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Why stop calling? A study of unison bout singing in a Neotropical treefrog

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Abstract. Chorusing males of the Neotropical treefrog, *Hyla microcephala*, call in distinct bouts punctuated by periods of silence, known as unison bout singing. Previous studies of the calling energetics of frogs do not support the idea that males periodically stop calling because of high muscle lactate levels. Three additional hypotheses of why calling periodically ceases were tested. A two-stimulus female choice experiment failed to support the hypothesis that females prefer males that call cyclically. Playback experiments with males in the field rejected the hypothesis that males stop calling because of high ambient noise. Rather, data indirectly supported the hypothesis that males cease calling to save energy. Male *H. microcephala* adjust the timing of notes in their multi-note calls when interrupted by notes of other males. The flexibility of note timing was tested by interrupting males with call-triggered computer-generated advertisement calls of 20–1000 ms. The vocal behaviour of the frogs reduced acoustic interference significantly for most stimulus durations and probably allows males to minimize note overlap with their closest neighbours in the chorus while continuing to call when chorus noise levels are high.

Individuals of many species of organisms that signal acoustically engage in group calling. This calling behaviour, known as chorusing, is common among anuran amphibians (Wells 1988). Within choruses, individual vocalizing males do not call randomly with respect to the calls of other males (Brush & Narins 1989). Rather, the timing of calls of neighbouring males may result from combined effects of acoustic stimulation, inhibition, refractory periods, central neuronal oscillators and stochastic processes (Loftus-Hills 1974; Lemon & Struger 1980; Schwartz & Wells 1985; Brush & Narins 1989; Moore et al. 1989). Male frogs may also respond to calling neighbours by increasing call or note production (Littlejohn 1977; Arak 1983; Ryan 1985; Wells 1988). Changes in call timing and call complexity can both enhance the ability of a male to attract a mate in an intensely competitive environment (Wells 1988) and reduce the probability of acoustic interference of signals (Zelick & Narins 1982, 1983; Schwartz 1987).

My study focuses on a particular pattern of call timing in choruses known as unison bout singing. Unison bout singing is collective calling by groups of males punctuated by variable periods of relative quiet and is common in anurans (Rosen & Lemon 1974; Whitney & Krebs 1975; Schwartz & Wells 1983) and chorusing insects (Alexander 1975; Otte 1977; Greenfield & Shaw 1983). Specifically, I address the question of why male Neotropical treefrogs, *Hyla microcephala*, in choruses periodically stop calling rather than call continuously throughout the evening.

Hyla microcephala has an elaborate vocal repertoire in which males use both multi-part advertisement and aggressive calls (Schwartz & Wells 1985). In choruses, males usually call within a few hundred milliseconds after the calls of neighbours, use more secondary notes than when isolated, and often approximately match note number of neighbours (Schwartz & Wells 1985; Schwartz 1986). Data from female choice experiments indicate that females prefer calls with more of these secondary notes (Schwartz 1986). Multi-note calls of vocally interacting males often overlap in time, although the constituent notes alternate so that acoustic interference is avoided. To facilitate this note alternation, males make very rapid fine-scale adjustments in note timing by increasing the duration of inter-note intervals when interrupted (Schwartz & Wells, 1985; Schwartz 1987). *Hyla microcephala* also engage in unison bout singing so that background noise levels in choruses fluctuate in intensity. At the initiation of a chorusing bout, a male typically starts calling and

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then is joined fairly quickly by more and more males in the aggregation. As the bout proceeds, males also tend to use calls with more notes (Wells & Taigen 1989). The chorus, therefore, soon becomes very noisy. After usually less than 30 s, however, the calling subsides (Schwartz & Wells 1983).

Whitney & Krebs (1975) tested the hypothesis that male Pacific treefrogs, *Hyla regilla*, periodically cease calling because of fatigue. However, they found no differences in ATP and lactate levels in the abdominal muscles of calling and control animals. More recent studies of calling energetics in frogs indicate that anaerobic metabolism does not contribute significantly to this activity and, therefore, also lend no support to the fatigue hypothesis (Ryan et al. 1983; Pough & Gatton 1984; Taigen & Wells 1985; Prestwich et al. 1989).

A second hypothesis invokes the role of predation to explain unison bout singing. It states that predators have difficulty localizing males in a chorus if they periodically stop calling. Tuttle & Ryan (1982) have shown that by calling in aggregations or making their calls overlap, male *Smilisca sila* may reduce predation risk by bats, but it seems unlikely that this finding can explain the evolution of unison bout singing in species that call for many seconds or minutes before becoming quiet. It is more likely that predator avoidance was an important factor in the evolution of group calling dynamics in species such as *S. sila* (Tuttle & Ryan 1982), which exhibit very brief calling bouts followed by long periods of silence.

Here I test three additional hypotheses to understand why choruses periodically stop. According to the female preference hypothesis, male *H. microcephala* may call cyclically because females discriminate against males that do not do so. In particular, females may find males calling periodically at a high rate more attractive than those calling steadily at a low rate.

The noise hypothesis states that males in the chorus call until noise levels become so high or acoustic 'air space' becomes so crowded with sound that effective communication becomes impossible. However, as a chorusing bout begins, and noise levels are low, males initially stimulate one another to call. The noise hypothesis predicts that males should stop calling sooner in denser choruses. *Hyla microcephala* choruses generate considerable background noise: sound intensities at 50 cm in front of a male can reach over 105 dB SPL (Schwartz & Wells 1984), and calls may have over 10 notes (Schwartz & Wells 1985). Males should try to avoid call interference because it can make communication between males more difficult and also reduce a male's chances of attracting a female (Schwartz 1987). Because it is obviously impossible for a male to time his notes finely so that no interference occurs when many other frogs are calling nearby, males may lose nothing and also save energy by becoming silent when noise levels are high.

