of maskers. First, we generated the amplitude envelopes of our fluctuating maskers. To create a randomly fluctuating envelope, we generated a 90-s low-pass noise with a cut-off frequency of 10 Hz and extracted its Hilbert envelope. To create naturally fluctuating envelopes, we used the Hilbert transform to extract the envelopes from 90-s recordings of green treefrog choruses and Cope’s gray treefrog choruses. Full details about recording equipment and protocols are described in Vélez et al. (in review) for green treefrogs and in Vélez and Bee (2010, 2011) for Cope’s gray treefrogs. Briefly, recordings of 50 choruses (25 per species) were obtained on nights and at times of high calling activity using a Marantz PMD 670 recorder and an omnidirectional Sennheiser M62 microphone. The microphone tip was placed 5 cm above ground or water level, between 4 and 15 m from the nearest calling male. We used only recordings in which other species were absent or calling at very low densities and well away from the microphone. Chorus recordings of each species were made on different nights, at different ponds, or at different times and locations within a pond. Naturally fluctuating envelopes were low-pass filtered with a cut-off frequency of 80 Hz.

Next, we generated four copies of a 90-s white noise (11,025-Hz sampling rate, 16-bit resolution). One copy was used to create a non-fluctuating masker. The other three copies were used to create maskers with fluctuating envelopes; one copy was multiplied by a random envelope, a second copy was multiplied by a green treefrog chorus envelope, and the final copy was multiplied by a gray treefrog chorus envelope. The non-fluctuating white noise and the three fluctuating white noises (i.e., one random and two natural) were then converted to the spectral domain with a fast Fourier transform (FFT; window size = 992,250), band-pass filtered between 500 Hz and 4,500 Hz by setting the Fourier coefficients outside this range to zero, and transformed back to the time domain with an inverse FFT. We created six-minute-long maskers of each type by consecutively appending multiple copies of each 90-s noise to itself at the nearest zero-crossings to the beginning and the end of the waveform. To avoid pseudoreplication (Kroodsma, 1989, 1990), we created 25 exemplars of each type of masker by repeating this process 25 times, each time using four copies of a different white noise, a different random envelope, and envelopes from a different chorus recording of each species. Each exemplar was used only once per treatment for each species. Masking treatment and exemplar were randomly assigned for each subject. In all treatments, maskers were broadcast from the overhead speaker with a long-term root-mean-square (RMS) amplitude of 76 dB sound pressure level (SPL re. 20 μ Pa; LCeq; 40 dB spectrum level) calibrated at the central release point of the arena.

Signal recognition thresholds—Using a between-subjects experimental design, we measured signal recognition thresholds for 25 subjects of each species in each of the four masker treatments described above: non-fluctuating, random, conspecific, and heterospecific (2 species $\times$ 4 treatments $\times$ 25 subjects per treatment; total N = 200). As in our previous studies, we operationally defined the signal recognition threshold as the minimum signal

level necessary to elicit positive phonotaxis to a target signal (Bee & Schwartz, 2009; Vélez & Bee, 2011; Vélez et al., in review). Positive phonotaxis to a target signal was scored when the subject (i) first touched the wall of the test arena in the hemi-circle containing the target speaker, (ii) made contact with the arena wall inside the 15° bin in front of the target speaker within 5 min of being released, and (iii) remained in a 30° arc centered in front of the target speaker for 30 s. Therefore, our signal recognition thresholds differ from more traditional signal detection thresholds because, for positive phonotaxis to occur, the target signal has to be (i) detected by the subject, (ii) recognized as a conspecific advertisement call, and (iii) successfully localized. Signal recognition thresholds are more akin to the speech reception thresholds used in studies of masked speech perception in humans (e.g., Bacon et al., 1998; Festen & Plomp, 1990).

