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# Female green treefrogs (*Hyla cinerea*) do not selectively respond to signals with a harmonic structure in noise

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**Summary.** 1. Females of the green treefrog, *Hyla cinerea*, communicate in noisy environments, with spectrally complicated signals. A previous study (Megela Simmons 1988), using the reflex modification technique, found that the masked threshold of green treefrogs to two-tone signals differed by about 10 dB depending on whether or not the two components were harmonically-related. The present study used the same two-component stimuli to test the prediction that gravid females would better detect harmonic sounds in noise than inharmonic ones.

2. We offered gravid treefrogs simultaneous choices between alternative two-component synthetic sounds: (1) an inharmonic sound of 831 + 3100 Hz, and a harmonic sound of 828 + 2760 Hz. We varied the sound pressure level (SPL in decibels [dB]) to which we equalized these alternatives at the female's release point (75 and 80 dB SPL), and we tested females in quiet conditions and in the presence of broadband background noise (52 dB/Hz at the female's release point).

3. At a signal playback level of 75 dB SPL, one-third of the females responded in the presence of background noise. Subtracting the spectrum level yields a critical ratio estimate of 23 dB, a value that is very similar to estimates for single pure tones in noise reported in other studies of this species (Ehret and Gerhardt 1980; Moss and Megela Simmons 1986). Females did not, however, choose the harmonic sound over the inharmonic sound in this condition, at the higher signal-to-noise ratio, or in either of the unmasked situations.

4. The failure of females to prefer harmonic to inharmonic signals in noise strongly suggests that there was no difference in the detectability of these two signals under the test conditions, which conservatively mimic those of a typical breeding chorus.

**Key words:** Masked threshold – Signal detection – Noise – Harmonic-inharmonic waveform

## Introduction

Green treefrogs (*Hyla cinerea*) communicate by means of stereotyped, acoustically complex vocalizations. A series of studies using synthetic calls has identified the major physical properties of natural signals that individuals of this species use to recognize other conspecifics and to differentiate between signals in the species repertoire (see review by Gerhardt 1988). A bimodal spectrum, with a low-frequency peak at about 900 Hz and a high-frequency peak centered at about 3000 Hz, and a waveform quasi-periodicity of about 300 Hz are characteristic of a typical advertisement call and of a synthetic call of equal attractiveness to gravid females. The quasi-periodicity is reflected in the spectrum as a series of components at intervals of about one-third of the frequency of the low-frequency spectral peak; some of these components are sidebands resulting from amplitude modulation and others are harmonics of the low-frequency peak (Oldham and Gerhardt 1975).

Psychophysical studies, using the reflex modification technique (Megela Simmons et al. 1985), and neurophysiological studies (Capranica and Moffat 1983) indicate that the frequency sensitivity of the green treefrog's auditory system is biased toward the two major spectral peaks in its vocalizations. More interestingly, Moss and Megela Simmons (1986) showed that the pure-tone critical ratio function was parallel in shape to the pure tone audiogram. Thus, the auditory system of this species very effectively extracts signals from noise in the same regions of the spectrum to which it is also most sensitive.

Megela Simmons (1988) recently presented psychophysical data from studies of *H. cinerea* suggesting that this species is sensitive to the harmonic structure of complex sounds as a specific acoustic feature. Specifically, she found that the masked threshold to combinations of two frequency components, one with a frequency at or near 900 Hz and the other with a frequency at or near 3000 Hz, differed by as much as 10 dB, depending on whether or not the combination of two tones had a stable, first harmonic periodicity of about 300 Hz.

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In the Introduction of her paper, Megela Simmons (1988) referred to the fact that the female treefrog must be able to isolate and locate the source of a single male's sounds within a noisy chorus of conspecific and heterospecific males. Her results predict that the harmonic structure of conspecific advertisement calls should make these signals more readily detectable in such natural, noisy situations than signals otherwise similar in their spectral composition but without harmonically-related components. The main aim of this study was to learn if the difference in detectability of these two kinds of signals, demonstrated in psychophysical experiments, is reflected in the natural behavior of the female under the conditions of higher signal and noise levels that exist in treefrog choruses.