A final hypothesis states that males periodically stop calling to conserve long-term energy reserves. Calls of many males become clumped in time because of the stimulatory effect calls have on males. Together, both phenomena result in unison bout singing. In contrast to the noise hypothesis, this energy conservation hypothesis does not predict a shift of male calling to quiet periods nor shorter calling bout lengths as choruses become noisier. *Hyla microcephala* males often call at a very high rate in choruses, and individuals can produce over 6000 notes an hour. Wells & Taigen (1989) measured calling metabolic rates in this species and obtained a peak value higher than that measured for any other ectothermic vertebrate (2·8 ml oxygen/g·h). It may be that a male just cannot call at a high rate for an entire evening. Alternatively, males may be conserving energy to enable them to call over a period of many successive nights. Therefore, by engaging in unison bout singing males could balance the selective imperative of remaining competitive with one another over the short term and maintain the potential for vocal activity over the long term.

In addition to testing hypotheses on unison bout singing, I tested the ability of male *H. microcephala* to modify the fine-scale timing of their notes in multi-note calls. Limits on such rapid vocal adjustments may help explain how call cessation is related to the problem of acoustic interference.

METHODS

Study Area

Experiments were conducted in July and August 1989 in Gamboa, Panama. The field site was a flooded meadow with ditches and scattered pools of deeper water containing stands of aquatic grasses (see Schwartz & Wells 1984 for further details). Temperatures during field work, which was conducted between 1930 and 2300 hours, ranged from 24 to 27°C.

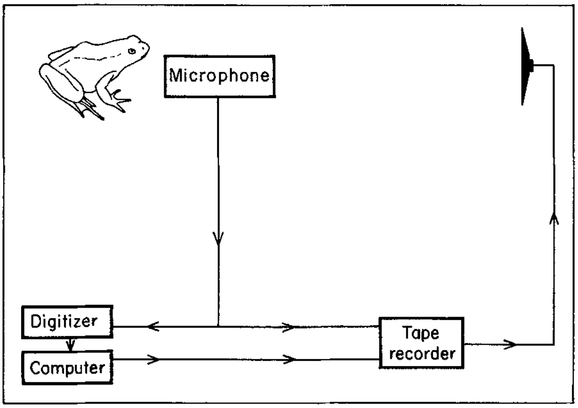

Figure 1. Schematic diagram of the stimulus presentation system used in the field with male *H. microcephala*.

Test with Females

Experiment 1 was designed to test the female preference hypothesis. Gravid female *H. microcephala* were captured in amplexus and tested in an indoor square arena measuring 2·0 × 2·0 m. Two mattresses and large cushions formed the walls of the arena and served to minimize reflection of sound. A 25-W red bulb suspended from the ceiling illuminated the testing arena. I placed individual females under a small plastic funnel, which was equidistant (75 cm) from two Realistic Minimus 0·3 speakers (amplitude-frequency response: ±3 dB, 1·0–12·0 kHz), which faced the centre of the arena. After 30 s of exposure to the test stimuli, I raised the funnel using a pulley and gave the female 10 min to approach the speaker. I scored a positive response if the animal made contact with or came within 10 cm of a sound source. Temperature during these experiments was approximately 26°C.

Stimuli consisted of two-note conspecific advertisement calls broadcast at an identical average rate of one call/s. From one speaker, calls were presented steadily. From the other, calls were broadcast in a cyclical pattern of 15 s of calls at two calls/s punctuated by 15 s of silence. The latter stimulus, therefore, was close to the average temporal calling pattern of males engaged in unison bout singing (Schwartz & Wells 1983). The stimuli were prepared by digitizing a recorded call (Marantz PMD 360 stereo cassette tape recorder, Realistic directional microphone) with an Amiga 2000 computer equipped with an Applied Visions FutureSound (TM) audio digitizer. The call was digitized at 20 kHz and low-pass filtered (Krohn-Hite model 3550) at 8 kHz. Digitized calls were output in stereo using the Amiga 2000 and software written by the author. Calls were timed so that stimuli alternated between channels (left two calls, right one call, left two calls, etc.), and no acoustic interference occurred. Test stimuli were recorded and played back during choice tests using a Marantz PMD 360 tape recorder. Playback intensities for each speaker were regulated by separate Realistic L-pads and were equalized at the female release point at 80±1 dB (peak SPL; dB re 20 μPa) with a calibrated Gen Rad 1982 precision sound-level meter. During successive tests, the cyclical stimulus was alternately started in calling and quiet periods, and on successive nights the two stimuli were shifted between speakers to neutralize any directional biases of the acoustic delivery system.