To determine the signal recognition threshold, we tested each subject in a series of 8–14 trials. Subjects were given a timeout period of 5–15 min inside the incubator between trials. Each series always began and ended with a test of a reference trial. In the reference trial, we broadcast the target signal at 85 dB SPL (LCF, measured at 1 m) and in the absence of masking noises. This signal level corresponds to natural call amplitudes measured at 1 m for both species (Gerhardt, 1975). We also performed a test of a reference trial after two consecutive test trials (described below) failed to elicit a response. We replaced subjects that failed to meet the response criteria in any reference trial ($N = 19$ green treefrogs and 13 Cope's gray treefrogs). This procedure ensured that all subjects included in the analyses were highly motivated to respond throughout all trials and validated test trials in which subjects failed to respond to the target signal (Bush, Gerhardt, & Schul, 2002).

After the first reference trial, we ran two control trials, a 'sham' trial and an 'attraction' trial, followed by a second reference trial. The sham and attraction trials are explained in detail below as experiments 2 and 3, respectively. After the second reference trial, we started a series of test trials designed to estimate a signal recognition threshold for each subject. In the first test trial, the target signal was broadcast at a level of 76 dB SPL (LCF), which was equivalent to a signal-to-noise ratio of 0 dB. In all subsequent test trials, the level of the target signal was varied contingent upon the response of the subject in the previous test trial. If the subject met the response criteria in the first test trial, the level of the signal in the following test trial was lowered by 3 dB. If, on the other hand, the subject failed to respond, the level of the target signal was increased by 3 dB. We continued decreasing or increasing the level of the target signal contingent on the subject's response in the previous test trial until there was a change in the subject's behavior between two trials (i.e., from response to no-response, or vice versa). Following the trial in which the subject's behavior changed, we ran a final test trial in which the direction of level change was reversed and the step size of the change was reduced to 1.5 dB. For each subject, we calculated the signal recognition threshold as the average between the minimum signal level that elicited positive phonotaxis and the maximum signal level that failed to do so. We calculated the signal recognition threshold in a linear scale and then converted it back to decibels.

Statistical analyses—We compared signal recognition thresholds using a 2 species $\times$ 4 masking treatment factorial analysis of variance (ANOVA). Signal recognition thresholds met the assumptions of normality, Kolmogorov-Smirnov tests: All P s $> .2$, and homogeneity of variance, Levene's test: $F(7, 192) = 1.86$, $P = .08$, for parametric testing. We used a priori planned comparisons to test our three specific hypotheses by setting the contrast weights differently to test each hypothesis of interest (Table 1). Based on the dip-listening hypothesis, we predicted lower signal recognition thresholds in all fluctuating treatments, compared to the non-fluctuating treatment. We tested this prediction by comparing threshold in the non-fluctuating treatment to the mean of the three fluctuating treatments combined and to each fluctuating treatment separately. According to the natural-soundscapes

advantage hypothesis, we predicted lower signal recognition thresholds in naturally fluctuating noise. We tested this prediction by comparing threshold in the random treatment to the mean of the two naturally fluctuating treatments as well as to each one separately. In the presence of naturally fluctuating treatments, the species-specific advantage hypothesis predicted lower signal recognition thresholds in the conspecific treatment. We tested this prediction by comparing signal recognition thresholds in the conspecific and heterospecific treatments.

Results and Discussion

Mean signal recognition thresholds varied within one dB across masking treatments. The main effect of masker treatment was not significant, ANOVA: $F(3, 192) = 1.27$, $P = .287$, partial $\eta^2 = 0.02$ (Figure 4A). Both the main effect of species, $F(1, 192) = 31.49$, $P < .001$, partial $\eta^2 = 0.14$ (Figure 4B), and the species $\times$ masking treatment interaction, $F(3, 192) = 5.65$, $P < .001$, partial $\eta^2 = 0.08$ (Figure 4C), were significant. Overall, signal recognition thresholds were 3.5 dB higher in green treefrogs than in Cope's gray treefrogs (Figure 4B). Because we found significant effects of species and species $\times$ masking treatment, but no effect of masking treatment, we compared signal recognition thresholds across masking treatments separately for each species. Results of individual planned comparisons testing each specific hypothesis are summarized in Table 1.

In green treefrogs, mean signal recognition thresholds varied within 2 dB (73.1 dB to 75.0 dB) across treatments and these differences were not significant, $F(3, 96) = 0.91$, $P = .439$, partial $\eta^2 = 0.03$ (Figure 4C). All three hypotheses of interest were rejected in green treefrogs, as individual planned comparisons revealed no differences in thresholds between the specified treatments (Table 1).

