

# Calling strategies of male túngara frogs in response to dynamic playback

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## Summary

Túngara frogs produce calls of varying complexity that consist of a whine followed by 0–7 chucks. In previous studies using static playbacks, males increase chuck number in response to calls with 1 versus 0 chucks but not in response to 2 or 3 versus 1 chuck. Here we use dynamic playback in which an automated interaction program counts the number of chucks present in a call and responds according to a specified calling strategy which determines the number of chucks in the response call. Males added progressively more chucks in response to the models' strategies of de-escalate, match or escalate; there was a significant difference in the focal males' responses to the de-escalate versus the escalate strategies. Similarly, males changed their calling strategy in response to the de-escalate strategy of the model. There was no evidence of change in calling patterns, as estimated by entropy, among treatments and between experimental and field data. Males produced significantly more chucks in these experiments than in the field, but in both contexts the mean chuck number is low and never approaches the maximum observed in nature. These data suggest that males are cost-sensitive when it comes to adding chucks and that they are more influenced by vocal competitors that de-escalate rather than escalate number of chucks. These are patterns of calling not previously revealed in studies using static playbacks, and this study is another demonstration of the usefulness of dynamic playback in studies of animal communication.

*Keywords:* túngara frog, animal communication, evoked vocal response, dynamic playback, interactive playback, acoustic behaviour, frog calls.

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## Introduction

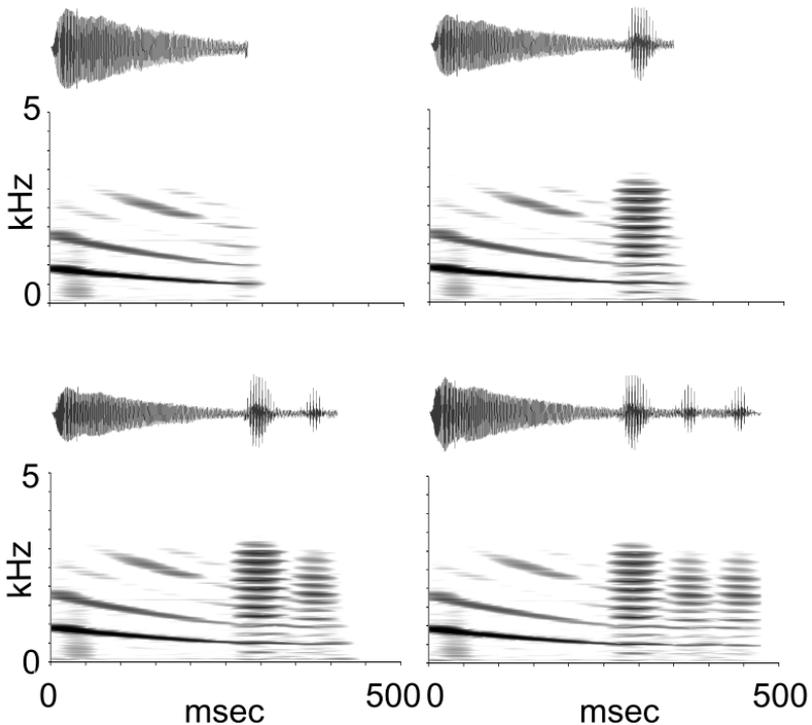
Animal signalling at its most basic is a dyadic interaction between a sender and receiver. In many cases each individual is both a sender and a receiver, and signals change dynamically during an ongoing interaction (reviewed in birds, Catchpole & Slater, 1995; frogs, Gerhardt & Huber, 2002; insects, Greenfield, 2002). When individuals engage in such interactions, there are several different signalling strategies that one individual might use in response to one another; these depend on the nature of the interaction, the costs and benefits of signalling, and various other biological features such as internal motivational state and working memory (Hurd & Enquist, 2005).

Despite the fact that signals often dynamically change during an interaction, many investigations of communication are based on playback studies that present static or looped displays which are insensitive to the focal animal's behavioural state. More recently, however, there has been an increase of studies that use dynamic or interactive presentations of signals to gain insights into the mechanisms and evolution of communication behaviour (reviewed in Schwartz, 1994, 2001; Dapplesteen & McGregor, 1996; McGregor, 2000). In some of these studies the signal presented to the focal animal is dynamically variable (e.g., Rosenthal et al., 1996). In others, however, the experimental stimulus not only varies but its variation is dependent upon the signals being produced by the focal animal (e.g., Otter et al., 1999; Langemann et al., 2000; Ord & Evans, 2002; Schmidt et al., 2006). One advantage of interactive playback is that the experimenter determines the precise algorithms for the playback's response and, thus, the model's 'communication strategy'. The strategy of a focal male can then be evaluated as a function of the strategy of the model. Such interactive playback has allowed increased insights into the dynamic and interactive nature of animal communication.