Tests with Males

To test the noise and energy conservation hypotheses, I conducted two field playback experiments with males using a computerized stimulus presentation system (Fig. 1). The system consisted of a Commodore Amiga 500 computer equipped with an Applied Visions FutureSound eight-bit sound digitizer and a Realistic LCD TV (model 16-156). The computer was powered with a custom built rechargeable battery pack that provided 150 V d.c. to the external switching regulator of the Amiga. The system is interactive, and the computer can be programmed to respond acoustically to the subjects in a variety of ways. During experiments, the audio outputs of the Amiga were fed into one channel of a Marantz PMD 360 stereo cassette tape recorder, amplified, and then broadcast from 1 m to the subjects using a University 4401 horn speaker mounted in a wooden baffle. Recordings of the frogs' responses were made using the second channel of the tape recorder and a Realistic 33-1062 directional microphone. The output of the microphone was split using a Y-cord, and the divided signal was fed into both the tape recorder and the sound digitizer. During an experiment, the computer simultaneously monitored the subject's vocal responses and recorded the timing of these calls along with relevant information on the stimuli. To minimize variation in stimulus and response timing, prior to tests, the gain on the digitizer was adjusted so that peak values of digitized calls were approximately the same for all subjects. Trigger thresholds were then entered in the program; these were the same for all frogs. To prevent other frogs or the stimulus output itself from triggering a response from the computer, the microphone was positioned as close as possible to the subject and facing 180 degrees from the speaker. A software routine was used to check that the peak values of the digitized output of the speaker were indeed below trigger thresholds. If not, the microphone was repositioned. Prior to experimental broadcasts, frogs were recorded for an initial 2-min no-stimulus control period.

Experiment 2 was designed to test whether males are inhibited from calling by chorus noise at high intensities. A chorus stimulus unit was created by digitally copying digitized *H. microcephala* calls using the sound editing software supplied with the sound digitizer. The stimulus unit was 1 s in duration and consisted of three three-note calls digitized at 20 kHz (eight bits per sample). The calls were timed so that the first primary note of each call fell between the second and third secondary notes of the previous call. In the field, this stimulus block was stored in the computer memory and presented to males as bouts of calling followed by periods of silence that were of random duration between 5 and 25 s. The stimulus, therefore, mimicked the pattern of background noise produced by a natural *H. microcephala* chorus. This chorus stimulus was presented at intensities of 90, 95 and 100 dB SPL. Order of stimulus intensity presentation was random. The modal sound intensity in front of a calling male *H. microcephala* is 106 dB SPL at a distance of 50 cm (Schwartz & Wells 1984). Therefore, the stimulus sound intensities approximate those produced by non-overlapping calls of three males 317, 178 and 100 cm from the subject. The number of males in natural choruses ranges from a few to dozens of individuals. These frogs may be spaced at a range of distances from any focal male. Because of this variation in male number and spacing, the stimulus intensity treatments qualitatively simulate different, rather than specifically known, chorus densities.

Experiment 3 was designed to test whether males are inhibited from calling when many other males call. The computer simulated choruses of different sizes that tracked the cyclical pattern of calling of the subject. Therefore, the calls of the test subject drove the stimulus. A digitized *H. microcephala* three-note call and blocks of silent buffer were stored in the computer memory and output using one, two, or all four channels of the sound chip in the Amiga to simulate the multi-note calls of one, two, or many frogs (up to 12). The calls of the two-frog stimulus, output using two sound channels, were spaced 100 ms apart so that their notes alternated like those of two interacting males. In the large chorus treatment, two additional calls, timed 103 and 120 ms after the first call were stored in computer memory and output using the two remaining sound channels. Each call was played three times. Therefore, some notes did overlap as would be expected in a real chorus of more than a few males. The sound output of the computer was call-triggered and the one- and two-frog artificial chorus ended almost immediately after the subject ceased calling (555 and 696 ms, respectively). The large chorus stimulus stopped after 2120 ms. If the frog continued to call during stimulus output, the simulated chorus was replayed. Stimuli were presented to males at 100 db SPL.

The flexibility in note timing of males was tested in experiment 4. The computer was used to interrupt the calls of nine subjects with synthetic advertisement calls of 20, 40, 60, 100, 200, 400, 800 and 1000 ms in duration. Therefore, these stimuli spanned the range in duration of conspecific and heterospecific calls to which *H. microcephala* males are most often naturally exposed. These interruptions were broadcast 55 ms after the end of the subjects' primary notes (as determined by the absence of above threshold digitized input). Therefore, they fell at the approximate midpoint of uninterrupted inter-note intervals. To create these stimuli, one pulse was synthesized (sampling rate = 20 kHz, eight bits per sample) using software written by the author. It was designed to resemble a natural advertisement call pulse in frequency, duration and shape of the amplitude envelope. There were two spectral components of 5·8 kHz + 2·9 kHz (−6 dB).

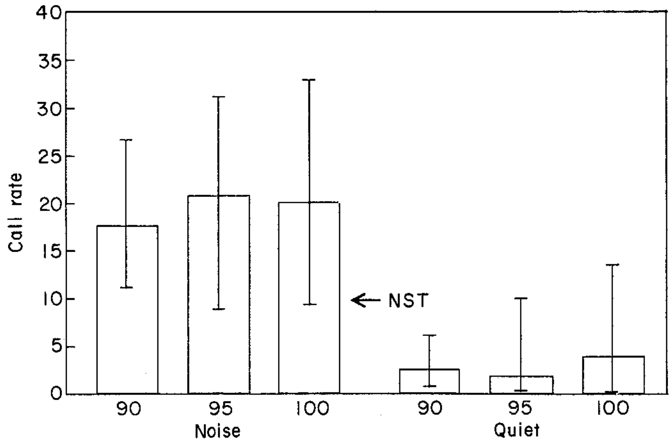

Figure 2. Call rates (calls per min) of 12 males during presentation of the conspecific chorus stimulus at 90, 95 and 100 dB SPL for both the noisy and quiet periods. The NST arrow indicates the median call rate during the no-stimulus periods. Data are presented as medians and approximate 95% confidence intervals.

