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Female Preferences for More Elaborate Signals Are an Emergent Outcome of Male Chorusing Interactions in Túngara Frogs

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ABSTRACT: In chorusing species, conspecific interference exerts strong selection on signal form and timing to maximize conspicuousness and attractiveness within the signaling milieu. We investigated how túngara frog calling strategies were influenced by varied social environments and male phenotypes and how calling interactions influenced female preferences. When chorusing, túngara frog calls consist of a whine typically followed by one to three chucks. In experimental choruses we saw that as chorus size increased, calls increasingly had their chucks overlapped by the high-amplitude beginning section of other callers' whines. Playback experiments revealed that such overlap reduced the attractiveness of calls to females but that appending additional chucks mitigated this effect. Thus, more elaborate calls were preferred when calls suffered overlap, although they were not preferred when overlap was absent. In response to increasing risk of overlap in larger choruses, males increased call elaboration. However, males overwhelmingly produced two-chuck calls in even the largest choruses, despite our results suggesting that additional chucks would more effectively safeguard calls. Furthermore, aspects of male phenotypes predicted to limit call elaboration had negligible or uncertain effects, suggesting that other constraints are operating. These results highlight how complex interrelations among signal form, signaling interactions, and the social environment shape the evolution of communication in social species.

Keywords: animal communication, communication networks, receiver psychology, sexual selection.

Introduction

Acoustic signals are essential for courtship in many insects and anurans, with courtship signals often sent and received within dense choruses (Gerhardt and Huber 2002). Acoustic signals are relatively omnidirectional and far-reaching, meaning that females navigating choruses often perceive several males' calls simultaneously or closely in time. Thus, the way temporally associated signals are parsed by female sensory systems has important consequences for female choice (Bee and Micheyl 2008). For instance, high background noise at choruses can hinder female abilities to recognize and localize calls or to discriminate among calls varying in salient properties (Wollerman and Wiley 2002; Vélez et al. 2013; Reichert and Ronacher 2015). Finer-scale interference patterns can also influence female choice; females may discriminate against choruses in which calls overlap rather than alternate (Schwartz 1987; Minckley and Greenfield 1995; Bosch and Márquez 2001) and may discriminate against one call in an overlapping call pair because of the relative temporal association of the calls. For example, certain types of call overlap can obscure attractive elements in one call and not in another, causing discrimination against the obscured call (Wells and Schwartz 1984; Grafe 1999). Similarly, females often exhibit precedence effects, preferring the first call heard of a call pair (the leading call) when calls overlap or occur closely in time (Greenfield and Roizen 1993; Greenfield 1994). When female preferences are influenced by the relative temporal associations of signals, this exerts strong selection on male signal-timing strategies (Greenfield et al. 1997, 2016).

When call overlap reduces callers' attractiveness, chorusing males avoid overlap and alternate calls with neighbors (Klump and Gerhardt 1992; Minckley and Greenfield 1995; Grafe 2005). Alternation is facilitated by inhibitory resetting; males are inhibited from calling while perceiving rivals' calls, instead initiating calls in the quiet gaps between these calls (Greenfield 1994). This "gap detector" neural mechanism is typically flexible, with intensity thresholds for inducing inhibition being adjusted relative to background noise levels (Zelick and Narins 1983; Greenfield and Rand 2000).

In alternating species, gap detection is sufficient for avoiding overlap when chorus-mates are few, but overlap becomes

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inevitable in large choruses (Brush and Narins 1989; Schwartz et al. 2002). Typical interonset intervals (IOIs; the time elapsing between the onset of successive calls by a caller) and call durations, as well as a species' flexibility in these properties, dictate the upper limit as to how many males can call together without call overlap. However, in choruses exceeding this upper limit, males can employ strategies to mitigate the detrimental effects of call overlap on attractiveness. For example, longer call durations may increase the probability that some notes of multinote calls remain free from overlap in fluctuating chorus noise (Martínez-Rivera and Gerhardt 2008; Schwartz et al. 2008; Love and Bee 2010). Additionally, males may increase IOIs as a means to reduce call overlap (Latimer 1981; Grafe 2003) and, when calls are pulsatile, may increase the duration of the silent intervals between pulses to allow interdigitation with the pulses of neighbors' calls (Schwartz and Wells 1985; Martínez-Rivera and Gerhardt 2008; Stirman and Pfennig 2019). Furthermore, when avoidance of overlap is impossible, males can prioritize overlap avoidance with only the most salient subset of neighbors within earshot ("selective attention"; reviewed in Greenfield et al. 2021). Males usually selectively attend to their nearest neighbors, whose calls they perceive as highest in amplitude and whose calls will be perceived as highest in amplitude relative to their own by approaching females (Brush and Narins 1989; Tárano and Carballo 2016).

Females visiting choruses often evaluate calls from nearby callers comparatively (Bateson and Healy 2005). This means that in addition to needing to maintain favorable call-timing relationships with neighbors, males must also produce calls that are relatively more attractive than those of nearby rivals to attract females. Accordingly, when calling against competitors, chorusing males typically exaggerate call properties that are attractive to females (Ryan and Keddy-Hector 1992; Dyson et al. 2013), such as increasing call amplitude (Lopez et al. 1988; Halfwerk et al. 2016), increasing call effort or rate (Wagner 1989; Wells and Taigen 1989; Morris et al. 2002), and embellishing calls with different note types (Wells and Schwartz 1984; Zhu et al. 2017; Oliva et al. 2018). These adjustments can increase the energetic demands of calling (Ryan 1988; Prestwich 1994), meaning that the degree of exaggeration may be constrained by aspects of male phenotypes (Zimmitti 1999; Voituron et al. 2012; Anichini et al. 2018). Thus, as competition increases and males signal nearer their upper limits, signal comparisons can become more informative to females in illuminating differences among males in their underlying quality (Wong and Candolin 2005; Gavassa et al. 2012).

We investigated how male túngara frog signaling strategies were influenced by the social environment and aspects of male phenotypes and investigated the effects of male signaling interactions on female choice. Although túngara frogs call within dense choruses in the wild, previous studies of male calling behavior have primarily investigated responses to a single rival or have focused on single focal callers within wild choruses. Thus, information regarding mutual signaling interactions and the granularity of male responses to changing social environments is lacking, as is information regarding how signaling interactions influence female choice. To address this, we conducted a series of experiments guided by three main aims. We briefly outline these aims and the logic connecting successive aims below, while specific hypotheses and predictions pertinent to each aim are found at the beginning of the relevant sections later in the text.