A common interaction during communication is when individuals escalate their signals in response to others. In great tits, for example, territorial encounters involve an increase in strophe length and song overlap. Langemann et al. (2000) used interactive playback with great success to study the dynamics of this interaction, finding that males used information from dyadic interactions to determine the level of threat. We take a similar approach to use interactive playbacks to study escalation in call complexity in male túngara frogs (*Physalaemus pustulosus*).

### The system

Male túngara frogs produce advertisement calls to attract potential mates, as do most anurans (Ryan, 2001; Gerhardt & Huber, 2002). Their advertisement call has two components (Figure 1). The initial and essential component is the ‘whine’, a frequency sweep typically of about 350 ms whose fundamental frequency descends from 800 to 400 Hz. This call component is necessary and sufficient to elicit phonotaxis from females and vocal responses from males. The whine can be produced alone (the simple call) or it can be followed by up to seven additional components called chucks (complex calls; Rand & Ryan, 1981; Ryan, 1985). The chuck is a shorter, multi-harmonic, higher-pitched element, ca. 45 ms in duration. Female túngara frogs are attracted preferentially to complex calls over simple calls (Ryan, 1985; Gridi-Papp et al., 2006), as are frog-eating bats (*Trachops cirrhosus*; Ryan et al., 1982) and blood-sucking flies (*Corethrella* spp.; Bernal et al., 2006).



**Figure 1.** Waveforms (top of each cell) and spectrograms (bottom of each cell) of a typical complexity series of the túngara frog illustrating a whine with 0, 1, 2 and 3 chucks (top left, top right, bottom left and bottom right, respectively).

Males facultatively vary their call complexity. When males are calling in isolation they often produce only simple calls, but in large choruses most males produce complex calls (Ryan, 1985). This suggests that males increase call complexity in response to calls of other males. Males call in bouts that contain an average of  $26.71 \pm 2.59$  calls per call bout, and last an average of  $66.03 \pm 5.25$  s (Bernal et al., 2007). Although the silent interval between bouts varies, we followed Pauly et al. (2006) in considering a bout complete after 10 s of silence. Recordings of call bouts of individual males in natural choruses show that more than half of the calls are simple ones, and 95% of the calls have zero, one, or two chucks (Bernal et al., 2007); in unusual cases, males may produce up to seven chucks. When males change the number of chucks they usually do so incrementally, either adding or subtracting a single chuck at a time (Bernal et al., 2009).

In the field, when a male is making a simple call a person can imitate a complex call and the frog will often respond with a complex call (Ryan, 1985). Several experimental studies have investigated in detail how male túngara frogs modulate call complexity in response to the calls of other males (Rand & Ryan, 1981; Ryan, 1985; Ryan & Rand, 1998; Greenfield & Rand, 2000; Bosch et al., 2002; Bernal et al., 2009). In all of these experiments calls were broadcast to males in a static rather than a dynamic manner; stimulus calls were not dependent on the male's response. These studies have shown that males increase the number of chucks in response to the initiation of signals and in response to signals with higher amplitudes or higher call rates; males, thus, appear to increase chuck production in response to neighbouring males who are closer, or in response to a larger number of males (Rand & Ryan, 1981). Males also produce more chucks in response to complex calls compared to simple calls (Ryan & Rand, 1998), and stimuli with lower-frequency chucks, which simulate larger males, elicit more chucks from focal males (Bosch et al., 2002). In addition, males also increase chuck number in response to the approach of females (Ryan, 1985).

Given data from the field and from the above-mentioned static playback experiments, males appear to escalate call complexity (number of chucks) in an arms race; call complexity results from males increasing the number of chucks in response to increasing numbers of chucks from other males. Recent experimental data, however, do not support this interpretation. Although males add more chucks in response to complex calls versus simple

calls, male calling behaviour does not vary in response to broadcasts of calls with one, two or three chucks (Bernal et al., 2009).