In contrast, the effect of masking treatment was significant in Cope's gray treefrogs, $F(3, 96) = 6.19$, $P < .001$, partial $\eta^2 = 0.162$ (Figure 4C), with mean signal recognition thresholds varying over a 5 dB range between 67.4 dB and 72.4 dB across treatments. In Cope's gray treefrogs, there was no difference between the mean threshold in the non-fluctuating treatment and the averaged threshold across the three fluctuating treatments (Table 1). Mean thresholds in each of the two naturally fluctuating treatments were also not different from that in the non-fluctuating control when tested separately. However, consistent with the dip listening hypothesis, signal recognition thresholds were significantly lower in the random treatment than in the non-fluctuating treatment (Table 1). The threshold difference between the non-fluctuating and random treatments was 3.3 dB, which a previous study of this species has shown is large enough to be biologically significant in terms of influencing female phonotaxis behavior (Bee, et al., 2012). Together, these results suggest that female Cope's gray treefrogs can listen in the dips of fluctuating maskers, but this ability provided little benefit in terms of reduced thresholds in the naturally fluctuating backgrounds tested in our experiment.

We found significant differences when we compared thresholds in the random treatment to those in the naturally fluctuating treatments (Table 1). However, in contrast to the natural soundscape advantage hypothesis, thresholds were actually higher in the naturally fluctuating treatments by 4 to 4.5 dB (Figure 4C). Hence, under the conditions tested in this study, signal recognition in fluctuating noise was actually better in the presence of maskers with artificial level fluctuations compared to natural ones. These results are inconsistent with the natural soundscapes advantage hypothesis, and we therefore reject this hypothesis as an explanation for our data. Similarly, we found no evidence to support the species-specific advantage hypothesis; signal recognition thresholds did not differ between the conspecific and heterospecific treatments (Table 1).

Together, our results suggest the following. There are species differences in dip listening abilities in frogs. While female green treefrogs did not listen in the dips of any of the fluctuating noise treatments tested in this study, Cope's gray treefrogs experienced dip listening in one of them. However, the ability of Cope's gray treefrog females to listen in the dips of fluctuating maskers appears to offer little benefit in naturally fluctuating backgrounds and was restricted to the random fluctuations treatment. Furthermore, our results suggest that dip listening in Cope's gray treefrogs is not tuned to level fluctuations of the soundscape of conspecific choruses.

Experiment 2: Behavior in the absence of target signals

Estimates of signal recognition thresholds could be affected if the maskers were not neutral stimuli and influenced female movement patterns. For instance, maskers that sound more like a conspecific breeding chorus could elicit higher levels of searching behavior, such as higher overall movements around the test arena, affecting the probability of recognizing low-level signals in phonotaxis tests. To assess the possibility that female behavior in our testing apparatus was differently affected by the maskers, we conducted a 'sham' trial in which we measured subject movement patterns in the presence of the same maskers used in experiment 1, but in the absence of any target signal.

Methods

During sham trials, each masker was broadcast continuously from the overhead speaker with a long-term RMS amplitude of 76 dB SPL (LCeq). Because no target signals were broadcast from speakers on the floor, there was no 'correct response' in the sham trial. Therefore, we did not apply any response criteria during sham trials and recorded subject behavior over 5 min of masker presentation after being released.

Statistical analyses—We analyzed movement patterns in two ways. First, we tested the hypothesis that the maskers did not affect subjects' tendency to move in the test arena. We scored whether or not subjects left the holding cage during the five minutes of masker presentation as a binomial response (1 = yes, 0 = no). We calculated the proportions of subjects leaving the holding cage in the four masking treatments and their corresponding exact 95% binomial confidence intervals. We reasoned that, if some maskers elicited more or less overall movements, then the proportions of subjects leaving the release cage would differ across treatments, as indicated by a lack of overlap among 95% binomial confidence intervals.