The rising edge of the pulse was concave down in shape and 1·0 ms in duration. The falling edge of the pulse was concave up in shape and 2·0 ms in duration. The pulse (duration = 3·0 ms) was followed by 1·4 ms of silence, so that by instructing the audio chip to play it a specified number of times, it was possible to generate calls with an appropriate pulse rate (227/s) and the desired stimulus durations. Interrupting stimuli were presented to most males at an intensity of 95 dB SPL for 2 min each. If a frog initially responded with a high proportion of aggressive calls the intensity was lowered to 90 dB.

Stimulus intensities (peak sound pressure level; dB SPL re 20 μPa) were predetermined at 1 m with a Gen Rad 1982 precision sound level meter set for flat weighting and a C-weighted Realistic sound level meter. Only the second meter was available for field use. The voltage level of a 2 kHz calibration tone, stored in the computer memory and measured at the 8-ohm speaker output of the Marantz tape recorder, was also determined for each stimulus–intensity combination. In the field, a Micronta (TM) pocket digital multimeter was used to adjust the output voltage during playback of this tone for each desired stimulus intensity level. Following tests, the stimulus intensity was directly measured using the Realistic sound level meter.

Data files from the field experiments were analysed using SAS (SAS Institute 1982). Call timing was cross-checked using the sound display software provided with the FutureSound digitizer. Oscillograms were prepared with a Kay Model 5500 Digital Sonograph.

RESULTS

Test with Females

Females in experiment 1 showed no discrimination for calls broadcast in cyclical bouts relative to calls broadcast at a steady rate. In fact, there was a non-significant bias in favour of the steady rate stimulus. All of the 20 females tested responded, but only seven chose the cyclical stimulus (P = 0·264; binomial test). During the tests, some females reversed direction during approaches to the speakers, suggesting that any temporary preference was a weak one.

Tests with Males

If high noise levels inhibit calling, subjects should have shifted their calling to the quiet intervals as the intensity of the chorus stimulus was elevated to 100 dB in experiment 2. However, call rates for the 12 males tested did not change significantly with increases in chorus intensity in either the noisy or quiet stimulus periods (Friedman test, Fig. 2). Nor was there a shift in the proportion of calls given in the quiet relative to noisy stimulus periods as stimulus intensity increased (Friedman test). These results are inconsistent with the predictions of the noise

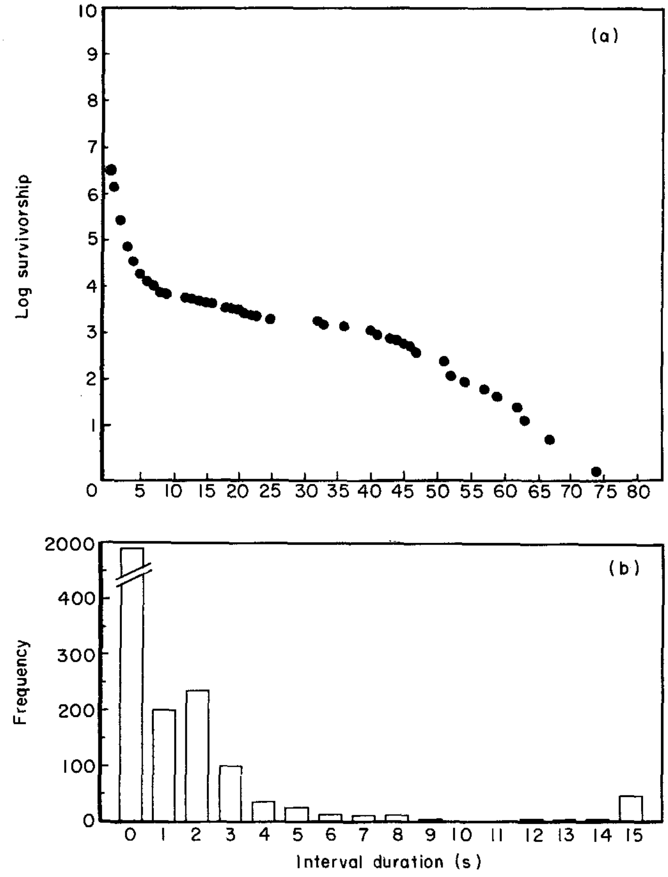

Figure 3. (a) The cumulative frequency of inter-call intervals of 15 male *H. microcephala* recorded during no-stimulus periods plotted as log survivorship versus interval duration ($N=654$, binwidth = 1 s). (b) The distribution of inter-note intervals of 15 male *H. microcephala* recorded during no-stimulus periods ($N=2660$). All bars but the first show the frequency of inter-call intervals. The last bar gives the frequency of all intervals greater than 14·5 s.

hypotheses but consistent with the predictions of the energy conservation hypothesis.

To analyse data from experiment 3 in which simulated chorus size was changed, bouts were defined as a block of calling punctuated by either a 5- or 9-s quiet period. The determination of what constitutes the end of a calling bout can be somewhat problematic (Slater & Lester 1982). One method involves calculating the logarithm of a reversed cumulative frequency distribution for inter-call intervals. The slope at any point along such a graph is proportional to the probability of a new call occurring at a particular time interval after the last call. Within calling bouts, there is a high probability of another call, and so the slope is steep. Between calling bouts, there is a lower probability of another call, and so the slope is more gradual. I chose the first temporal criterion because 5 s was about where the slope of this log survivor function for intervals changed most rapidly (Fig. 3a). Over 95% of all inter-note intervals (approximately 90% of inter-call intervals) during no-stimulus periods were less than 5 s (Fig. 3b). I conducted a second analysis using a 9-s criterion to assess the robustness of the earlier results. This value was obtained using the non-linear least-squares procedure of Sibly et al. (1990), which fits a curve to log frequency rather than log survivorship data. Using either criterion, there was a significant treatment effect ($N=15$ males; Friedman test; 5 s: $P<0.01$; 9 s: $P<0.05$). In direct opposition to the prediction of the noise hypothesis, in response to the large

