

Temporal updating during phonotaxis in male túngara frogs (*Physalaemus pustulosus*)

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Abstract. In acoustically advertising anurans the male courtship call elicits species-typical responses from conspecifics – usually phonotactic approach and mate choice in gravid females and an evoked vocal response in adult males. Males in several species, however, are also known to perform phonotaxis, sometimes with the same acoustic preferences as females. Female túngara frogs are known to update their phonotactic approach as male advertisement signals change dynamically in attractiveness. Here we show that males also perform such temporal updating during phonotaxis in response to dynamic playbacks. While males exhibit slower phonotactic approaches than females, their responsiveness to dynamic changes in call complexity does not differ significantly compared to females. These results demonstrate that males are sensitive to the location of preferred call types on a moment-to-moment basis and suggest that similarities between male and female sexual behaviour in anurans might often be overlooked. We suggest that anuran phonotaxis is more widespread and serves different functions in reproductive females and males. Lastly, these temporal updating results suggest that male frogs are highly selective about site selection in a chorus.

Keywords: acoustic communication, male phonotaxis, *Physalaemus pustulosus*, temporal updating.

Introduction

Acoustic signaling is a dynamic process that typically occurs over brief timescales. In many anuran amphibians, males gather in leks during the breeding season to attract females to mate using advertisement calls that indicate a male's sexual receptivity and location (reviewed in Wells, 2007). Volumes of research have shown that females use courtship signals to localize, approach (i.e., exhibit phonotaxis) and select amongst males on the basis of call attributes (reviewed in Ryan, 2001; Gerhard and Huber, 2002). A smaller number of studies have shown that males often perform phonotaxis as well (Hödl, Amézquita and Narins, 2004; Bernal, Rand and Ryan, 2009; Baugh and Ryan, 2010a). In previous mate choice studies (Baugh and Ryan, 2009, 2010b) we showed that female tún-

gara frogs (*Physalaemus pustulosus*) respond to sudden changes in male courtship signals during phonotactic approach: under a two-choice playback condition, females reversed phonotaxis if the approached signal suddenly became less attractive while the unapproached signal became more attractive. This temporal updating behaviour during mate choice occurred in approximately one-half of trials. In the remaining trials females continued on their initial trajectory, thereby committing to their initial choice despite a sudden reduction in call attractiveness. Here we apply this approach to examine if male túngara frogs exhibit the same behaviour despite the fact that phonotaxis is not associated with mate choice in male túngara frogs.

Túngara frogs are small anurans (ca. 30 mm snout-to-vent length) with a distribution that includes much of Mesoamerica (Weigt et al., 2005). Males advertise to females during the breeding season (May-December) using a species-typical call, known as the “whine” or simple call (Ryan, 1985). Males can embellish the whine with one to seven acoustic ornaments known as “chucks”, thereby producing what is known as the complex call or whine-chuck. Vo-

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calizing males are also known to adjust their call amplitude over the course of a call bout (Pauly et al., 2006) and, therefore, call amplitude has passive and active dynamic properties. In laboratory two-choice phonotaxis tests, females and males prefer the whine-chuck calls to whine calls (Ryan and Rand, 2003; Bernal, Rand and Ryan, 2009; Baugh and Ryan, 2010a). Túngara frogs also show preferences for calls of higher amplitude over lower-amplitude alternatives, which presumably results in attraction towards nearer males and thus reduces travel time (Ryan and Rand, 1990). Phonotaxis in male anurans is examined less frequently but it is assumed that in lek-breeding species (e.g., túngara frogs) this behaviour functions to guide males to aggregations for the selection of a calling site and in territorial species to repel rival male intruders (Hödl, Amézquita and Narins, 2004).

Temporal updating, or dynamic reproductive decision-making, provides a practical solution for animals that make decisions in social environments that are in constant flux. In lek-breeding animals, such as many insects, anurans and birds, females and males make reproductive decisions in the midst of temporally variable social signals. For females, the challenge is to identify a suitable mate whereas males must participate in a chorus. To do so, a male must first locate a lek and select a territory from which to call. In this study we performed a dynamic phonotaxis experiment with males to determine if they exhibited updating behaviour similar to that of females. In females, selection of the complex call is an expression of mate preferences – in males, such behaviour might increase the likelihood of selecting a high quality calling site because complex calls are indicative of high density choruses which confer increased per capita mating success for males and lower predation risk (Ryan, Tuttle and Taft, 1981; Bernal et al., 2007).