Second, we tested the hypothesis that movement patterns in the test arena of the subjects that left the holding cage did not differ across masking treatments. We used the software Ethovision XT (Noldus, 2010) to measure each individual's total distance moved (in cm), the average velocity of their movements (in cm/s), the average angle of their turns (in degrees), and a composite measure called meander (in cm/s), which is a measurement of the amount of change in direction relative to the distance moved (see Bee & Riemersma, 2008). The last two measurements were included because they are potentially related to sound localization behaviors (Rheinlaender & Klump, 1988). Because the assumptions of normality and homoscedasticity for parametric testing were not met, we used non-parametric tests to investigate the effect of masker type (Kruskal-Wallis tests) and species (Mann-Whitney U tests) on median values of these movement measures.

Results and Discussion

The number of subjects leaving the holding cage across treatments varied between two and nine in green treefrogs, and between 17 and 25 in Cope's gray treefrogs (Figure 5A). Within

each species, the 95% binomial confidence intervals of the corresponding proportions exhibited considerable overlap across the four types of maskers. These results suggest that subject tendencies to move in our testing apparatus were not differently affected by the maskers. The 95% confidence intervals do not overlap between species in any treatment, suggesting Cope's gray treefrogs left the holding cage more often than green treefrogs.

For all the subjects that left the holding cage during sham trials, we calculated the total distance moved and velocity. Turn angles and meander could not be calculated for three green treefrogs and one Cope's gray treefrog because the distance moved was very short and in a straight line. Analyses of movement patterns revealed no differences across masking treatments for either species in total distance moved (Figure 5B, Table 2), velocity (Figure 5C, Table 2), turn angles (Figure 5D, Table 2), and meander. Because there were no differences across treatments and sample sizes are very small for green treefrogs, we pooled data across treatments to investigate between-species differences in each of the four variables measured. We found no differences between species in turn angles (Mann-Whitney U Test: $U = 822.0$, $P = .85$) or meander ($U = 758.0$, $P = .48$). However, the total distance moved ($U = 303.0$, $P < .001$) and velocity ($U = 647.0$, $P < .001$) were significantly higher in Cope's gray treefrogs (medians = 482 cm; 1.7 cm/s) than in green treefrogs (medians = 248 cm; 1.3 cm/s).

Together, results from these two analyses indicate that the maskers by themselves did not differentially affect subject movement patterns in our test arena (Figure 5). Hence, the effect of the maskers on subject behavior had little potential to confound our estimates of signal recognition thresholds within each species. The between-species differences in the proportion of subjects leaving the holding cage and in the total distance moved might account for the 3.5 dB difference in overall signal recognition thresholds between green treefrogs and Cope's gray treefrogs (Figure 4B). At low signal levels, females of both species may not recognize the target signal while in the holding cage. However, because Cope's gray treefrog females tend to wander around the test arena, they have a greater probability of reaching places in the arena where the signal-to-noise ratio is higher than in the holding cage. Consequently, Cope's gray treefrogs also have a higher probability of recognizing the signal at lower levels in our experimental setup.

Experiment 3: Maskers as Potential Signals

Another potential confound in estimating signal recognition thresholds stems from the possibility that our maskers acted like salient and biologically relevant signals that perceptually competed with the target signals. By 'compete,' we mean that some of our maskers may have been perceived inadvertently as attractive signals and not simply as masking noise. For instance, males and females of some frog species can use the sound of conspecific choruses as acoustic beacons to locate breeding aggregations (Gerhardt & Klump, 1988; Bee, 2007a; Swanson, Tekmen, & Bee, 2007; Christie, Schul, & Feng, 2010). Therefore, the aim of the attraction trials was to evaluate the possibility that our maskers competed with the target signal. Specifically, we tested the null hypothesis that the maskers used in experiment 1 were not attractive to females.

Methods

In attraction trials, we broadcast each noise used as a masker in experiment 1 from a speaker on the floor, just outside the wall of the arena, centered in the 15° bin that was 180° away from the speaker used to broadcast the target signal in experiment 1. Noises were broadcast continuously during a trial and their long-term RMS amplitudes were calibrated to 76 dB SPL (LCeq). After release, we measured the angle at which subjects first touched the wall of the arena in 15° bins. As in previous similar studies (Swanson et al., 2007; Vélez & Bee,

2010, 2011; Vélez et al., in review), we ended the attraction trials as soon as subjects touched the wall anywhere around the test arena or after five minutes of stimulus presentation.