## Methods

We synthesized experimental stimuli via software and an AT & T 6300 microcomputer, output them via a custom-designed D/A interface board (G. Klump, Munich), and recorded them on magnetic tape (Sony Tc-D5M cassette, Dolby C). One stimulus, consisting of phase-locked harmonically-related components of 828 and 2760 Hz of equal amplitude, was recorded on one channel of the recorder. The other stimulus, consisting of the inharmonic combination of 831 and 3100 Hz of equal amplitude, was recorded on a second channel of the recorder. The frequencies and relative amplitudes of these stimuli were the same as in two of the stimuli used by Megela Simmons (1988). Each signal lasted 160 ms, had the same rise-fall characteristics, repetition rate (0.8 s) and timing relationship as in previous studies of phonotaxis (e.g. Fig. 2 in Gerhardt 1974). Specifically, the timing relationship of the two stimuli was fixed such that there was an equal period of silence between the end of one signal and the start of the other signal. Broad-band noise was generated by a General Radio 1390B noise generator and recorded on both tracks of a second Sony cassette recorder.

The test stimuli were amplified with a Quad 303 stereo-amplifier and were broadcast from a pair of Realistic Minimus-7 speakers separated by 2 m. Background noise was amplified by a pair of Nagra DH amplifiers and played back from two Analog-Digital-Systems (ADS) 200 speakers. An ADS speaker was placed immediately adjacent to each of the Minimus speakers. The frequency response of the playback systems was checked with a General Radio 1933 sound level meter and a Brüel and Kjaer 1621 tunable filter. The amplitudes of the spectral peaks of all of the stimuli were equal ($\pm 1$ dB) at the point midway between the speakers where the females initially were released. The noise spectrum was flat ($\pm 3$ dB) from 0.1 to 3 kHz and above 3 kHz rolled off gradually at about 3 dB/octave to 10 kHz. Noise levels were not measurably different at the two regions of the spectrum (0.80–0.85 and 2.7–3.1 kHz) corresponding to the frequencies of the spectral peaks in the synthetic signals.

Overall levels of the synthetic signals were equalized at 75 or 80 dB SPL at the release point midway between the speakers. The noise level from each speaker was adjusted to be 77 dB in the 1 kHz-octave band at the same point. When both speakers were activated, as in the experiments, the noise level was 80 dB in the 1 kHz-octave band at the release point. This was equivalent to a spectrum level of 52 dB/Hz.

We collected females of *H. cinerea* in amplexus in ponds in Savannah, Georgia during June 1988. After capture, we refrigerated them at about 4 °C to inhibit oviposition and tested them the following day. Experiments took place at $25 \pm 2$ °C in part of a dimly illuminated (25 W red bulb) room that was lined with acoustic foam and anechoic wedges to reduce sound reflections. After each female had warmed to room temperature, we placed her individually in a small hardware cloth cage covered with a plexiglass top in the center of a 2 m diameter circle. After the stimuli and noise (if used) had been played back for about 30 s, we remotely removed the top of the cage by a string and observed the movements of the female from a small opening in a black cloth curtain that covered the entrance to the testing arena.

We recorded a response if the female left the cage and moved to within 30 cm of a speaker within 5 min. If she failed to move during this time or wandered slowly about the arena without orienting to a speaker that broadcast a signal, then we considered this to be a 'no response'. The latter criteria were same as those used by Ehret and Gerhardt (1980) to define masking. We switched the sources of the two different signals periodically in order to avoid any directional biases of the animals to the testing arena. None were detected. Usually there was one trial per female in any one condition (signal levels of 75 or 80 dB, masked, or unmasked signals). Two exceptions are discussed below. A minimum of 5 min elapsed from the test of a female in one experiment and a subsequent test in another experiment. The order of the experiments varied; 5 females were first tested in a masked situation; 12 were first tested with unmasked signals.