Materials and methods

We conducted this experiment during the breeding season between the months of July and August in 2007 at facilities for the Smithsonian Tropical Research Institute in Gamboa, Panamá (9°07.0'N, 79°41.9'W). We collected frogs as amplexant mated pairs from breeding aggregations between 1900 and 2200 h and performed behavioural testing between 2000 and 0500 h. Animals were held in small plastic containers in dark, quiet conditions before testing. To prevent resampling we marked individuals with a unique toe-clip combination, measured the mass and snout-to-vent length and returned them to their original site of collection within 12 h. In marking frogs, we followed the Guidelines for the Use of Live Amphibians and Reptiles in Field Research, compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR), and our methods were approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin and La Autoridad Nacional del Ambiente in Panamá. In total, 47 males were tested and of these 20 males responded in both of two consecutive trials (a prerequisite), resulting in 40 successful choices.

We used two stimuli: a synthetic whine and the same whine with one chuck appended (fig. 1a). Stimuli were matched for the peak amplitude of the whine before playback and broadcast from small speakers (ADS L210) located at the two poles of an arena (fig. 1b). The chuck on the complex call is twice the peak amplitude of the whine. Information on these stimuli including the synthesis procedure can be found in Ryan et al. (2003).

Male frogs were tested for phonotaxis under infrared light in a sound-attenuating chamber (2.7 × 1.8 × 1.78 m, L × W × H; Acoustic Systems, Austin, TX). Before each subject was tested, we calibrated each speaker to 82 dB SPL (re. 20 μ Pa) at the centre of the arena (1.35 m from each speaker) using the whine stimulus (GenRad 1982 SPL meter, peak amplitude, flat weighting). Trials began with the subject placed under a cone at the centre of the acoustic chamber (i.e., release point; fig. 1b) for 3 min while the two stimuli were broadcast antipodally at a rate of one call per 2 s from each of two speakers opposite one another. The cone was then lifted remotely and the phonotactic behaviour was monitored via a ceiling-mounted infrared camera and television monitor outside the chamber. The chamber was divided symmetrically by boundaries (hereafter "approach boundaries") at a minimum distance of 75 cm from the speakers (dashed line, fig. 1b). To the subject, this boundary represents a minimum distance of 20 body lengths on average from the release point. These dashed lines were visible only to the human observer (outlined by transparencies on the monitor). In each trial one of the two speakers initially broadcast the whine-chuck (this "target" speaker was selected randomly and then alternated between tests and subjects to minimize potential side bias; see Results) while the opposite speaker broadcast the whine. Frogs almost always approach the whine-chuck. When the subject crossed the approach boundary nearest the whine-chuck, the human observer pressed a key on the playback computer's keyboard

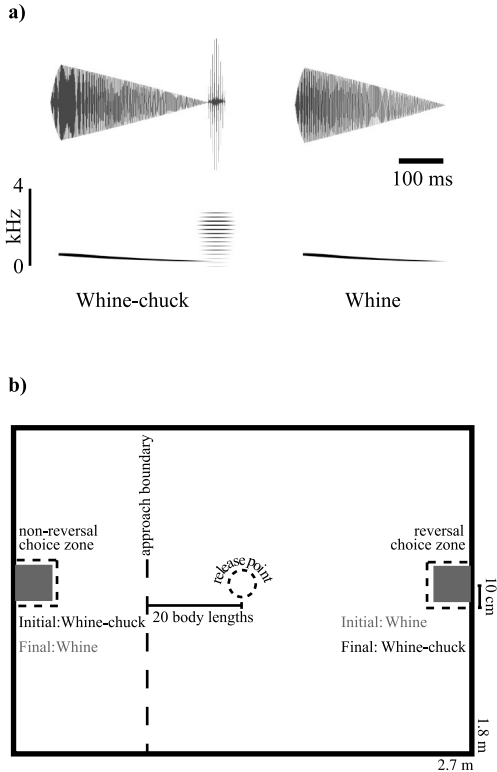


Figure 1. (a) Synthetic whine and whine-chuck oscillograms (top) and spectrograms (bottom). (b) Phonotaxis arena showing dimensions and position of release point (midpoint of chamber), speakers, choice zones, and approach boundary (75 cm from speaker, ca. 20 body lengths from release point). This figure depicts one of two symmetrical configurations for simplicity.