Statistical analyses—We investigated subjects' behavior in attraction trials using a categorical analysis. We first grouped the angles at which subjects reached the arena wall in eight 45° bins. In response to attractive sounds, subjects typically reach the arena wall in the bin in front of the speaker or one bin to either side (Vélez & Bee, 2010). We then grouped all responses into two categories. The first category included responses in which subjects first touched the arena wall inside the 45° bin in front of the target speaker. The second category included all other response angles as well as trials in which subjects failed to touch the arena wall. The inclusion of the latter trials is important because these data are biologically relevant and cannot be included in analyses using circular statistics. We used a Fisher's exact test to compare categorical responses in all treatments to those in the absence of noise, and calculated the phi coefficient as a measurement of the effect size. Our reasoning was that if maskers were attractive, then more females than expected by chance should touch the wall inside the 45° bin centered in front of the speaker. From two previous studies (Vélez & Bee, 2010, Vélez et al., in review), we reanalyzed data and calculated the proportion of subjects first touching the arena wall inside the 45° bin in front of a designated, but silent, target speaker. These null expected proportions are 0 (0 out of 24 subjects) and .083 (two out of 20 subjects) for green treefrogs and Cope's gray treefrogs, respectively (Vélez & Bee, 2010, Vélez et al., in review).

Results and Discussion

The proportion of female green treefrogs reaching the arena wall inside the 45° bin in front of the target speaker (Figure 6A) was not significantly different from the null expectation in the non-fluctuating, conspecific, and heterospecific treatments (two-tailed Fisher's exact test, all P s = 1.0). However, the proportion of green treefrogs approaching the target speaker was significantly higher than the expected by chance in the random treatment ($P < .01$). In Cope's gray treefrogs (Figure 6B), the proportion of subjects reaching the target speaker was not significantly different from the null expectation in the non-fluctuating ($P = .269$), random ($P = .269$), and heterospecific ($P = .437$) treatments. In the conspecific treatment, however, the proportion of subjects that reached the target speaker was significantly higher than the null expectation ($P = .004$).

Green treefrogs were attracted to noises with random level fluctuations and Cope's gray treefrogs were attracted to those with conspecific level fluctuations. Consequently, these maskers had some potential to 'compete' with the target signal during test trials of experiment 1. For instance, at low signal-to-noise ratios, females may have recognized the target signal but not responded to it because they were more attracted to the overhead maskers (which they could not reach) than to the target signals. However, we believe the degree of 'competition' for subjects' responses between these maskers and the target signals was likely small. In two previous studies we measured female responses to low-level signals (< 76 dB SPL) in the absence of background noise. In green treefrogs, 21 out of 24 females reached the wall inside the 45° bin in front of the speaker at signal levels between 42 dB and 75 dB (mean signal level = 55.3 dB SPL; Vélez et al., in review). Out of 20 Cope's gray treefrog females tested in the absence of noise and at a signal level of 61 dB SPL, 19 approached the target speaker (Vélez & Bee, 2010). These proportions of subjects that responded to low-level signals are much higher than the proportions of subjects that responded to the noises of the present study. Hence, the attractive maskers in the present study should be regarded as marginally attractive at best.

Together, our results from experiments two and three suggest that the potential confounding effects of the attractiveness of the random and conspecific maskers in green treefrogs and Cope's gray treefrogs, respectively, were probably small. More importantly, we can exclude the potentially confounding treatments and still test the specific hypotheses of this study. With green treefrogs females, we can test both the dip-listening and species-specific advantage hypotheses after excluding the random treatment. Likewise, with Cope's gray treefrog females we can test the dip-listening and natural sounds advantage hypotheses after excluding the conspecific treatment. The results of these analyses parallel those reported above for the full data set (Supplementary Material 2). Therefore, our study offers strong evidence allowing us to reject both the natural soundscape advantage and the species-specific advantage hypotheses. Our study also offers strong evidence for the operation of dip-listening mechanisms in Cope's gray treefrogs under some noise conditions, such as those present in our random fluctuations treatment.