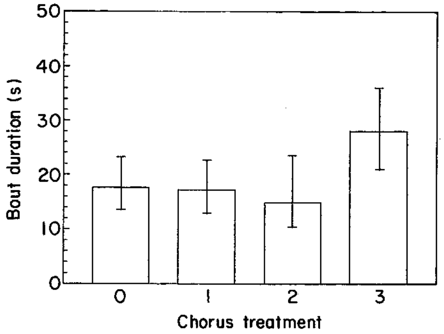

Figure 4. The duration of chorusing bouts of 15 male *H. microcephala* during presentation of the call triggered simulated choruses. Data are presented as medians and approximate 95% confidence intervals. Treatments: 0, no-stimulus; 1, one frog; 2 two frogs; 3, 12 frogs.

chorus stimulus, males increased the lengths of their calling bouts (Fig. 4). There was no significant change in bout length in response to either the one- or two-frog simulated chorus (non-parametric multiple comparisons; Noether 1976).

In experiment 4, synthetic advertisement call interruptions of all durations elicited a significant increase in inter-note intervals relative to the no-stimulus condition (sign test on median values; $P<0.035$, Figs 5 and 6). However, for interruptions longer than 200 ms, males made adjustments in note timing that were often insufficient to prevent overlap with the stimulus unless a male terminated his call (Fig. 7). Moreover, median inter-note intervals were no longer for 400-ms interruptions than for 200-ms interruptions (sign test, $P=0.637$). With the exception of the increase from 800–1000 ms, males responded to all other increases in stimulus duration with an increase in interval duration (sign test, $P<0.05$). Figure 8 illustrates how shifts in note timing by *H. microcephala* males can reduce note overlap with interrupting calls. The expected level of acoustic overlap was determined for each male by calculating the percentage of inter-note intervals during the no-stimulus period that were too brief to prevent acoustic interference with a stimulus, had it been broadcast. Only inter-note intervals following primary notes were used in the calculations. For all interruptions greater than 20 and less than 1000 ms, there was a significant reduction in the actual acoustic overlap relative to that expected (sign test, $P<0.01$). There also was a sharp rise in actual note overlap as stimulus duration increased above 200 ms.

DISCUSSION

In a two choice situation, female *H. microcephala* will discriminate in favour of the higher of two call rates (Schwartz 1986). If this kind of preference is sufficiently strong, it might explain why males call in bouts. Males could attract more females by calling periodically at a high rate than by calling constantly at a slow rate. However, experiment 1 failed to support the idea that by calling cyclically, males render themselves more attractive to potential mates. Although I only used one set of call timing parameters out of an essentially unlimited number of those possible, the result constitutes a solid refutation of this hypothesis for two reasons. The call rate and bout duration values tested were close to those that females must commonly encounter as they approach natural choruses (Schwartz & Wells 1983; Fig. 4). Moreover, there was a bias for the non-periodic stimulus, although it was not significant. It is possible that the absence of significant discrimination in this experiment is related to the ability of these frogs to integrate sound, since the long term sound energy of the cyclical and non-cyclical stimuli were equal. Results of a previous experiment using stimuli with different call rates, but equalized total sound energy, also suggests that female *H. microcephala* are able to integrate the sound energy in multiple calls (Schwartz 1986).

Results of experiments 2 and 3 with males clearly refute the hypothesis that males stop calling because of noisy conditions in the chorus. In response to increases in stimulus intensity, males did not shift calling into the quiet periods between bouts of broadcast calls. Therefore, their behaviour was quite different than that displayed by male *H. ebraccata* when exposed to the chorus sounds of *H. microcephala* (Schwartz & Wells 1983). Instead, the calls of large numbers of conspecifics stimulated vocal responses in male *H. microcephala*, and lengthened their calling bouts (Fig. 4). The results obtained during the call interruption experiment (experiment 4) also indicated that males may call even when acoustic overlap is a consequence (Fig. 7, 400 and 1000 ms). Given the important problems for communication created by background noise, and, in particular, conspecific chorus sounds (Gerhardt & Klump 1988) the question of why this should be the case arises. In fact, acoustic interference may not be an important problem among those males most likely to degrade one another's calls seriously: those in close proximity in the chorus. By adjusting