that initiated a program in the acoustic software program SIGNAL[®] (Engineering Design, Belmont, MA, USA). This program introduced an initial 500 ms delay to prevent the premature occlusion of a stimulus, and then subtracted the chuck from the approached speaker and added a chuck to the unapproached speaker. Simultaneously the program amplified the distant stimulus (the one that was not initially approached) by +2 dB, thus equilibrating the mean peak amplitude along the approach boundary (this was determined empirically by averaging the peak amplitude differential at six equally spaced points along the approach boundary transect). This amplification was done so that call complexity alone could be examined without the confounding effects of call amplitude. In each trial we recorded two variables: (1) whether the frog made a choice and if so, whether it involved a reversal choice (final selection was for the initially unapproached speaker) or a non-reversal choice (maintained initial trajectory selecting the initially approached speaker); and (2) the latency to the choice zone.

We used the same criteria to score a phonotactic choice by the male frogs as we do in the females (e.g., Baugh and Ryan, 2010b): a choice occurred when the male approached

one of the speakers within a 10 cm radius without simply following the wall. Frogs that were motionless for the initial 5 min after the cone was raised or during any 2 min interval thereafter, or that failed to make a choice within 15 min after the cone was raised were scored as failing to show a phonotactic choice. A prerequisite for these tests was that males must initially approach the preferred stimulus (whine-chuck). Frogs that were unresponsive in any experiment or that failed to initially approach the preferred stimulus and instead initially approached and selected the less preferred stimulus (simple call), were scored as showing “no response”. In the uncommon instances in which males were responsive but approached the less preferred stimulus initially, we re-tested these subjects. Almost invariably these subjects approached the preferred stimulus on a subsequent trial.

We tested each male twice in the same condition (two replicates) and used the nonparametric Mann-Whitney *U*-test (two-tailed) to compare the frequency of reversal choices (frogs could show reversal of choice 0-2 times within a test condition) made by males compared to the frequency of reversal choices made under an identical protocol using females from a previous study (Baugh and Ryan, 2010b). This two-replicate design was used in order to compare across these two studies and ensures that all subjects are responsive. We have shown previously that consecutive trials do not influence the behavioural outcome of the tests (Baugh and Ryan, 2009, 2010b).

Results

We tested for a side bias and found no evidence of one (see Baugh and Ryan, 2009). We found that males exhibited reversal behaviour with a frequency of 35.0% (14 reversal choices, 26 non-reversal choices). This result does not differ from that of females (44.3%; Baugh and Ryan, 2010b) under identical experimental conditions ($U = 597$, $P = 0.276$). Latency to choice for males, however, was slower than for females on reversal trials (Mean \pm SE (s): males: 329.9 ± 46.7 ; females: 163.8 ± 121.1) but not for non-reversal trials (males: 169.7 ± 19.3 ; females: 162.2 ± 131.2).

Discussion

In túngara frogs, temporal updating during phonotaxis is not limited to females – males also exhibit this flexibility and do so with the same frequency as females. Under standard

(non-dynamic) playback conditions broadcasting whine versus whine-chuck, we have not witnessed males reverse direction after beginning an approach (of 20 body lengths) toward the whine-chuck (Baugh, 2009). This is also true for females, which reverse in 0% of trials under non-dynamic whine versus whine-chuck conditions (Baugh and Ryan, 2010b). Therefore, the reversal behaviour in males reported here appears to be a specific response to the stimulus manipulation.

It is important to bear in mind that mate choice is only one class of reproductive decisions. Because túngara frogs are a lek species, females and males must also select and approach a lek – a relatively understudied topic of investigation. The fact that males also perform temporal updating might illustrate the role of lek selection that the sexes share. In other words, one interpretation of temporal updating in male frogs is that it guides males to the most attractive lek, toward which females will also be attracted. Following the selection of a lek, male updating toward complex calls might function to guide a male to a relatively attractive local calling site.