General Discussion

Results from this study suggest that female green treefrogs and Cope's gray treefrogs may not be adapted to exploit level fluctuations of the chorus noise to recognize male advertisement calls. As in a previous study (Vélez et al., in review), we show here that level fluctuations in the background noise have little effect on signal recognition in green treefrogs. While Cope's gray treefrog females possess an ability to listen in the dips of maskers with random (this study) or sinusoidal (Vélez & Bee, 2011) level fluctuations, this ability appears not to be tuned to the physical properties of the natural soundscape. These results with Cope's gray treefrogs parallel those with human listeners showing that sinusoidal and random amplitude modulations provide greater dip-listening benefits than level fluctuations of multi-talker environments (Gustafsson & Arlinger, 1994; Bacon et al., 1998).

Our results are, to some extent, in line with results from previous studies on signal recognition in fluctuating noise in both species (Vélez & Bee, 2011; Vélez et al., in review). In those studies, we investigated the effect of level fluctuation rate on signal recognition using maskers that either lacked level fluctuations or were sinusoidally amplitude modulated (SAM) at rates ranging from 0.625 Hz to 80 Hz, in octave steps. Female green treefrogs did not experience dip listening in the presence of any SAM masker (Vélez et al., in review). In Cope's gray treefrogs we found (i) dip listening when the fluctuation rates of the SAM maskers were equal to or slower than 2.5 Hz, (ii) no effect when masker fluctuation rates were between 5 Hz and 20 Hz, and (iii) additional masking when fluctuation rates were 40 Hz and 80 Hz (Vélez & Bee, 2011). The additional masking was explained by a process known as 'modulation masking,' which describes increased difficulty to detect fluctuating signals in the presence of fluctuating maskers (Bacon & Grantham, 1989).

Why Cope's gray treefrogs did not experience dip listening in maskers with level fluctuations of conspecific choruses might be explained by the rates at which these sounds fluctuate. Sound levels in Cope's gray treefrog choruses fluctuate both at rates below 3 Hz and at rates between 40 Hz and 60 Hz (Vélez & Bee, 2010; Figure 2D). The slow rates of level fluctuation could lead to dip listening while fluctuation rates between 40 Hz and 60 Hz could lead to modulation masking (Vélez & Bee, 2011). Therefore, the lack of dip listening in the conspecific treatment could be due to an interaction between dip listening and modulation masking. An interaction of this type has been invoked to explain some observed patterns of human speech recognition in the presence of fluctuating backgrounds (Kwon & Turner, 2001).

It is important to note that we generated conspecific maskers using chorus recordings in which conspecific males were the only or the most common vocally active individuals. While this is the case for part of the breeding season of both species at our study sites, these species often form mixed-species choruses. Green treefrogs can be vocally active with eastern gray treefrogs (*H. versicolor*), squirrel treefrogs (*H. squirrela*), fowler's toads (*Bufo fowleri*) and Gulf Coast toads (*B. nebulifer*). Cope's gray treefrogs are sometimes active with eastern gray treefrogs (*H. versicolor*), American toads (*B. americanus*) and Boreal chorus frogs (*Pseudacris maculata*). The advertisement calls produced by all of these heterospecific species are pulsed and have frequency spectra that overlap with that of the background noise of green treefrog and Cope's gray treefrog choruses (Rose & Capranica, 1984; Gerhardt & Doherty, 1988; Sullivan & Wagner, 1988; Taylor, Buchanan, & Doherty, 2007; Bee et al., 2010). Furthermore, green treefrogs and Copes' gray treefrogs may be found in sympatry in some parts of their geographic ranges (Conant & Collins, 1998). Studies on call interference suggest that heterospecific call overlap can disrupt the perception of important species-recognition cues, affecting a female's ability to recognize appropriate mates (Marshall et al., 2006; Nityananda & Bee, 2011). Due to partial spectral and temporal overlap, heterospecific signals in mixed-species choruses are therefore likely to have a negative effect on call recognition.