## Results

We tested 17 females. Six females were tested in all 4 experiments, 4 females, in 3 experiments, and 6 females, in 2 experiments. One female was not tested further after it failed to respond to either signal in the unmasked situation.

In the masked situation (Table 1 A), 5 of 15 females responded to a stimulus when the signal levels were 75 dB SPL. Ten of these same frogs, and one additional frog were tested when the signal levels were 80 dB SPL; all 11 responded. Eight females were first tested in the masking situation with 80 dB signals; 7 were first tested in a masking situation with 75 dB signals.

In the unmasked situation (Table 1 B), 10 of 12 frogs responded when the alternative signals were 75 dB SPL. All of these frogs had also been tested first in a masking

**Table 1.** Responses of 17[a] females of *H. cinerea* in two-stimulus playback experiments

| | Signals: 75 dB SPL | | | Signals: 80 dB SPL | |
|---|---|---|---|---|---|
| No. frogs tested | No. frogs responding to: | | No. frogs tested | No. frogs responding to: | |
| | inharmonic stimulus | harmonic stimulus | | inharmonic stimulus | harmonic stimulus |
| A. Background masking: 100–10000 Hz at 52 dB/Hz spectrum level | | | | | |
| 15 | 3 | 2 | 11 | 8 | 3 |
| B. No masking | | | | | |
| 12 | 5 | 5 | 11 | 5 | 6 |

[a] One response per female in any one two-stimulus experiment; one female did not respond in any test. See the text for additional details about the number of tests per female and the order of tests

experiment, and 5 of those that responded had also done so in a test of unmasked signals at 80 dB SPL. All 11 of the frogs tested with unmasked signals at 80 dB SPL responded; this was the first test for 7 of these females; 4 had been previously tested in a masked situation.

The females did not prefer either kind of signal in any of the experiments. The sample size was inadequate for meaningful statistical tests, but the choices of females in one test did not appear to affect their choices in subsequent tests. For example, the choices of 3 females to unmasked signals at 75 dB SPL were the same as they previously made to unmasked signals at 80 dB SPL, but 2 other females chose a different signal in the second test. Similarly, 3 females chose the same unmasked signal (at 80 dB) that they previously chose in the masked situation, whereas 3 other females chose a different unmasked signal. Gerhardt (1981, appendix) provides an extensive analysis of this issue; there was no evidence that females of *H. cinerea* tested multiple times with the same stimulus were biased one way or another by their experience in the first test, even when two tests occurred closely together in time, i.e., without a time-out period of 5 min or more as in the present study.

Two females were tested twice in the masked condition with signal levels at 80 dB SPL. One responded both times to the harmonic stimulus. The other first responded to the inharmonic sound and second, to the harmonic sound. The second responses of these animals are excluded from the tallies in Table 1 A.

## Discussion

The hypothesis addressed in the present study, and suggested by the results of Megela Simmons (1988) was that the harmonic sound would be detected at a lower signal-to-noise ratio (about 10 dB) than the inharmonic sound. We assume that this would have manifested itself as a preference for the harmonic sound at signal-to-noise ratios near or just above the masked threshold for the harmonic sound; the inharmonic sound would still be masked under these conditions.

A preference for the harmonic sound in the masked condition could have been unequivocally interpreted as a difference in detectability if, in addition, the animals had failed to select the harmonic sound in the unmasked condition. Indeed, in a previous study of the phonotactic behavior of the same species, Gerhardt (1978) showed that in quiet conditions females did not preferentially respond in a choice between two-component synthetic calls, one with the species-typical waveform periodicity of 300/s and an alternative of 900/s. As in the present study the animals would have had to detect differences in the fine details of the waveform itself in order to make such a discrimination. Other experiments in the phonotactic study (the discrimination of beats and amplitude modulated noise) indicated that discrimination of differences in periodicity depended on concomitant differences in the amplitude-time envelope of the signals.