In addition to the studies discussed above (Baugh and Ryan, 2009, 2010b), a few studies have pursued dynamic mate choice in other female anurans. In reed frogs, *Hyperolius marmoratus*, Dyson, Henzi and Passmore (1994) found that females will reverse course after initially approaching (one body length) a preferred stimulus (leading call) if the stimuli are switched. Likewise, Gerhardt, Dyson and Tanner (1996) tested the preference strength for pulse number in *Hyla versicolor* and found that females, after initially approaching a high pulse number call, reversed directions when this preferred stimulus was suddenly switched with a less preferred low pulse number alternative. By varying the pulse number of competing calls across experiments within the natural range of variation, and adjusting the distance and amplitude of the sources, the authors obtained reversal frequencies that averaged about 50%,

and peaked at approximately 75% under conditions of greatest signal contrast. Also, Márquez, Bosch and Eekhout (2008) examined the related issue of mate choice as a function of call amplitude, using a treadmill and amplitude ‘setpoints’ to derive another metric of dynamic preference in midwife toads. These previous studies provide strong evidence that decision-making involves temporal updating in several species of frogs. To our knowledge the present study is the first to report on temporal updating behaviour in male anurans.

Virtually all anuran phonotaxis studies have examined females that are in reproductive condition – with the implication that phonotaxis is an expression of mate choice. This is not, however, the only interpretation. There are now several studies, including the present one, demonstrating that adult male frogs (Bush, Dyson and Halliday, 1996; Lea, Dyson and Halliday, 2002; Baugh and Ryan, 2010a), metamorphic subadults (Lea, Dyson and Halliday, 2002; Baugh and Ryan, 2010a) and non-reproductive (post-mated) adult females (Lynch et al., 2005) exhibit positive phonotaxis to conspecific mating signals. One such study (Baugh and Ryan, 2010a) provides evidence that the motivations for phonotaxis are not the same across these subject categories. Reproductively competent females exhibit a trademark component of the phonotactic response (‘perseverance’) in túngara frogs that differs markedly from juvenile frogs of both sexes and adult males. Perseverance behaviour consists of highly localized movement around the conspecific sound source (speaker or calling male), and persists for the duration of a playback. Reproductive female túngara frogs will travel several meters distance around the sound source in only a few minutes provided the signal continues to be broadcast. Males and juveniles, however, remain nearly stationary following arrival at the sound source. This marked difference suggests that reproductive females perform phonotaxis to seek out a mate (which necessarily involves finding and making physical contact), whereas the motiva-

tion for juveniles and adult males likely does not necessitate contact with the calling male. There are also differences in latency to phonotactic response (Baugh and Ryan, 2010a; present study) for juveniles, adult males and females – this difference, however, is a difference in degree, whereas the presence and absence of perseverance behaviour is a difference in kind, and as such strongly suggests motivational differences between these subject categories.

We suggest that reproductive decision-making in frogs is not simply a two-step process of evaluating signals and applying a decision rule, as it is commonly framed in behavioural ecology (Valone et al., 1996); such conceptualizations ignore the role of executing the decision, including the iterative process of temporal updating during choice. By taking a psychophysical approach and dynamically manipulating signals during phonotactic approach, we show that the decision-making process is iterative and that both females and males exhibit this updating behaviour. This further supports the idea that phonotaxis is a more widespread behaviour and not simply limited to reproductive females. Thus, we provide additional evidence that anuran phonotaxis, an archetypical sexual behaviour, serves functions beyond simply selecting a mate (Lea, Dyson and Halliday, 2002). We propose that dynamic phonotaxis in male túngara frogs provides a sensitive mechanism for high-density lek selection, with which complex calls are associated (Ryan, Tuttle and Taft, 1981; Bernal et al., 2007), and the selection of highly attractive calling sites within the chosen lek. By actively approaching complex calls, males might take advantage of the increased per capita mating success and reduced predation risk associated with high-density choruses. This might also suggest that male positioning in a chorus changes throughout a night of calling activity as males enter and exit the chorus, or simply modify their calling patterns. A promising direction for ongoing and future studies involves mapping the detailed movements of multiple calling males within a natural breeding ag-

gregation through the use of microphone arrays (Jones and Ratnam, 2009). In addition to providing a tool to test hypotheses about vocal interactions in anurans, microphone array studies might yield insight into the dynamics of male orientation and phonotactic interactions on a scale relevant, but previously too difficult to measure accurately, for anuran choruses.

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