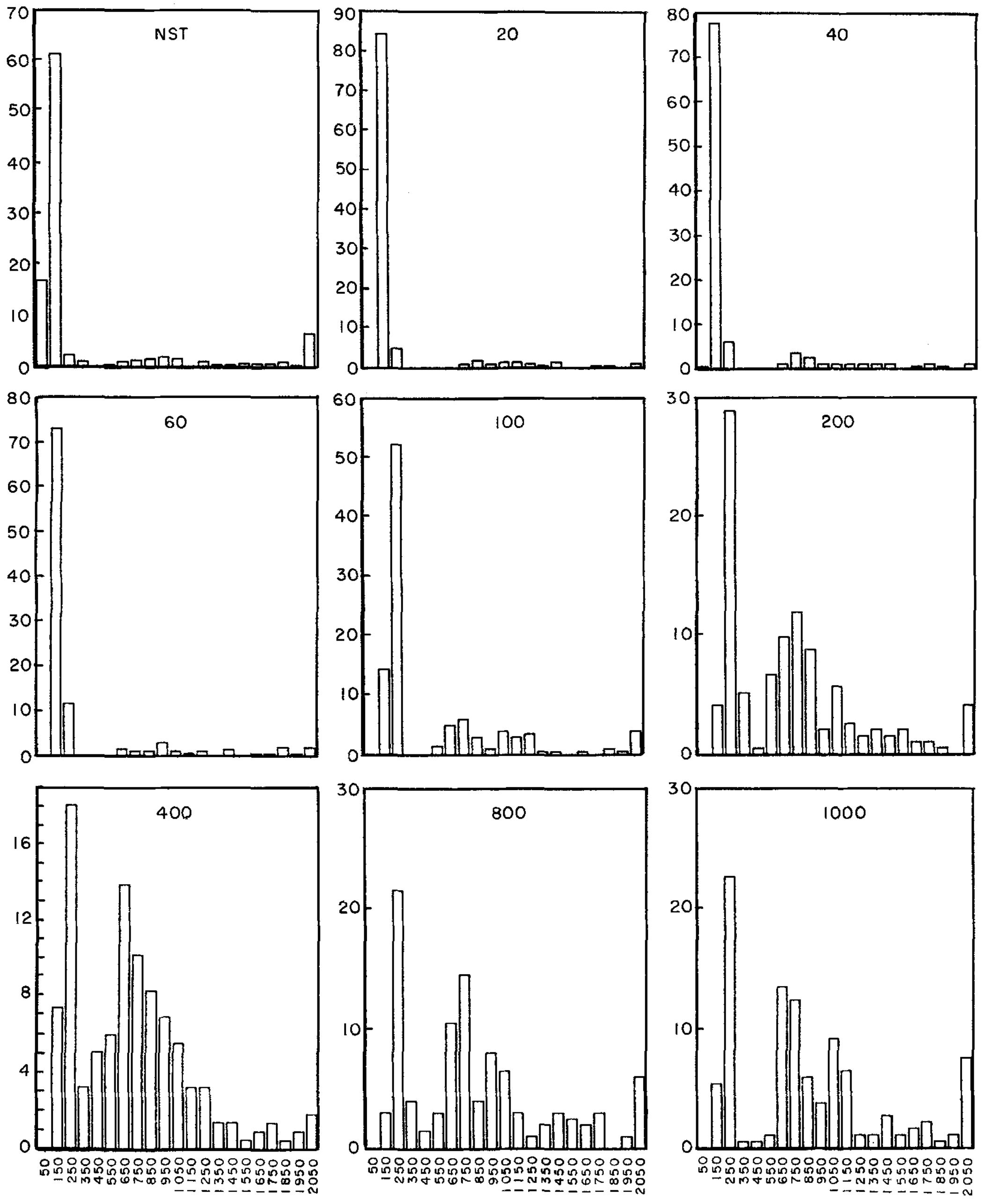

Figure 5. The distributions of inter-note interval durations of nine *H. microcephala* males during the no-stimulus control period (NST) and when interrupted by calls of 20–1000 ms duration. Data include all intervals of less than 5 s between the primary note and the first secondary note as well as inter-call intervals if no secondary note was given after an interruption.

note timing during call overlap with one or two neighbours, a male can minimize note interference with the most intense sound sources while at the same time overlapping the less intense notes of more