There are at least two possible explanations for the insensitivity to more versus fewer chucks. It is possible that males are sensitive to chuck number but this cannot be uncovered in static playback experiments. If males are escalating in a truly dyadic interaction, they might be sensitive to the calling strategy of their vocal competitors and not just the absolute number of chucks. Thus, static playback would fail to elicit responses in focal males that vary in response to more versus fewer chucks. On the other hand, males might be insensitive to chuck number because of costs associated with producing more chucks. Males might be reluctant to escalate due to the increased risk of predation from frog-eating bats, who tend to prefer more chucks to fewer chucks (Page, unpublished data), as well as other unanticipated costs of making large numbers of chucks (e.g., biomechanical constraints).

### *Objectives*

In this study we use a dynamic playback system in which a computer-automated interaction program (the model) sums the number of chucks in a focal male's call and responds with a chuck number that is dictated by one of four strategies: match, escalate, de-escalate or random. We have two objectives in this study. The first is to determine if males are sensitive to the calling strategies of others. The second is to determine if males employ specific calling strategies that vary with the calling strategies of others. As part of both objectives, we will also determine how males increase chuck number when confronted by a calling strategy of a model that is not constrained by costs of escalation. Will focal males escalate chuck number towards the maximum we observe in nature (7), or instead will they continue to produce the relatively low number of chucks (0–2) more commonly observed in the wild and in experiments with static playbacks?

## **Methods**

### *Study site and subjects collection*

This study was conducted between June and August 2008 in Gamboa, Republic of Panama (9°07.0'N, 79°41.9'W). Experiments were performed in laboratory facilities of the Smithsonian Tropical Research Institute (STRI),

which are located near areas where túngara frogs are common. Calling male túngara frogs were collected at night at different sites in the area and tested on the same night. After completing the tests, each male was measured, weighed, given a unique toe-clip to prevent them from being tested again, and released at its site of capture.

### *Experimental design*

We conducted dynamic playback experiments in darkened sound-attenuation boxes that each contained both a microphone and a speaker connected to a computer via a multi-channel data acquisition (DAQ) device. Experiments were conducted following Bernal et al. (2007). Each male was placed in an acoustically transparent plastic bag with approx. 150 ml of rainwater. Bags were inflated with air and closed, then placed in one of eight darkened sound-attenuation boxes measuring  $30.5 \times 46.0 \times 30.5$  cm. Each bag had sufficient standing water to allow a male to call, and was placed on a ledge that allowed the male to emerge from the water.

Each of the sound-attenuation boxes had a small microphone (Radio Shack, Electret model 33-3013) that was connected to a computer via a National Instruments® USB-6221 Multifunction DAQ device which allowed us to monitor and record sounds within each box. We were also able to broadcast stimuli to each box from the sound card of the computer, through the DAQ device, to two amplifiers (10 W Realistic SA-10 stereo amplifier) which split the signal to each of the eight small speakers (Cambridge Soundworks), one inside each box.

At the beginning of the night, ca. 2000 h, all of the boxes containing males were closed. A 60-s recording of a male túngara chorus, the screening stimulus, was broadcast at approx. 82 dB SPL (re. 20  $\mu$ Pa) measured at the location of the focal male within each box. Males were not tested if they did not respond to the screening stimulus. The sound in each box was monitored simultaneously and independently. When a male in a box began to call the chorus stimulus ceased and testing of that male was initiated. Although we were able to monitor all of the males and boxes simultaneously, we tested each one sequentially.

When a male called, the automated interaction program recognized the signal as a túngara frog call, and noted the number of chucks (see details below). At 1300 ms after the onset of the male's call the model responded with a call dictated by one of four calling strategies. This call and response

interaction continued until 50 responses were recorded for each individual. Upon reaching 50 calls, the male was tested with the next calling strategy, repeating this cycle until all calling strategies were tested for a male. If a male ceased responding, there was a break of silence in stimulus signals (chorus or interactive) before resuming with the screening stimulus until the male called one again (details below).

We used four treatments or calling strategies. Match: the model's response to the focal male contained the same number of chucks as the male's previous call. Escalate: the model's response to the focal male contained one more chuck than the male's previous call. De-escalate: the model's response to the focal male contained one less chuck than the male's previous call with a minimum of zero. Random: the number of chucks in the model's response to the focal male was randomly determined but did not exceed five.