In natural settings, recognition of advertisement calls by female green treefrogs and Cope's gray treefrogs may be influenced by spatial, spectral, and temporal relationships between the target signals and competing sounds. As is the case for receivers in other taxa (reviewed in Bee & Micheyl, 2008), female green treefrogs and Cope's gray treefrogs experience a release from auditory masking when the sources of signals and noise are spatially separated (Bee, 2007b, 2008; Schwartz & Gerhardt, 1989; Nityananda & Bee 2012). Cope's gray treefrog females also exploit spectral differences as a cue to perceptually separate signals from distracting sounds (Nityananda & Bee, 2011). Therefore, synergistic interactions between spatial, spectral, and temporal cues might contribute to ameliorating the problem of signal recognition in noisy natural settings.

Why Cope's gray treefrogs and not green treefrogs listen in the dips of some fluctuating backgrounds remains an open question. The difference in the duration of the target signal might account, in part, for these results. In humans, the ability to detect signals in fluctuating maskers increases with signal duration (Schooneveldt & Moore, 1989; but see Klump & Langemann, 1995 for a negative result in birds). Another possible explanation for between-species differences in dip-listening capabilities concerns differences in temporal resolution of the auditory system. Because important evolutionary processes such as mate choice and species isolation are predominantly mediated by spectral and temporal properties of the advertisement call in green treefrogs and Cope's gray treefrogs, respectively (Gerhardt, 1982, 2001; Höbel & Gerhardt 2003, Schul & Bush, 2002), temporal resolution of the auditory system may be better in Cope's gray treefrogs than in green treefrogs. Comparative physiological and behavioral studies on temporal processing in the context of signal recognition in noise are therefore necessary to elucidate potential between-species differences in the underlying mechanisms for communication in noise. To date, most physiological studies on temporal processing in anurans have focused on how amplitude modulation is encoded along the auditory pathway and how species-specific temporal features of signals are processed (reviewed in Feng, Hall, & Gooler, 1990; Klump, Benedix, Gerhardt, & Narins, 2004; Rose & Gooler, 2006).

Dip listening appears to be a widespread hearing mechanism shared by animals of different taxa (birds: Bee, Buschermöhle, & Klump, 2007; Hofer & Klump, 2003; Jensen, 2007; Klump & Langemann, 1995; Langemann & Klump, 2001, 2007; Nieder & Klump, 2001; cats: Nelken et al., 1999; dolphins: Branstetter & Finneran, 2008; fish: Fay, 2011; frogs:

Goense & Feng, 2012; Vélez & Bee, 2011; grasshoppers: Ronacher & Hoffmann, 2003). However, this is the first study, to our knowledge, to investigate the ability of nonhuman animals to listen in the dips of natural soundscapes. Our results provide little support for the hypothesis that receivers are adapted to exploit specific level fluctuations typical of the natural soundscape to recognize communication signals (Langemann & Klump, 2005). Whether this is just the case for frogs or whether it is a widespread phenomenon is still an open question. We suggest that, in order to understand the evolution of auditory processing mechanisms for communication in noisy environments, it is of great importance to (i) take comparative approaches in behavioral and physiological studies, (ii) investigate the properties of natural soundscapes and how they affect signal recognition, and (iii) use both natural and artificial stimuli to better understand the capabilities and limitations of the auditory system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Waveform of 0.8-s segments (top panel) and frequency spectrum (bottom panel) of the synthetic call used as a target signal for tests with green treefrogs (A) and Cope's gray treefrogs (B). Frequency spectra were calculated with 256-point Hamming windows. For reference, we also show here the frequency spectrum of the maskers (gray line).

Figure 2.