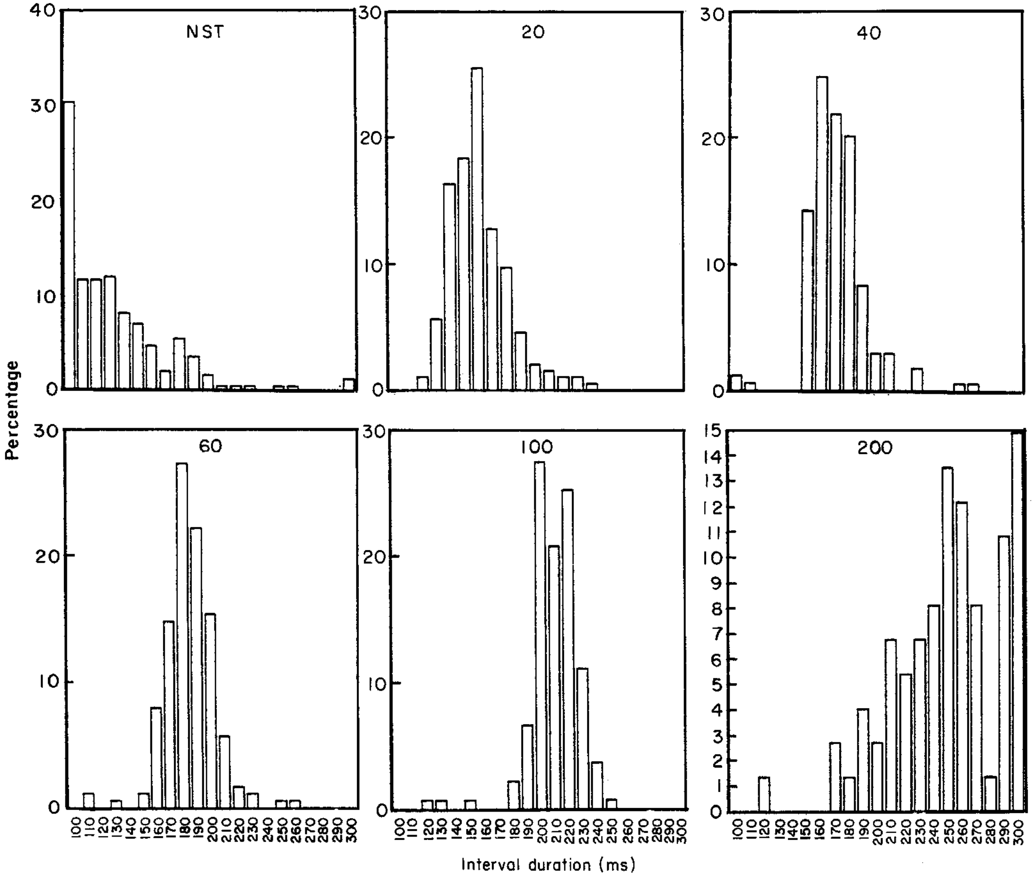

Figure 6. Distributions of inter-note interval durations of less than 400 ms of nine *H. microcephala* males during the no-stimulus control period (NST) and when interrupted by stimulus calls. Data show just the increase in the intervals between primary and secondary notes with increasing stimulus duration up to 200 ms.

distant males. It is important, however, that future work clarify how differences in the relative intensity of interfering calls influence female behaviour.

Another likely reason why males call at about the same time is the intense level of competition for mates. Operational sex ratios on most evenings in the chorus are highly male biased (personal observations), and if a male failed to call when other males did so, he might severely jeopardize his chances of attracting a female. That males append secondary notes to their calls when the calls of other males are detected also underscores the high level of competition and the importance of maximizing the relative attractiveness of signals to females on a fine time scale (Schwartz & Wells 1985). If a male adopted a strategy of only calling after other males ceased calling, he could theoretically minimize acoustic interference, keep energy expenditure down, and continue to attract females. In an actual chorus, however, such a strategy probably could not be realized. The calls of such a hypothetical male would stimulate other animals to call and obviate the potential advantages of such behaviour.

In contrast to the predictions of the noise hypothesis, the predictions of the energy conservation hypothesis were consistent with the data obtained during stimulus presentations to males. Therefore, there is indirect support for the idea that males punctuate their bouts of calling to save energy. Although it will be very difficult to do, this hypothesis should be tested directly. Additional indirect support for the energy conservation hypothesis is available from recordings of test males when stimuli were not broadcast. Mean note rates of subjects during

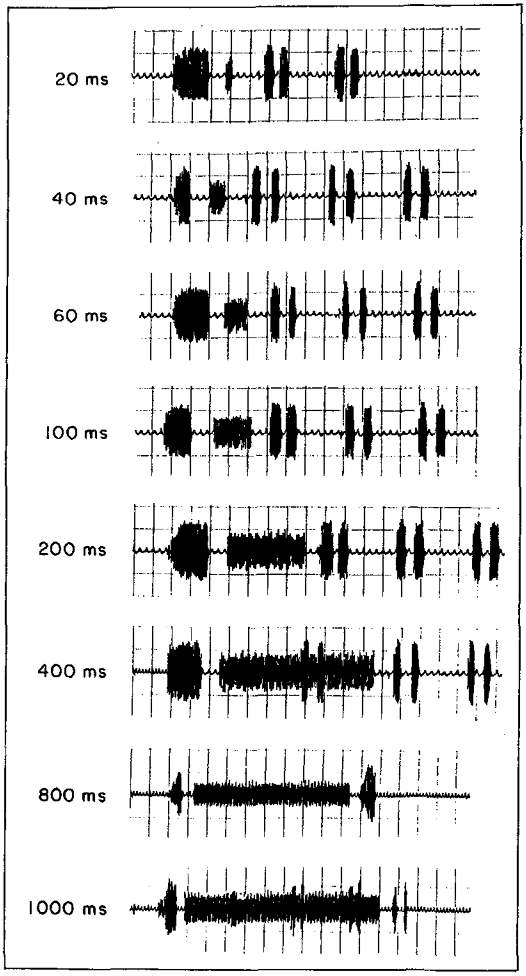

Figure 7. Oscillograms of responses of a *H. microcephala* male to stimulus interruptions following his primary notes. The responses to the 400- and 1000-ms stimuli were insufficient to prevent acoustic overlap. In response to the 800-ms stimulus, the male terminated his call. Scale: for 20–400 ms, 1 division = 50 ms; for 800–1000 ms, 1 division = 100 ms.

no-stimulus periods were 2297/h, and quiet periods greater than 5 s in length constituted an average of 60% of their chorusing time. If note rates are recalculated without these pauses, the average jumps to 5648/h. Therefore, if males did not punctuate their calling and continued to call at rates equal to their rates during bouts, mean note rates would be very high. In fact, they would be close to the maximum of 6720/h ever recorded for an individual of this species during stimulus playback (Wells & Taigen 1989). Some males would exceed this value considerably (Fig. 9).

Male *H. microcephala* show much individual variation in note rate and, therefore, in estimated rates of energy expenditure (Wells & Taigen 1989). These authors suggest that males conserve energy by adjusting their calling effort to the level of competition present in the chorus. This idea is consistent with the data from this study, that males increased bout durations during exposure to large simulated choruses. Adjustments in calling effort may allow males to either (1) maintain periodic high rates throughout an evening or (2) prolong tenure in the chorus during the course of many nights. The ability to do either or both of these are probably very important because mating success of male *H. microcephala* may be highly correlated with call rate and chorus attendance. Although direct field data on mating success are not available for this species, in choice tests, females prefer higher call rates (Schwartz 1986), and the importance of chorus attendance has been demonstrated for a number of other species of frogs (Greer & Wells 1980; Woodward 1982; Arak 1983; Ryan 1985; Gerhardt et al. 1987; Morris 1989).