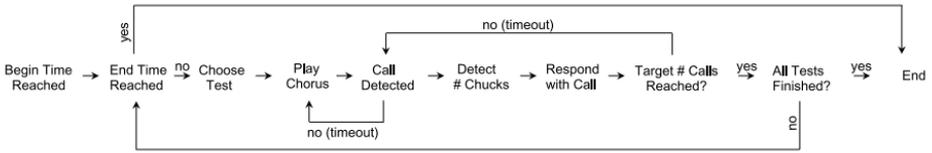
The test stimuli were derived from 20 natural whine-chuck calls of 20 different males drawn from a sample of 300 calls of 50 males in the Gambia population from a previous study (Ryan & Rand, 2003). Each of these calls was manipulated to produce calls with 0–5 chucks. The chucks were removed from the calls to produce whine-only calls, and the same chucks were copied and added to the original call with 4 ms silent intervals to produce calls with one to five chucks. This resulted in a pool of 120 test stimuli, 20 calls each with 0–5 chucks. When selecting a call for playback, the computer randomly chose a call with the appropriate number of chucks from this stimulus pool.

The order of the four treatments was randomized among males. The program produced a sound file of the male's call and the response call, as well as a text file listing both the number of chucks and the time of the calls of the focal male and the model. For each test, the text file information was compared to the accompanying sound file. The model occasionally made errors, and these call-model interactions were eliminated for most tests (see below).

Males who failed to respond on one night were tested the following night and released the third night if they never responded to the screening stimulus. Males who responded were released the night of their tests. All testing ceased between 0200–0300 h. The sound attenuation boxes were open during the day allowing the males to experience a natural photoperiod.

### *Computer program*

Túngara frogs respond relatively rapidly to calls of other males; they produce one call about every two seconds. This precludes a manual interactive



**Figure 2.** Flow chart of TECSIS.

playback system (e.g., Mennil & Ratcliff, 2000). Therefore, we implemented a system that both recognizes the male's call characteristics and generates a response based on a programmed calling strategy.

The Túngara Evoked Call with Simultaneous Interaction Software (TECSIS) was programmed in the National Instruments LabVIEW<sup>®</sup> version 8.5 environment. The program was designed to monitor a single channel of real-time audio input to detect the presence of a túngara frog call, classify a detected call based on the number of chucks it contains, and generate a response call dependent upon this classification. All settings described below are determined by the user before beginning a battery of tests. A flowchart of TECSIS is shown in Figure 2.

Upon commencement of testing, TECSIS records audio from a single input channel on the DAQ device. Concurrently, the program initiates output of an audio file, the screening stimulus, and randomly selects one of the four different calling strategies described in the experimental design section to determine the pool of response calls. In this study, the screening stimulus was a túngara frog chorus with a 60-s duration.

TECSIS monitors 800-ms segments of the input audio at 100 ms intervals until an amplitude threshold is crossed within the first 100 ms of the 800-ms segment. Once the amplitude threshold is crossed, the 800 ms block is considered a call candidate. The call candidate is subsequently analyzed to distinguish it from background noise. A 50-ms slice of the call candidate, beginning 100 ms from the time the amplitude threshold was crossed, is analyzed with a Fast Fourier Transform. The amount of energy in the five harmonics with largest amplitudes are summed and compared to the total amplitude of the call. If this ratio is greater than the threshold, then the call candidate is designated a call and analysis of the rest of the call is performed. This amplitude comparison guards against noise within the box, such as the frog jumping into the plastic bag, which could trigger a recognition error.

The number of chucks in the call is determined by analysis of the peaks occurring after a preset delay to account for the duration of the whine. A peak

is determined to be a chuck if its amplitude exceeds some amplitude threshold and its duration exceeds some duration threshold. Both thresholds are adjustable by the user to avoid false recognition of chucks due to background noise and chucks that are not fully formed. We set the amplitude threshold of the chuck to be 0.5 that of the peak amplitude of the whine. Phonotaxis experiments show that this is the minimum amplitude of the chuck relative to the whine that results in females responding to chuck; that is, in preferring the whine-chuck to the whine-only.

Once the number of chucks is determined, a response call is randomly generated from one of the six pools, each pool containing 20 natural calls with a specified number (0–5) of chucks. The response call is generated by the DAQ device and TECSIS resumes analysis of the audio stream for the focal male's response.

If TECSIS does not receive a response during a specified duration, the program pauses to allow the focal frog time to rest, then restarts the screening stimulus. TECSIS analyses a given number of calls for each calling strategy. Once this number of calls is acquired, the interaction ceases and, after a time delay, the program selects one of the remaining tests and resumes with the screening stimulus.