In aim 1, we observed intercaller interaction patterns in experimental choruses of different sizes (two to six males). Túngara frog calls consist of a whine typically followed by one to three chuck notes, and we found that intercaller dynamics in larger choruses led to prevalent stereotyped call overlap in which the chucks of males' calls were overlapped by their chorus-mates' whines.

In aim 2, we used phonotaxis experiments to investigate the consequences of this stereotyped call overlap for female choice. We found that calls whose chucks were overlapped in this way were less attractive to females, suggesting that calling strategies that safeguard calls against costs of overlap may be favored by selection.

In aim 3, using female phonotaxis experiments and reanalysis of our experimental chorus data, we investigated whether appending greater numbers of chucks to calls represents a means to mitigate the attractiveness costs suffered during call overlap. Furthermore, we investigated whether the capacity for adding greater numbers of chucks was constrained by male body size and condition.

General Methods

Here, we outline background information and general methods relevant to the multiple aims listed above. More detailed methods specific to each aim are provided in the relevant sections.

Túngara Frogs

Túngara frog males call while floating in water, with breeding sites typically being shallow pools such as puddles and drainage areas. Operational sex ratios at breeding sites are heavily male biased, and choruses can reach high densities (Ryan 1983; Bernal et al. 2007; fig. S1; figs. S1, S2, S4, S7, and S8 are available online). Females visit choruses during the night, moving among callers and appearing to sample several males before selecting a mate (Ryan 1985).

Túngara frog calls begin with a whine—a continuous descending frequency sweep whose fundamental frequency changes from ~1,000 to ~450 Hz over ~330 ms (Wilczynski et al. 1995; Ryan and Rand 2003). Simple, whine-only calls are sufficient to attract females. However, males can also produce complex calls by appending a number (typically one to three; Bernal et al. 2009) of short (~35 ms) harmonically structured chuck notes to whines (fig. 1). Complex calls are fivefold more attractive to females than simple calls (Ryan et al. 2019), although complex calls with greater numbers of chucks are more attractive than those with single chucks only under certain experimental conditions (Ryan 1985; Bernal et al. 2009; Akre and Ryan 2010*a*, 2010*b*; Akre et al. 2011; Tárano 2015; Stange et al. 2017). Túngara frogs are unison bout callers, with males calling approximately every 2 s and alternating calls with their neighbors (Ryan 1985; Bosch et al. 2000). Males increase call rate (Green 1990), call amplitude (Halfwerk et al. 2016), and number of chucks (Bernal et al. 2007) in response to rivals.

Experimental Subjects

Between August and December 2021 and 2022, we collected túngara frogs from urban breeding sites around Gamboa, Panama (9°070″N, 79°419″W). All male and female frogs were collected in amplexus, increasing the likelihood that females would be receptive and responsive to playback and that males would call in our experimental setups. After trials, we weighed subjects (g), measured their snout-vent length (SVL; mm), and gave them a unique toe clip. We designed toe-clip codes so that the fewest toes possible were clipped, and we never clipped the innermost digits on the front limbs, which may be important for amplexus. All protocols followed the *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research* (Beaupre et al. 2004). After trials, we reunited male and female pair-mates and returned them to their collection site that same night.

Experimental Chorus Recordings

To investigate call-timing patterns in choruses of different sizes, we constructed choruses of two to six males in a darkened room ranging in temperature from 25.2°C to 27.7°C. Males were placed in individual acoustically transparent enclosures containing water in which they could call (fig. S2) and haphazardly arranged as vertices of a hexagon with 1-m sides. We used repeated playback of a one-chuck call (a whine followed by one chuck) to stimulate calling, then ceased playback and began recording once several males called. Each chorus-mate was recorded onto a separate channel of a Zoom F6 multitrack recorder via an individual Synco LavS6R tie clip microphone. Chorus participation varied over time, meaning that certain males/choruses were represented multiple times at different chorus sizes. We included choruses in the dataset if they persisted at a given size for at least 5 min without caller identities changing (chorus size [number of choruses in dataset]: six males [six]; five males [eight]; four males [five]; three males [six]; two males [five]). In total, 69 individual males arranged in 16 unique choruses were included in the dataset. We extracted time stamps for all chorus-mates' whines and chucks from recordings using Python scripts that utilized the Librosa package (McFee et al. 2015). Chucks can vary in amplitude and must exceed

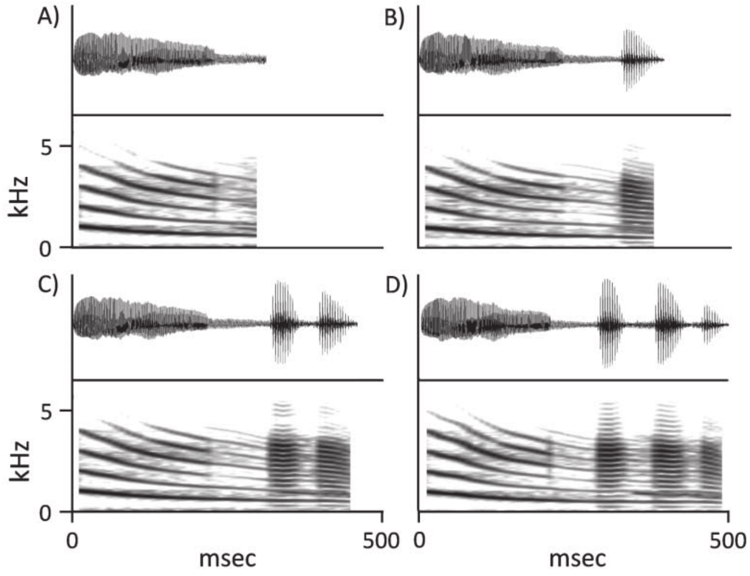

Figure 1: Waveforms and spectrograms of calls from the same túngara frog male varying in number of chucks. *A*, A simple, whine-only call. *B–D*, Complex calls with one to three chucks.

half the amplitude of their associated whine to be salient to females (Baugh and Ryan 2011). Thus, we only considered chucks exceeding this threshold. Time stamps were visually validated and cleaned prior to analysis.