Anurans employ a number of behavioural and neural mechanisms to reduce the problem for communication posed by the high ambient noise levels in choruses (Narins & Zelick 1988). The data from this study demonstrated that *H. microcephala* males can dramatically lower the probability of overlapping notes with neighbouring males by modifying the timing of their notes. This is accomplished to a large extent by increasing the spacing between their notes in multi-note calls. In a previous analysis of pairwise natural interactions, no acoustic interference occurred in 74 of 100 instances of call overlap (Schwartz & Wells 1985). My study of the phenomenon was specifically designed to assess the sensitivity of the response to changes in stimulus duration. The data indicate that synthetic calls as brief as 20 ms elicited a significant increase in note spacing. However, for stimulus durations above 200 ms, the timing changes were often not sufficient to prevent acoustic interference. Since the primary and secondary notes of conspecifics (with mean durations less than 100 ms) are the most frequent acoustic stimuli to which a male *H. microcephala* is naturally exposed the limits of the behaviour are more than sufficient to prevent overlap in the vast majority of potential cases. In fact, it is likely that the ability of males to modify note timing

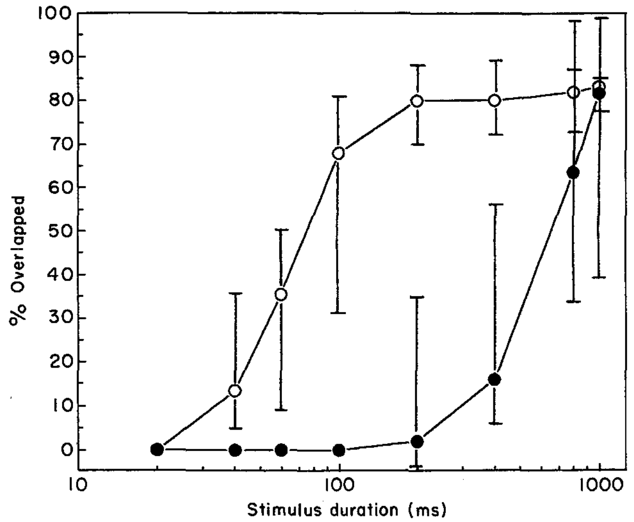

Figure 8. Percentage of actual (●) and expected (○) note overlap by stimuli of 20–1000 ms duration. The expected values were calculated from inter-note intervals during the no-stimulus periods. Data are presented as medians with approximate 95% confidence intervals. Tick marks on the duration axis are logarithmically spaced.

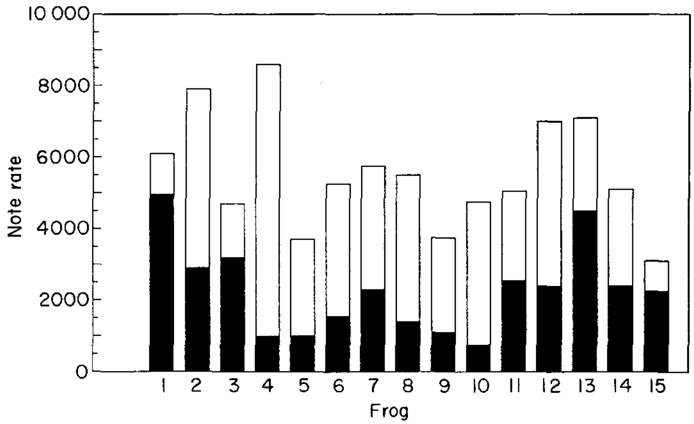

Figure 9. Note rates (notes per h) of 15 male *H. microcephala* during no-stimulus periods. □: rates calculated with quiet periods greater than 5 s excluded; ■: unadjusted rates.

evolved to facilitate the alternation of such short notes during overlap of advertisement calls of vocally interacting males. In spite of the increased probability of interference during interruptions of longer duration, the level of acoustic overlap was still usually lower than that predicted in the absence of timing shifts (Fig. 8). Therefore, the vocal flexibility of *H. microcephala* males should also enhance their ability to communicate during agonistic interactions with conspecific and heterospecific males when longer aggressive calls are detected (Schwartz & Wells 1985).

Future empirical work on the acoustically mediated note-timing adjustments of *H. microcephala* males should focus on the sensitivity of the behaviour to the time of occurrence of the stimulus as well as its underlying neuronal mechanisms. Narins (1982) found that the synchronized response rate of male *H. ebraccata* and *Eleutherodactylus coqui* was a function of the timing of an acoustic stimulus relative to the previous call of the frog. He identified both a behavioural refractory period, during which time the subjects were unresponsive, and a relative refractory period, during which time the subjects' responsiveness increased with stimulus delay. Rosen & Lemon (1974), Lemon & Struger (1980), Loftus-Hills (1974) and Moore et al. (1989) have discussed models of call timing in other species incorporating elements of excitation and inhibition. Both call stimulation and the inhibition of motor output in *H. microcephala* may be associated with periods in the call cycle like those identified by these researchers. However, the relevant neuronal circuitry may be elaborated since male *H. microcephala* adjust timing of both calls and the notes within calls relative to auditory input. Such circuitry could also incorporate both an integrator that would sum the output of a note generator and an inhibitory pathway which could prevent note production. Therefore, after a long enough inter-note delay, acoustic input would fail to block calling. Of course, these ideas are speculative and must be tested. In addition to enhancing our understanding of the calling dynamics of *H. microcephala*, future research of this kind could provide insights on the behaviour of other chorusing species.

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