### *Statistical analysis*

Chucks from the focal male were manually re-counted from the sound files and compared to the interaction program's analysis; any discrepancies were excluded from analysis. TECSIS did occasionally make errors in counting the number of chucks in the male's call. Moreover, as we were interested in the response of the male to the playback, and the male always initiated testing with his call, the first call of each bout was excluded from analysis. The first twenty calls were extracted from the 50 calls recorded for each test. We used these data to address the following questions.

#### Differences in chuck production in response to calling strategies

All analyses were conducted with SYSTAT version 12<sup>®</sup>. We evaluated within-male differences in chuck production in response to different calling strategies. We first calculated the average number of chucks from 20 calls produced by each of 29 males who called during all four playback strategies. These averages were compared in a one-way repeated-measures

ANOVA, where all treatments were repeated with each male. Post hoc tests with Bonferonni corrections were used to compare individual treatments if the omnibus ANOVA revealed a statistically significant difference among treatments.

### Differences in entropy in response to calling strategies

Besides comparing the number of chucks produced in response to the model, we also compared the focal males' patterns of calling within a call bout. We used Shannon's measure of entropy to quantify the variability in transition among call types in response to each calling strategy. We used the transition probability between call types (i.e., calls with varying numbers of chucks) to calculate the transition entropy, which is defined as:  $\sum - p_i * \log^2(p_i)$ , where all transitions among calls types are summed and  $p_i$  is the probability of the  $i$ th transition (e.g., see Sakata & Brainard, 2006). Thus, there is one entropy value for the male's response to each calling strategy. As with the analysis of chuck number, we used a repeated-measures ANOVA to compare entropy among the calling strategies.

The sample of calls used in the chuck number and chuck variability analyses differed slightly. In the former, we eliminated any calls in which the computer made errors in counting number of chucks. Thus, the 20 calls analyzed were not necessarily consecutive. In the analysis of entropy it was critical that the calls of the focal male are consecutive as we are analyzing their sequential pattern. Of the 2320 calls analyzed for entropy (20 calls \* 29 males \* 4 treatments) the model erred in accurately counting the number of chucks 77 times, or about 3.32%. In most of the cases in which there was an error, the computer's assessment of chuck number was off by one.

### Entropy and mean chuck number in the field

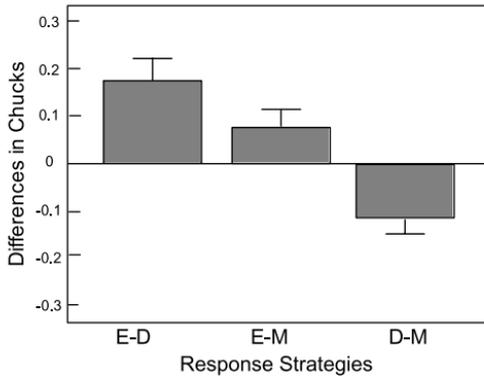
For comparative purposes we calculated the average number of chucks and the entropy of call bouts recorded from focal males in the wild. These calls are from a subset of calls used in a previous study (Bernal et al., 2007), but these analyses were not reported in that study. We conducted these analyses for call bouts of 20 males who had bouts of at least 20 calls. We analyzed the first 20 calls in each call bout to make the data more comparable to the experimental data. We used these data to compare values of mean chuck number and call entropy of natural choruses to our experimental data.

## Focal male calling strategies in response to computer strategies

We determined if males adopted specific calling strategies in response to the calling strategies of the model. Although different calling strategies could result in differences in mean chuck number, the two are not synonymous. Males could vary chuck number in response to treatments without employing statistically distinguishable strategies. For example, if a male occasionally or haphazardly added chucks in response to an escalate strategy and decreased chucks in response to a de-escalate strategy, there could be differences in mean chuck number but not in the strategies, at least as we define them below. Thus, both the analyses and the interpretations of changes in chuck number and calling strategy are addressed separately.

We quantified a male's strategy by subtracting the number of chucks in the male's call from the number of chuck's in the previous call produced by the model. A value of 0 would be akin to the call matching strategy (the male produced the same number of chucks he just heard), while values less than 0 would indicate a de-escalation strategy (he produced fewer chucks than he just heard) and positive numbers an escalation strategy (he produced more chucks than he just heard). We did this for all 20 calls the males produced in response to each computer strategy. We averaged these values for each male in each treatment to compare male strategies in response to computer strategies.