General Phonotaxis Experiment Rules

All phonotaxis experiments were conducted in a dark sound-attenuating chamber (Acoustic Systems; ETSLindgren, Austin, TX) ranging in temperature from 25.9°C to 28.1°C. At the start of phonotaxis trials, females were confined beneath a funnel and exposed to 2 min of playback of the pertinent treatment (details in subsequent sections). After 2 min, we remotely raised the funnel as playback continued, allowing females to make a choice (defined in subsequent sections). Females were disqualified from trials if they climbed the chamber wall, moved along the wall for 2 min, or failed to make a choice within 10 min. Treatment order and stimuli sides were randomized for each trial, and individual females were tested in multiple different treatments. We used two-tailed binomial tests to test for female preference (H_0 = probability of 0.5 of choosing either stimulus). To ensure that preferences could be solely attributable to experimental manipulations, all phonotaxis stimuli used synthetically generated calls representing the centroid of call parameters (e.g., frequency, duration, and frequency/amplitude modulation over time) for this population (Ryan and Rand 2003). Females respond to these synthetic calls as to natural calls (Ryan et al. 2019); however, we also verified certain results using natural calls.

Aim 1: Investigate Call-Timing and Overlap Patterns at Different Chorus Sizes

Aim 1 Brief Introduction and Predictions

Previous studies found that túngara frog males increase call rate in response to rivals (e.g., Green 1990). Thus, we predicted that males would decrease their IOIs (the time elapsing between the onset of successive calls by a caller) as chorus size increased. We also predicted that the prevalence of call overlap would increase with chorus size (Brush and Narins 1989) and that when overlap was unavoidable, males would preferentially avoid overlap with nearer chorus-mates whose calls they would perceive as higher in amplitude (Greenfield and Rand 2000).

Aim 1 Methods

General Call-Timing and Overlap Patterns by Chorus Size. To investigate whether males altered IOIs of their calls as choruses changed size, we randomly selected 100 IOIs for each male at each experimental chorus size he occupied (n = 12,400). IOIs chosen were constrained to be shorter than 5 s, to avoid mistaking interbout intervals for IOIs (Pauly et al. 2006). We then built a linear mixed effects model (LMM) using the lme4 package (Bates et al. 2007) in R (ver. 4.1.2; R Core Team 2021), with IOI as the response variable, chorus size as a fixed effect, and frog ID nested within chorus ID as random intercepts. To determine whether IOIs became more varied in larger choruses, we built a similar LMM with the coefficient of variation of IOIs for each male as the response variable (n = 124). For all modeling done in this study, we checked model diagnostics using the DHARMa R package (Hartig 2022), and when refining models we compared nested models with likelihood ratio tests (LRTs).

To determine whether the temporal relationship between chorus-mates' calls changed with chorus size, we randomly selected 100 calls per male at each chorus size he occupied (n = 12,400). Then, for each call we calculated the temporal relationship between the onset of this call and the onset of the call (by any chorus-mate) that directly preceded it (time of onset of focal call − time of onset of preceding chorus-mate's call).

Call Overlap Patterns by Distance. To test whether the prevalence of call overlap was correlated with the distance between chorus-mates, we used the quadratic assignment procedure, a permutation procedure suitable for social networks (Farine and Whitehead 2015). We selected 10-min recording segments from sufficiently long chorus recordings (chorus size [n]: six males [five]; five males [five]; four males [four]) and used these to create weighted social networks, with males as nodes and a directed index score of call overlap prevalence as edges. This index was inspired by the spike time tiling coefficient (Cutts and Eglen 2014) and controlled for call rates of both dyad members (for details, see supplemental information [SI] 3 in the supplemental PDF). The necessity of selective attention likely differs by chorus size, so we ran analyses separately for each chorus size and combined networks for all same-sized choruses into one matrix. For each of these combined matrices, we constructed linear regression models with call overlap score as the response variable and distance between chorus-mates (m) as the predictor variable. To generate P values, we compared t-statistics from observed models to the distribution of t-statistics from 1,000 models in which the data had been row/column permuted using the sna R package (Butts 2008), with values permuted only within, not between, separate choruses.

Aim 1 Results and Discussion

General Call-Timing and Overlap Patterns by Chorus Size. Male IOIs were largely unresponsive to chorus size,

Table 1: Median interonset intervals (IOIs), mean coefficients of variation of IOIs, and proportions of complex calls with different numbers of chucks (relevant to aim 3) for choruses of different sizes

	IOIs		Proportion of complex calls with no. chucks			
Chorus size	Median IOI (s)	Mean CV(IOI)	1	2	3	4
2	1.71	.13	.56	.44	.01	0
3	1.71	.14	.42	.58	0	0
4	1.72	.16	.37	.61	.02	0
5	1.73	.15	.26	.72	.02	0
6	1.74	.15	.17	.78	.05	.001

Note: Values are generated from pooled randomly drawn calls (100 per male) from all males in all same-sized choruses. CV = coefficient of variation.

showing a negligible average decrease of 10 ms with each chorus-mate added (LMM, β estimate $= -0.01 \pm 0.003$, $P < .001$), and there was no effect of chorus size on the coefficient of variation for IOIs (LMM, β estimate $= -0.003 \pm 0.005$, $P = .55$; table 1). Insensitivity of IOIs likely reflects selection on males to maintain high call rates regardless of rival density (Bosch et al. 2000).

In experimental choruses of two and three males, silent gaps between chorus-mates' calls were frequent and of sufficient duration to allow a typical male to insert his calls without overlap (fig. 2). Males predominantly called in these silent gaps, leading to alternation (fig. 3). However, available silent gaps became shorter than typical call durations for any chorus larger than three males, making call overlap inevitable beyond this threshold (fig. 2). As choruses became larger and silence became increasingly scarce, males exhibited a shift in the way they timed their calls relative to those of their chorus-mates. Males called simultaneously more often in larger choruses (growing peak at ~0–50 ms; fig. 3), likely due to chorus-mates simultaneously resetting their calls based on the same chorus-mate's call (Aihara et al. 2011), and they increasingly inserted calls just before the chucks of their chorus-mates' calls (peak at ~320 ms). Overlap by a follower's call did not induce interruption of leading calls.