Waveform of 10-s segments (top pannel) and mean (solid line) $\pm$ 1 standard deviation (dashed lines) modulation spectra (bottom pannel) of 90-s maskers with no level fluctuations (A), synthetically generated random fluctuations (B), or natural level fluctuations characteristic of green treefrog choruses (C) or Cope's gray treefrog choruses (D). Mean modulation spectra were calculated from the 25 90-s exemplars of each masker and are shown on a logarithmic x axis. Modulation spectra were generated by first extracting the Hilbert envelope of the waveform. To correct for the DC offset, we subtracted the mean value of the envelope from each sample of the envelope. The modulation spectrum of each masker was calculated as the fast-Fourier transform (FFT) of the envelope (sampling rate = 11025 Hz, Hamming window size = 65536 points, overlap = 25%). We normalized the spectrum of each 90-s segment to the maximum value of the magnitude of the FFT and calculated the mean and standard deviation of the 25 modulation spectra of each type of masker. Mean and $\pm$ 1 standard deviation modulation spectra were transformed to a dB scale ($20 \cdot \log_{10}(\text{FFT magnitude})$) and smoothed for plotting purposes using a running average of 11 points.

Figure 3. Schematic representation of the method used to generate the different maskers. This process was repeated 25 times, each one with a different low-pass (10 Hz cut-off) random noise, a different chorus recording of each species, and a different white noise. See text for additional details.

Figure 4.

Mean ($\pm 95\%$ confidence intervals) signal recognition thresholds in Experiment 1 as a function of masking treatment (A), species (B), and masking treatment for each species (C).

Nf = Non-fluctuating, Consp. = Conspecific, Heterosp. = Heterospecific, and Rand. = Random. In (C), open circles represent *H. cinerea* and filled circles represent *H. chrysoscelis*.

Figure 5.

(A) Proportions ($\pm 95\%$ confidence intervals) of females leaving the holding cage during sham trials of Experiment 2 as a function of masking treatment. The total number of females tested in each treatment was 25. Median (point), inter-quartile range (box), and non-outlier range (whiskers), values for total distance moved (B), velocity (C), turn angle (D), and meander (E). Nf = Non-fluctuating, Consp. = Conspecific, Heterosp. = Heterospecific, and Rand. = Random. Open bars and boxes represent *H. cinerea* and filled bars and boxes represent *H. chrysoscelis*.

Figure 6.

Angles at which subjects reached the arena wall in attraction trials of Experiment 3 for green treefrogs (A) and Cope's gray treefrogs (B) in the nonfluctuating (far left), random (middle left), conspecific (middle right) and heterospecific (far right) treatments. Each point depicts where a subject first touched the wall, in 15° bins (solid, short lines), relative to the position of the target speaker (top of each circle). Dotted lines represent 45° bins. See text for additional details.

Table 1

Loadings and results of planned comparisons testing each specific hypothesis for each species. Significant P values in bold

Hypothesis	Treatment loadings				Green treefrogs			Cope's gray treefrogs		
	Non-Fluctuating	Random	Conspecific	Heterospecific	F(1, 96)	P	partial η^2	F(1, 96)	P	partial η^2
Dip listening	-3	1	1	1	0.07	0.798	0.001	0.18	0.669	0.002
	-1	1	0	0	0.53	0.469	0.005	7.76	0.006	0.075
	-1	0	1	0	0.34	0.563	0.004	1.57	0.213	0.016
	-1	0	0	1	0.59	0.441	0.006	0.23	0.631	0.002
Natural-Soundscapes advantage	0	-2	1	1	2.63	0.108	0.027	17.79	<0.001	0.156
	0	-1	1	0	1.71	0.194	0.017	16.31	<0.001	0.145
	0	-1	0	1	2.25	0.137	0.023	10.68	0.002	0.100
Species-Specific advantage	0	0	-1	1	0.04	0.847	<0.001	0.59	0.443	0.006

Table 2

Results from analyses of movement patterns in the test arena during sham trials of Experiment 2

Variable	Green treefrogs		Cope's gray treefrogs	
	Kruskal-Wallis	<i>P</i>	Kruskal-Wallis	<i>P</i>
Total distance moved	$H(3,22) = 0.35$	.95	$H(3,90) = 0.96$	.81
Velocity	$H(3,22) = 1.77$	.62	$H(3,90) = 0.41$	.99
Turn angles	$H(3,19) = 0.6$	.93	$H(3,89) = 1.87$	.60
Meander	$H(3,19) = 0.74$	.86	$H(3,89) = 2.76$	.43