A matter complicating the analysis is that the male's strategy is a property of both his and the model's calling behaviour. For example, if a male produced the identical calls in response to each model strategy, the analysis would indicate that the focal male's strategy did indeed change in response to the different model strategies despite their being no difference in the calls made by the male in response to each strategy. Thus, we tested the null hypothesis that the male did not vary his call. If such were the case, the null hypotheses are that the male's strategy would be 0 in response to the match strategy of the model, +1 in response to the model's de-escalate strategy, and -1 in response to the escalate strategy. We compared the mean response of each male for each treatment to these null hypotheses with a one-sample *t*-test. Only if a male's calling pattern differed from that of the null hypothesis did we conclude that the male's calling strategy is influenced by the model's calling strategy. There are no bases to generate a null hypothesis for the focal male's null response to the random strategy of the model; therefore, those data are not included in this analysis.



**Figure 3.** The mean differences for each male in the number of chucks between the following strategies: escalate minus de-escalate (E–D), escalate minus match (E–M) and de-escalate minus match (D–M).

## Results

### *Differences in chuck production in response to calling strategies*

The total number of chucks produced in the experiments ranged from 0–4. On average, males produced 1.350 (SD = 0.46) chucks in response to the de-escalate strategy of the model, 1.454 (0.51) chucks in response to the matching strategy, 1.533 (0.53) chucks in response to the escalate strategy, and 1.46 (0.48) chucks in response to the random strategy. Figure 3 shows the repeated-measures or within-male differences in chucks in response to the different non-random calling strategies. The repeated-measures analysis reveals a significant difference in the mean number of chucks produced among the four treatments ( $F_{3,84} = 4.96$ ,  $p = 0.003$ ). Post hoc tests with Bonferroni corrections showed a significant difference between escalate and de-escalate strategies ( $p = 0.003$ ) and a trend for a difference between the match and escalate strategies ( $p = 0.069$ ). There were no significant differences between the number of chucks produced in response to the match and de-escalate ( $p = 0.397$ ), match and random ( $p = 1.0$ ), escalate and random ( $p = 0.272$ ) and the de-escalate and random strategies ( $p = 1.0$ ; Figure 3).

### *Differences in entropy in response to calling strategies*

The entropy of call bouts was similar among treatments: match (mean = 0.406, SD = 0.32), escalate (0.514, 0.33), de-escalate (0.393, 0.32) and random (0.476, 0.38). The repeated-measures analysis did not reveal a significant difference among the treatments ( $F_{3,84} = 1.371$ ,  $p = 0.257$ ). As the

omnibus ANOVA did not reveal significant differences we do not report the results of post-hoc tests.

### Entropy and mean chuck number in the field

The mean entropy in natural call bouts in the field was 0.374 (SD = 0.42) and the mean chuck number was 0.553 (0.61). In the 20 males analyzed, there was no correlation between entropy and average chuck number (Pearson's  $r = 0.309$ ,  $p = 0.185$ ), entropy and call bout length ( $r = -0.089$ ,  $p = 0.709$ ), or mean chuck number and bout length ( $r = 0.036$ ,  $p = 0.881$ ). These results show that analyses of the first 20 calls of a bout were not biased by bout length, and also indicate that a male's long-term behaviour, in terms of bout length, is not predicted by his short term behaviour.

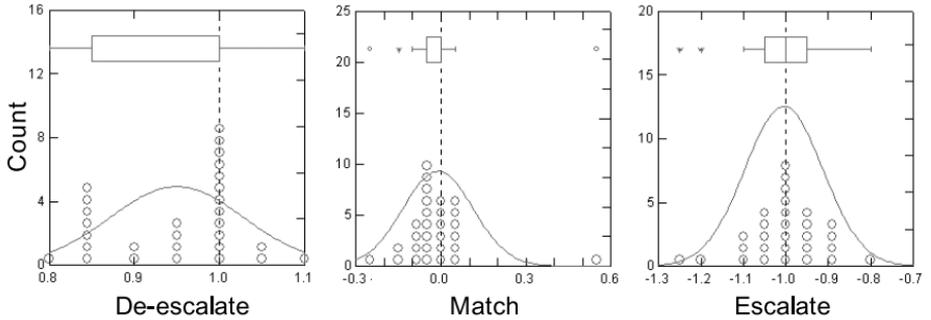
Males produced fewer chucks in the field (mean = 0.552) than in the four experimental treatments (mean = 1.449; two-sample  $t$  test, separate variance,  $t = 6.283$ ,  $df = 23.5$ ,  $p < 0.001$ ). There were no differences, however, in the average entropy of call bouts in the field (0.374) and in the experiments (0.447;  $t = 0.751$ ,  $df = 23.6$ ,  $p = 0.460$ ).