What appears to be a qualitative shift in call-timing strategies as choruses become larger may in fact not be. Rather, stereotyped call overlap in larger choruses may be the result of the gap detection algorithm that is expressed in small choruses interacting with the high background noise levels of larger choruses. When possible, males call in the silent gaps following rivals' calls, resulting in alternation in smaller choruses where silence is plentiful (fig. 3). However, as choruses grow, true silence becomes increasingly scarce (fig. 2). Whines decrease in amplitude over time, reaching minimum amplitude just before ending and giving way to

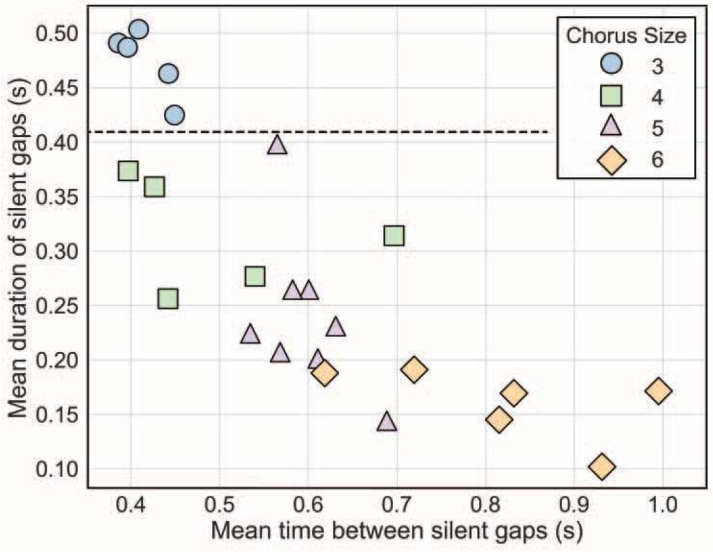

Figure 2: Mean duration of silent gaps and mean time between silent gaps available to a randomly chosen focal male calling in experimental choruses of different sizes. Calculated from 2-min periods when all chorus-mates called continuously. The dashed line indicates the average duration of calls in this population (Ryan and Rand 2003). Thus, call overlap is inevitable for a typical male calling in choruses below this line.

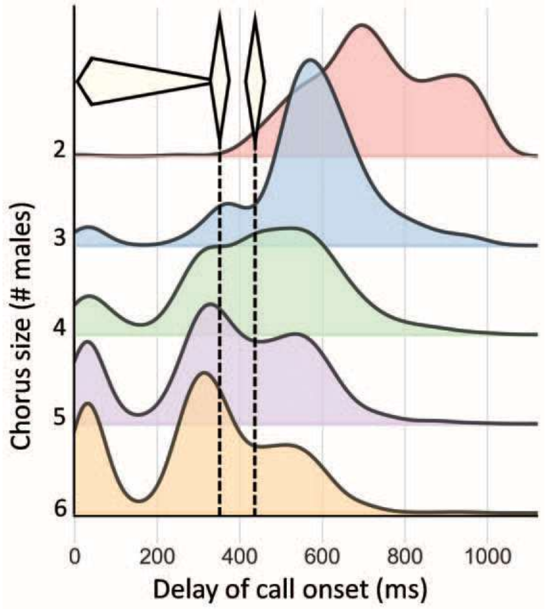

Figure 3: Kernel density plots showing the distribution of call onsets relative to the onset of the most recent call by another chorus-mate, shown by chorus size. Made with the Joypy Python package (Taccari 2021). Two-chuck calls predominated in most chorus sizes (table 1). Thus, the preceding chorus-mate's call is approximated by a typical duration two-chuck call (Ryan and Rand 2003) to illustrate likely overlap. Density peaks for choruses: two males = 695.7 ms, three = 570.6 ms, four = 522.5 ms, five = 329.3 ms, six = 314.3 ms.

the high-amplitude chucks (fig. 1). As silence becomes scarcer in the continuous din of larger choruses, the low-amplitude ends of chorus-mates' whines will increasingly become the most common amplitude minima perceived by males calling in these choruses, making them attractive lulls in which to call (fig. S4). Therefore, the same general heuristic, calling in relatively low-amplitude lulls following inhibition, can result in alternation in small choruses and the stereotyped call overlap seen in larger choruses as long as males adjust acceptable amplitude thresholds relative to background noise levels (Zelick and Narins 1983; Greenfield and Rand 2000).

Call Overlap Patterns by Distance. Greater distance between chorus-mates predicted more frequent overlap in five-male choruses (LM, β estimate $= 0.082 \pm 0.019$, $P < .001$) and six-male choruses (LM, β estimate $= 0.047 \pm 0.016$, $P < .001$) but not in four-male choruses (LM, β estimate $= -0.022 \pm 0.038$, $P = .825$). This lack of a relationship in four-male choruses could be due to less overlap occurring generally in these relatively smaller choruses (fig. 3), or it could be an artifact of increased variation in intermale distances due to males in four-male choruses occupying an arbitrary subset of the six possible hexagonal vertices. Acoustic signals attenuate with distance (Naguib and Wiley 2001); thus, more distant calls will be perceived as lower in amplitude and so are more likely to be perceived as acceptably low to call over (Greenfield and Rand 2000). Overlap, even with more distant chorus-mates, was still predominantly of the stereotyped form mentioned above—the following call beginning just before the leading call's chuck (fig. 3). Calls beginning ~50 to ~225 ms after onset of any chorus-mates' whine were exceedingly rare in all chorus sizes, suggesting strong inhibition by the high-amplitude beginning of all chorus-mates' whines. Behavioral outcomes of selective attention are typically conceived of as binary, with callers being inhibited or not by different chorus-mates (Greenfield et al. 2021). However, in túngara frogs we see gradations: (i) inhibition by any part of another caller's whine, (ii) inhibition by only the beginning of the whine, and (iii) no inhibition at sufficient intercaller distances (Taylor et al. 2019).

Aim 2: Investigate the Influence of Call-Timing and Overlap on Female Choice

Aim 2 Brief Introduction and Predictions

The way in which temporal relationships among signals influence female preferences drives male chorusing strategies (Greenfield et al. 1997). However, in túngara frogs such effects have been tested only for a subset of possible leading/following call associations (Tárano 2015; Legett et al. 2020), and this subset excludes those most commonly observed in our experimental choruses (fig. 3). First, we investigated general female preferences for leading/following calls across a range of temporal associations and generated a preference function. Second, as call overlap in larger experimental choruses was highly stereotyped (leading calls' chucks being overlapped by the beginning of following calls' whines; fig. 3) but occurred most often among more distant chorus-mates, we tested whether such overlap was detrimental to leading call attractiveness at various intercaller distances. Based on our preference function, we predicted that overlap would reduce leading call attractiveness but that this effect would decrease as the overlapping following call came from greater distances beyond the leading call.