Our results indicate that the masked threshold for responses to either of the two-component synthetic signals was about 75 dB SPL. About one-third of the animals responded to a signal at this playback level, and in these simultaneous choice experiments they did not prefer the harmonic stimulus to the inharmonic stimulus.[1] The critical ratio for both harmonic and inharmonic sounds was thus 23 dB, which is the difference between masked threshold and the noise spectrum level of 52 dB. This value is very similar to the previous estimates of critical ratios for single pure tones in the same range of frequencies. Moss and Megela Simmons (1986) and Megela Simmons (1988) used the reflex modification procedure; Ehret and Gerhardt (1980) used phonotactic tests involving both single speaker (detection) and two-speaker tests. Moreover, another study (Schwartz and Gerhardt 1989), which used the phonotactic procedure with green treefrogs under the very same conditions and in the same test arena as in our present study, yielded estimates of masked thresholds of between 72 and 78 dB SPL (and corresponding critical ratios of 20–26 dB) for synthetic calls made up of 3 harmonically-related components (0.9 + 2.7 + 3.0 kHz) having a waveform periodicity of 300 Hz in the form of beats.

What might account for the differences between the results of our experiments and those of the study by Megela Simmons (1988)? There are several major differences in the two procedures. The reflex modification technique yields a measurement after each single presentation of a stimulus; the technique does not depend on the motivation of the subject. In the phonotactic procedure a gravid female listens to a succession of alternations of two sounds before moving to one of the sound sources. Megela Simmons (1988) mixed the tonal stimuli with broadband masking noise and played back the combination from a single speaker. She held the spectrum level of the noise constant at 25 dB/Hz, and varied the signal levels in 10 dB steps from 30–70 dB. Thus the absolute values of both signals and noise were lower in the psychophysical study than in the present study. We think that this was the most likely reason for the difference in the results of the two studies, and may reflect the fact that CR-bands widen as masker levels exceed about 40 dB/Hz (Ehret and Gerhardt 1980). One problem with attempting to repeat our present experiments at lower signal and noise levels is that under conditions of no masking only about one-third of green treefrog females respond to signals at playback levels of less than about 60 dB SPL (Gerhardt 1981).

The higher signal level (80 dB SPL) used in the present study was just within the normal range of variation of the amplitudes of conspecific signals at a distance of 1 m (80–87 dB SPL, Gerhardt 1975). The noise level

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[1] An alternative view is that at signal levels of 75 dB in the masked situation, the 5 females did not, in fact, respond, but moved near a speaker by chance. This interpretation would yield an estimate of a masked threshold above 75 dB, but below 80 dB, a signal level at which all females responded. This alternative interpretation does not change our main conclusion.

over the frequency range of conspecific calls was slightly lower than that produced by a chorus of conspecific males as measured within the chorus but not next to any individual male (Gerhardt and Klump 1988). Thus, our results indicate that the phenomenon described by Megela Simmons (1988) apparently does not play a role in selective phonotaxis in the usual situations in which acoustic communication takes place in nature.

Finally, we point out that all of the components of the advertisement calls of *H. cinerea* are not strictly harmonically-related. As discussed by Oldham and Gerhardt (1975), the rate of amplitude modulation of the advertisement call is usually fixed at one-third of the frequency of the low-frequency spectral peak. This generates side-bands around the low-frequency peak and its second and third harmonics. However, the rate of modulation sometimes changes during the course of the call, and the sideband nature of many components then becomes obvious in sonagrams because the frequencies of the sidebands change whereas the low-frequency spectral peak and its harmonics do not (see Fig. 5 in Oldham and Gerhardt 1975). As a result, the complex waveform of the advertisement call does not repeat exactly, hence the term 'quasi-periodicity.' We thus suggest that the acoustic waveform of many 'typical' natural advertisement calls might as easily be termed inharmonic as harmonic, and males frequently produce variants on the advertisement call that are distinctly aperiodic.

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