### Focal male calling strategies in response to computer strategies

The male calling strategy did not differ from the null hypothesis in response to the interaction program's match strategy ( $H_0 = 0$ , mean =  $-0.009$ , SD = 0.124,  $t = -0.374$ ,  $p = 0.711$ ) or its escalate strategy ( $H_0 = -1$ , mean =  $-1.00$ , SD = 0.092,  $t = -0.303$ ,  $p = 0.764$ ). The male calling strategy did differ from the null hypothesis when the interaction program employed a de-escalate strategy ( $H_0 = 1$ , mean = 0.948,  $t = -3.715$ ,  $p = 0.001$ ). As can be seen in Figure 4, there is a strong skew in the strategies of these males towards de-escalation. Thus, when the model reduces the number of chucks, males change their calls and they tend to follow the de-escalation.

## Discussion

Male túngara frogs engage in dynamic calling interactions in which they vary the number of chucks in their calls in response to changes in the social environment. Experiments with static playbacks have shown that males change their calling behaviour with the initiation of a playback stimulus and



**Figure 4.** The calling strategy (the number of chucks in a male's call minus the number of chucks in the previous response call by the model) of each male in response to each of the three response strategies of the model. The null hypotheses for the male's strategy (if he did not change his calls during the calling bout), denoted by the dashed vertical line, are 1.0 (De-escalate), 0 (Match),  $-1$  (Escalate). The upper bars in each graph represent a box plot of the males' strategies.

in response to simple versus complex calls. Static playback experiments have not, however, shown consistent variation in the number of chucks that males produce in response to stimuli with one, two, or three chucks (Bernal et al., 2009). Other studies have shown that in species with dynamically variable signaling, responses can be sensitive to dynamic changes in competitors (reviewed in Schwartz, 1994, 2001; Dabelsteen & McGregor, 1996; McGregor, 2000; Schwartz et al., 2002). Our goal in this study was to determine if male túngara frogs modulate their calling behaviour in response to other males with different calling strategies. We also attempted to determine how observations in the field correspond to the strategies that males might use in calling within an environment where there are both different benefits and costs associated with variation in calling behaviour.

### *Chuck number*

Our results show that males vary chuck number in response to variation in the model's calling strategy. As predicted, males produced fewer chucks in response to the de-escalate strategy, more chucks in response to the match strategy and the greatest number of chucks to the escalate strategy. The numbers of chucks in response to the random strategy was intermediate between the responses to the match and escalate strategies. There was a significant difference only between the two extremes, the mean numbers of chucks in response to the de-escalate and escalate strategies. Thus, the general notion that

males vary the number of chucks in their calls in response to escalation of vocal competitors appears to be generally true. Although this pattern seemed apparent in nature, it could not be verified in static playback experiments. In those experiments males did not increase number of chucks in response to one versus two versus three chucks. Although they did produce more chucks when they were challenged by six chucks, this is a rare occurrence in nature and probably does not account for the apparent variability in male behaviour in the field (Bernal et al., 2009). Our study reveals a calling pattern that is both consistent with our intuition from naturalistic observations and differs from the results of the less realistic paradigm of static playback.

The result that males are sensitive to calling strategies based on chuck number might suggest an arm's race in which males continually and quickly increase chuck number until they reach the limit of the call production system. This is clearly not the case. The number of chucks in response to the model strategies ranged from a total of 0 to 4, but only averaged between 1.35 and 1.53 among treatments. Males produced more chucks, on average, in the experiments than they did in the field. But in nature males can (rarely) add up to 7 chucks. All these data lead us to suggest that the maximum chuck number is not imposed by a biomechanical constraint of calling.

The low number of chucks produced in response to the model is characteristic of this communication system. The mean number of chucks in the experiments, however, was significantly higher than that in the field. This might be because in the safe environs of the calling chamber the focal males were not exposed directly to predators or to the behaviour of other frogs that indicated the presence of predators (Jennions & Backwell, 1992; Phelps et al., 2007).

Another explanation for more chucks in the experiments than in the wild is that focal males were competing with calling strategies that were not sensitive to risks of producing additional chucks or to other costs that might be associated with producing more chucks. For example, although the rate of oxygen consumption does not increase for calls with versus without chucks (Bucher et al., 1982), we know nothing about costs of anaerobic metabolism or tissue deterioration that might occur with adding chucks. We do know that there are increased predator costs associated with producing more versus fewer chucks due to call preferences of frog-eating bats (R. Page unpublished data). All of these costs influence not only the focal male but his vocal competitors.