Aim 2 Methods

Female Preference Function for Temporal Call Associations. To establish female preferences for leading/following calls across a range of temporal associations, we broadcast call pairs (both one-chuck calls) to females in which the delay between the onset of leading and following calls ranged from 50 to 750 ms ($n = 35$ females per treatment). Calls were 400 ms long; thus, delays less than 400 ms represent degrees of call overlap. Females began trials equidistant (1.35 m to each) between two speakers (setup A in fig. 4). Peak whine amplitude of calls from both speakers registered at 82-dB sound pressure level (SPL; re. 20 μPa) at this starting location, and call pairs repeated every 2 s (mean IOI for this population; Bosch et al. 2000). A choice was scored when females approached to within 10 cm of one of the speakers.

Influence of Distance on Attractiveness Costs of Call Overlap. Call overlap in túngara frog choruses typically manifests as leading calls' chucks being overlapped by the start of following calls' whines (fig. 3). Our preference function revealed that such overlap would reduce leading call attractiveness when leading and following calls were perceived by females at the same amplitude (fig. 5). However, call overlap tends to occur between more distant chorus-mates (aim 1 results). Thus, to test whether overlap from more distant chorus-mates still reduces leading call attractiveness, we conducted phonotaxis experiments with following call amplitudes simulating overlap coming from chorus-mates at various distances (setup B in fig. 4). Two speakers were placed 1 m apart on one side of the chamber (the choice speakers) antiphonally broadcasting identical one-chuck calls every 2 s. Peak whine amplitudes were 82-dB SPL at the female starting location, 1.35 m from each speaker. In each treatment, a third speaker (the interference speaker) played a one-chuck call that overlapped one of the choice speakers, beginning 20 ms before the chuck of the overlapped choice call (stimulus C in fig. 4). This approximated the most common degree of overlap seen in our six-male experimental choruses (fig. 3). The interference speaker was mounted on the ceiling directly above the choice speakers, pointing downward, ensuring that it was highest in amplitude directly at the choice speakers but that females could not perform phonotaxis toward it (Lea and Ryan 2015). In different treatments ($n = 35$ females per treatment), we varied the peak whine amplitude of the interference speaker so that it would be perceived by females as coming from 1, 2, or 3 m beyond the choice speakers (for amplitude calculations, see SI5 in the supplemental PDF).

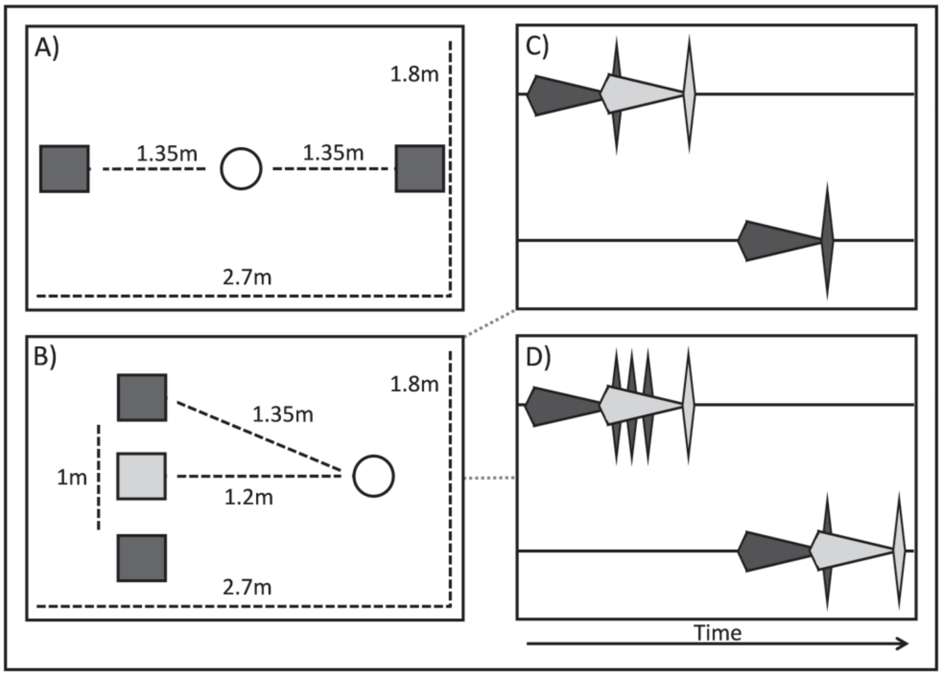

Figure 4: Phonotaxis experiment details. *A* and *B* show different acoustic chamber setups. Dark gray squares represent speakers for females to choose between, the light gray square represents the ceiling-mounted interference speaker, and the circle represents female starting location. *C* and *D* show stimuli used in setup B. *C*, Stimuli to test for female preferences for nonoverlapped calls (aim 2). *D*, Stimuli to test for female preferences for more chucks when calls are overlapped (aim 3). Competing stimuli are shown on separate lines (dark gray calls) at their approximate temporal relationships. Overlap from the interference speaker is shown by light gray calls. To obtain desired sample sizes (35 per treatment), 54 females were tested overall in the experiment using setup A, and 71 were tested overall in the combined experiments using setup B.

We scored a female choice when she remained within 10 cm of a choice speaker for 3 s, to rule out incidental travel.

Aim 2 Results and Discussion

Female Preference Function for Temporal Call Associations. Females strongly preferred leading calls when following calls began 50 or 400 ms after the leading call (fig. 5). Some weaker preferences also remained for leading calls when leading/following calls did not overlap. However, females switched to strongly preferring following calls when following calls began 250 and 350 ms after the leading call, that is, when any part of the first 150 ms of the following call's whine overlapped the leading call's chuck. To verify that this was not an artifact of using synthetic calls, we confirmed these results using natural calls (see SI7 in the supplemental PDF).

Generally, in species exhibiting precedence effects, female preferences for leading calls are consistent at various delays, although preference strength may vary (e.g., Grafe 1996; Bosch and Márquez 2002; Marshall and Gerhardt 2010; but see Grafe 1999). Three features of túngara frog communication can explain the reversals in preference seen at certain delays: (i) whines begin at high amplitude, then amplitude steadily decreases (fig. 1); (ii) whines with chucks are more attractive than whines, but only if chucks are of sufficiently high amplitude (Baugh and Ryan 2011); and (iii) females prioritize call complexity over call order, preferring complex following calls over simple leading calls (Tárano 2015). The first 150 ms of an overlapping following whine may then be sufficiently high amplitude to at least partially mask the leading call's chuck (cf. Wells and Schwartz 1984), devaluing the usual attractiveness boost induced by this chuck and causing females to discriminate against the leading call (Wilczynski et al. 1999; Baugh and Ryan 2011). Thus, in subsequent discussion, we designate the first 150 ms of the whine (the portion capable of masking chucks) as the "detrimental interval" and refer to overlap of this nature as the "detrimental overlap."

Influence of Distance on Attractiveness Costs of Call Overlap. Females discriminated against leading calls suffering detrimental overlap even when the overlapping following call was perceived as coming from up to 3 m beyond the choice speakers (probability of preferring nonoverlapped call [95% confidence interval (CI), P value]: 1 m = 0.82 [0.66–0.93, $P < .001$]; 2 m = 0.91 [0.77–0.98, $P < .001$]; 3 m = 0.71 [0.54–0.85, $P = .017$]). Detrimental overlap becomes common in any chorus larger than three males (fig. 3), suggesting that males in any chorus denser than three males within (at least) 3 m are at risk. Túngara frogs commonly exceed this threshold (Ryan 1983; Bernal et al. 2007), suggesting ubiquitous risk. Peak amplitude of chucks in our synthetic stimuli is nearly twice that of the whines. That chucks can be at least partially masked by this lower-amplitude call part, even when its amplitude is lowered further by our distance adjustments (see SI5 in the supplemental PDF), suggests that female cognitive biases beyond simple amplitude masking may underlie

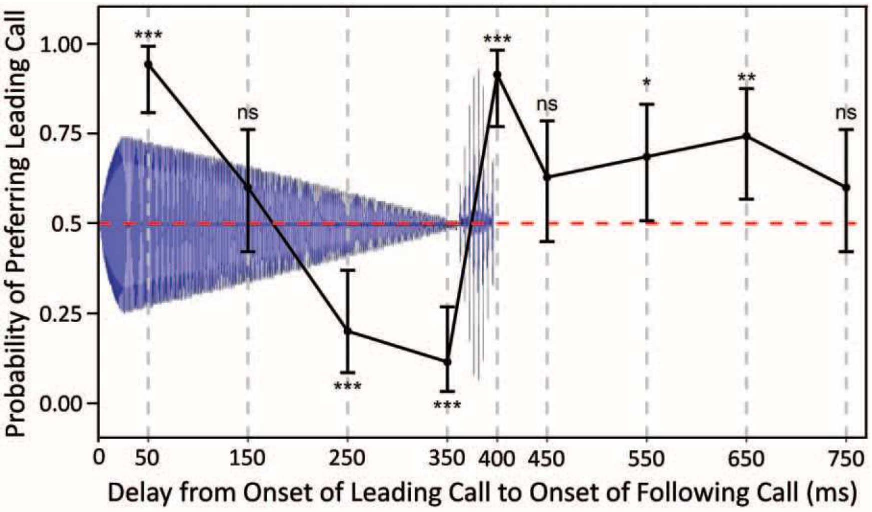

Figure 5: Preference function of túngara frog females for leading or following calls at various delays. The blue call represents temporal placement of the leading call, while each vertical gray line represents when the identical following call began relative to this leading call for that treatment. Statistical results are presented in table S6, available online.

these masking effects (see discussions in Wilczynski et al. 1999; Farris et al. 2005). For example, in túngara frogs the frequencies contained from 50 to 150 ms into the whine are sufficient and necessary to elicit female phonotaxis (Wilczynski et al. 1995) and so may be prioritized during simultaneous processing.

If producing following calls at specific delays effectively reduces rivals' attractiveness, why do we see alternation rather than targeted detrimental call overlap in smaller túngara frog choruses? Indeed, in frog species in which males compete to mask one another's calls via targeted overlap we see overlap even in isolated pairs (Schwartz and Wells 1984; Wells and Schwartz 1984; Grafe 1999; Reichert 2011). To our knowledge, female preferences for alternating versus overlapping choruses have not been tested in these species. However, in species that have been tested, chorusing strategies track female preferences—males alternate when females discriminate against overlapping choruses but may overlap/synchronize if they do not (Schwartz 1987; Bosch and Marquez 2000; Legett et al. 2019, 2021). Túngara frog females strongly prefer alternating call pairs to overlapping ones (Legett et al. 2019). Thus, when possible, alternation may better facilitate female approach to choruses and increase male per capita mating success (Ryan et al. 1981), making targeted overlap counterproductive.

But might the timing shift we saw in larger choruses (aim 1 results) represent males switching from a strategy of inhibition-based gap detection in smaller choruses to one of targeted overlap in larger choruses when call overlap becomes unavoidable anyway? We are skeptical of this possibility. For one, an effective targeted overlap strategy would be to preferentially target nearest neighbors that pose the greatest reproductive threat (Greenfield et al. 1997; cf. Reichert 2011). Instead, call overlap in túngara frogs occurs primarily among more distant chorus-mates (aim 1 results; Greenfield and Rand 2000). Targeted overlap would also suffer from similar theoretical issues as altruistic punishment if it entails any costs (Clutton-Brock and Parker 1995)—particularly here, where males are targeting more distant chorus-mates, thus primarily benefitting the target's nearest neighbors. Finally, such a qualitative strategy shift would necessitate drastic alterations to the properties of male neural call-timing mechanisms in different social environments (Greenfield 1994). This seems less parsimonious than our hypothesis that alternation and overlap both emerge from the same general gap detection mechanism interacting with the varied acoustic environments present in differently sized choruses (aim 1 discussion). However, even if prevalent detrimental overlap is not the result of selection for targeted overlap, our preference function revealed that this call placement is advantageous for preserving the relative attractiveness of following calls (fig. 5).