The analyses of changes in male calling strategies also suggest that low chuck number is due to cost-sensitive behaviour. Males only exhibited a calling strategy that deviated from the null of no change when they interacted with a model's de-escalation strategy. The focal males de-escalated in these situations. All of these results show that males are reluctant to escalate to the maximum number of chucks they can produce.

The changes in calling strategies, as we define them, do not necessarily encompass all of the variation in male calling behaviour. We show that males de-escalate in response to a de-escalate strategy but do not employ a calling strategy different from the null expectations when they are challenged with a match or an escalate strategy. This might lead one to predict that all the differences in chuck number should reside only in responses to the de-escalating model. But this seems not to be the case. Differences in chuck number differ between de-escalate and escalate but not between de-escalate and the other two calling strategies, match and random.

Under what situations do males produce many chucks ( $>3$ )? Recently, K. Akre (unpublished data) demonstrated that males add chucks in response to water vibrations, as would be produced by an approaching frog, and that females incite males to produce additional chucks with swim-by behaviour as well as physically nudging the males. Thus, vocal competition among males is not the only factor influencing call complexity, and the fact that females can incite increases in call complexity suggests a strong reluctance of males to make more attractive calls, and speaks legions to the importance of the costs of call complexity.

Not only could high costs select against producing more chucks, but relatively low benefits are also a critical variable. When females are given a choice between calls with zero and one chuck there is a five-fold preference for calls with one chuck (Ryan, 1985; Gridi-Papp et al., 2006) but no general preference for multiple versus single chucks (Bernal et al., 2009). These preference experiments were conducted so that the call of the male was 82 dB SPL at the release point of the female, mimicking a situation in which the calling male was about 1.35 m away. When these experiments were repeated at 90 dB SPL at the site of the female, which would indicate a closer male, females preferred three chucks to both two chucks and to one chuck (K. Akre, unpublished data). Thus, there is a benefit to making more versus fewer chucks but only when females are in close proximity, which is

when males add more chucks in nature. Given the predation costs of complex calls, it seems that the benefit of producing more versus fewer chucks, without some evidence that a female is nearby, might not outweigh the costs of doing so.

In summary, this study, combined with other studies of the túngara frog communication system, suggest a system that is conservative in that males only rarely escalate to maximum performance, and that the communication system appears to be quite cost sensitive and prone to de-escalation.

### *Entropy*

Besides changing their chuck number, males can also change their pattern of calling. But males are as recalcitrant to changing calling pattern as they are to changing chuck number. There were no significant differences in entropy in response to the varied calling strategies of the model.

The relatively low variation in entropy that we see in the experiments is mirrored in the field; there was no significant difference between the two settings. As noted above, we suggested that the limit on chuck number in the experiments and in the field is not due production constraints. In contrast, we think that entropy is influenced by biological constraints. Bernal et al. (2009) examined the transitional probabilities of chuck numbers in the field. In that sample, males usually changed chuck number one at a time. For example, when males changed from producing 1 chuck the combined probability of changing to 0 or 2 was 1.0. Males did not change from 1 chuck to 3 or more chucks. When males changed from 2 chucks, the combined probability of changing to 1 or 3 was 0.91, and from 3 chucks the probability of changing to 2 chucks was 1.0. This does not demonstrate that males cannot vary chuck number more unpredictably, but it is possible that the biomechanics of chuck production biases males to adding or subtracting single chucks at a time. In addition, we know that females are not sensitive to variation in chuck number independent of mean chuck number. For example, females do not show a preference between one speaker that broadcasts a whine +1 chuck versus another that cycles through a whine, whine +1 chuck, whine +2 chucks and, thus, has a mean of a whine +1 chuck (Ryan, unpublished data). Until more is known about the details of chuck production we can only speculate that males are constrained from more variable calling patterns than we see in nature.

## Conclusions

We expose here details of the calling behaviour of túngara frogs that were previously unrevealed by static playback but yield to studies using dynamic playback. Males vary call complexity in a manner predicted by their behaviour in the wild, increasing or decreasing calls in response to that of vocal competitors, but doing so in a conservative manner. Despite the ability to produce up to seven chucks, the mean number of chucks in these experiments was less than two. Although this is greater than the mean number of chucks produced in the wild, it highlights the conservative nature of call escalation in this species. We suggest that males are risk sensitive due to the presence of eavesdropper who predate upon and parasitize calling males.

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