Aim 3: Investigate Multiple Chucks as a Condition-Dependent Means of Mitigating Risks to Attractiveness

Aim 3 Brief Introduction and Predictions

Males are at risk of suffering attractiveness costs due to detrimental call overlap (having their chucks masked by the first 150 ms of chorus-mates' whines) in even moderately dense choruses, suggesting ubiquitous risks. That leading calls are disadvantaged during such overlap makes defending against it difficult; after a male calls, the eventual temporal relationship between his call and that of the male calling after him is entirely up to the follower. Males can vary the degree of elaboration of their complex calls by appending variable numbers of chucks (fig. 1). Thus, we investigated whether appending additional chucks represents a strategy to safeguard calls by extending the duration of the chuck-containing call section, thereby decreasing the probability that every chuck of a call suffers detrimental overlap (fig. 6). We predicted that males would append more chucks to calls as choruses became larger and call overlap

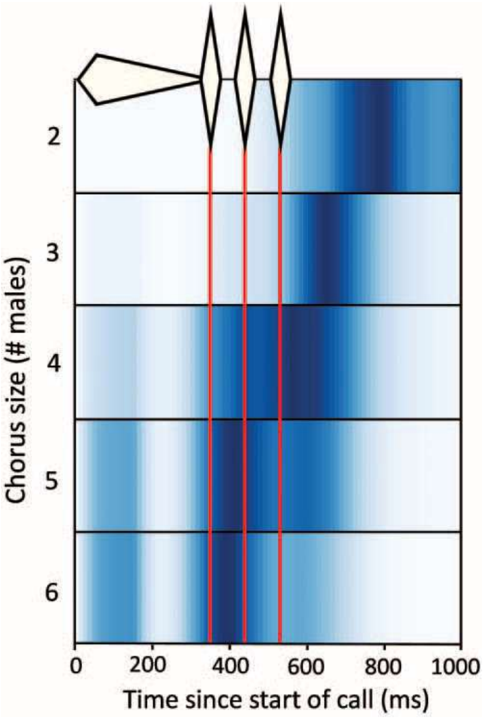

Figure 6: Same data as in figure 3 but presented as heat maps showing the distribution of coverage by the 150-ms detrimental interval of calls relative to the chorus-mate's call preceding them, shown by chorus size. Darker shades indicate intervals relatively more commonly spanned. A typical duration, three-chuck call (Ryan and Rand 2003), has been added to visualize the risk of detrimental overlap for chucks at different positions.

became more common, that calls with more chucks would be more likely to have some chucks remain free from detrimental overlap (especially in larger choruses), and that when calls suffered detrimental overlap, calls with more chucks would be preferred by females. Finally, upper limits of signal exaggeration are often constrained by aspects of male phenotypes (Dougherty 2021), meaning that highly competitive social environments can produce tighter correlations between signal magnitude and male phenotypes (Candolin 2000; Gavassa et al. 2012). All males can produce complex calls (Ryan 1985), but we predicted that larger males and those in better body condition would append greater numbers of chucks to calls, especially in larger choruses.

Aim 3 Methods

Benefits of Multiple Chucks for Avoiding Complete Chuck Overlap. Using our experimental chorus recordings, we investigated whether calls with greater numbers of chucks appended had a higher probability of having some chucks remain free from detrimental overlap and whether this varied with chorus size. For each male at each chorus size, we randomly selected 10 calls per call elaboration category he produced (one, two, or three chucks; $n = 2{,}140$ calls). Using time stamps of individual call elements, we identified whether each individual chuck from each call was or was not overlapped by the 150-ms detrimental interval of any chorus-mate's whine. We built a mixed effects logistic regression model (model 1 in table 2) with whether each call had at least one chuck unmasked by chorus-mates' detrimental intervals as a binary response variable (yes/no) and number of chucks, chorus size, and their interaction as fixed effects. We included frog ID nested within chorus ID as random intercepts. Additionally, as individuals differ in call properties that might influence risk of complete chuck overlap, such as chuck duration and interchuck intervals (Ryan and Rand 2003), we included a random slope for number of chucks. We tested the significance of this random slope using the simulateLRT function from the DHARMa R package (Hartig 2022) with 250 simulations. Final reduced model results for all models in this section are presented in table 2.

Preferences for Multiple Chucks in the Presence and Absence of Call Overlap. To test whether additional chucks mitigate the attractiveness costs of detrimental overlap, we gave females a binary choice between a one-chuck call and a three-chuck call using phonotaxis setup B (stimulus D in fig. 4). This time, both choice speakers were overlapped by the interference speaker, with interfering calls again beginning 20 ms before the first chucks of choice

Table 2: Results from our final generalized linear mixed models

Response variable, fixed effect	Estimate	SE	*Z*	95% CI	*P*
	Model 1 (conditional R^2: .308; marginal R^2: .152)				
Logit(≥1 chuck unobscured (yes/no)):					
Intercept	5.47	1.13	4.83	3.34 to 7.71	**<.0001**
Number of chucks	−.47	.69	−.69	−1.79 to .9	.49
Chorus size	−1.04	.22	−4.68	−1.46 to −.62	**<.0001**
Number of chucks × chorus size	.26	.13	1.98	.02 to .52	**.048**
	Model 2 (conditional R^2: .74; marginal R^2: .14)				
Logit(>1 chuck (yes/no)):					
Intercept	−2.24	.45	−4.97	−3.14 to −1.4	**<.0001**
(Body condition)	−.17	.37	−.47	−.97 to .59	.64
Chorus size	.96	.04	25.77	.88 to 1.05	**<.0001**
(SVL)	.6	.34	1.77	−.07 to 1.33	.08
Chorus size × (body condition)	.08	.04	2.06	−.01 to .17	**.04**
	Model 3 (conditional R^2: .72; marginal R^2: .1)				
Count(≥3-chuck calls produced):					
Intercept	−5.45	1.06	−5.13	−9.62 to −3.86	**<.0001**
Chorus size	.4	.09	4.48	.09 to .7	**<.0001**
(SVL)	1.22	.51	2.4	.23 to 2.18	**.02**

Note: Models 1 and 2 are logistic regressions, and model 3 is a Poisson regression. Fixed effects contained in parentheses were standardized ((x-mean(x))/SD(x)). *P* values presented here are those output from models by the lmerTest R package (Kuznetsova et al. 2017), whereas *P* values from likelihood ratio hypothesis tests are presented in the text. Boldface type indicates statistical significance. Confidence intervals (CIs) were calculated via bootstrap using the confint.mermod function with 1,000 simulations. R^2 values for models are given in the cut-in heads. SVL = snout-vent length.

speaker calls. Again, we varied interference speaker amplitude to simulate overlap coming from 1, 2, and 3 m beyond the choice speakers and, as a control, also included a treatment in which neither choice speaker was overlapped ($n = 35$ females per treatment).

Male Phenotype and Social Environment as Drivers of Call Elaboration. We investigated whether the propensity to produce more elaborate calls was influenced by interactions between chorus size and aspects of male phenotypes that may both constrain call elaboration and be important targets of female choice: SVL and body condition. We calculated body condition using the scaled mass index (Peig and Green 2010), which has been validated as a good proxy for amphibian energy reserves (MacCracken and Stebbings 2012). We randomly selected 100 complex calls per male from our experimental choruses ($n = 12{,}400$). We then built a mixed effects logistic regression model (model 2 in table 2) with a binary measure of call elaboration (one chuck/more than one chuck) as our response variable (few calls exceeded two chucks; table 1; see SI8 in the supplemental PDF). We included standardized body condition, standardized SVL, and chorus size as fixed effects and included interactions between SVL and chorus size and between condition and chorus size. We included frog ID nested within chorus ID as random intercepts. Additionally, to investigate whether production of calls with three or more chucks was influenced by these same factors, we built a mixed effects Poisson regression model (model 3 in table 2) with the count of three or more chuck calls each male produced (out of his randomly chosen 100) as the response variable and the same fixed and random effects as the previous model.

Aim 3 Results and Discussion

Benefits of Multiple Chucks for Avoiding Complete Chuck Overlap. Extending the duration of the chuck-containing part of calls by appending additional chucks seems to effectively safeguard calls against complete detrimental chuck overlap in larger choruses. The probability that a call had at least one chuck left unobscured by chorus-mates' detrimental intervals was predicted by a near-significant (LRT, $P = .052$, 95% CI = 0.02–0.52) interaction between number of chucks and chorus size (fig. 7; model 1 in table 2). Rescuing effects of additional chucks became increasingly pronounced as choruses grew larger, paralleling increasing chuck overlap risks as chorus size increased (figs. 3, 6). Additionally, the random slope for the influence of number of chucks on avoidance of complete chuck overlap was highly significant (simulated LRT, $P < .001$), suggesting that males differ in the magnitude of protective effects additional chucks provide or differ in their baseline risk of being overlapped by their chorus-mates.

Preferences for Multiple Chucks in the Presence and Absence of Call Overlap. Our playback experiments confirmed that additional chucks mitigated the attractiveness costs of detrimental overlap. Calls with three chucks were preferred by females over those with one chuck, but only when both calls suffered detrimental overlap coming from 1–3 m away (probability of preferring three-chuck

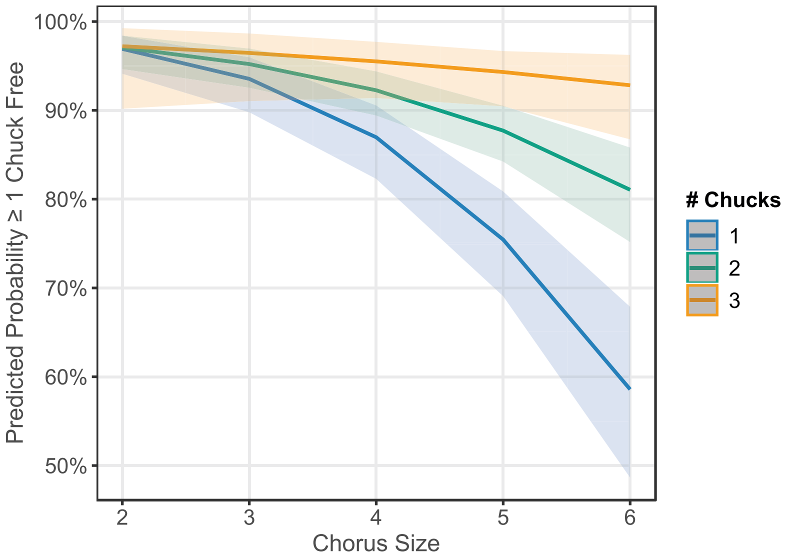

Figure 7: Marginal effect plot showing the interaction between call elaboration and chorus size in predicting the probability that a call has at least one chuck free from detrimental overlap. Plot produced using the sjPlot R package (Lüdecke 2021).

call over one-chuck call) [95% CI, P value]: 1 m = 0.743 [0.567–0.875, $P = .006$]; 2 m = 0.657 [0.478–0.809, $P = .09$]; 3 m = 0.743 [0.567–0.875, $P = .006$]). When neither call was overlapped, there was no preference for greater numbers of chucks (0.571 [0.394–0.737, $P = .5$]). Evidence that calls with additional chucks beyond one are more attractive to females is mixed, with some studies finding a preference at certain broadcast amplitudes or call-timing configurations (Akre and Ryan 2010*b*; Akre et al. 2011; Tárano 2015) and others finding no preference (Ryan 1985; Bernal et al. 2009; Stange et al. 2017). Our results reveal an important function of additional chucks, protection against complete chuck overlap, that becomes apparent only when contextualizing male signaling strategies and female preferences within the milieu of calling interactions occurring within the dense choruses túngara frogs form in the wild. Thus, additional chucks may not increase call attractiveness per se. Rather, the stark attractiveness dichotomy between calls with zero and one or more chucks (Ryan 1985) may remain the critical contrast, with additional chucks increasing the probability that a nonzero number of chucks remain free from detrimental overlap in these choruses.

These results highlight that varied sensory conditions arising from male signaling interactions in different social environments have important consequences for receiver preferences. Túngara frog females prefer lower-frequency whines and chucks, which are associated with larger and heavier males (Ryan 1985; Bosch et al. 2000, 2002; James et al. 2021). However, high levels of chorus noise seem to abolish established call preferences (Taylor et al. 2021). Conversely, our experiments showed that conspecific interference typical of large choruses induced a female preference for increased call elaboration. Thus, conspecific interference does not invariably flatten female preference functions for all call properties (Vélez et al. 2013). Rather, preference functions for different properties can change shape due to conspecific interference in divergent ways (Reichert and Ronacher 2015), with important implications for the evolution of signals and signaling strategies.

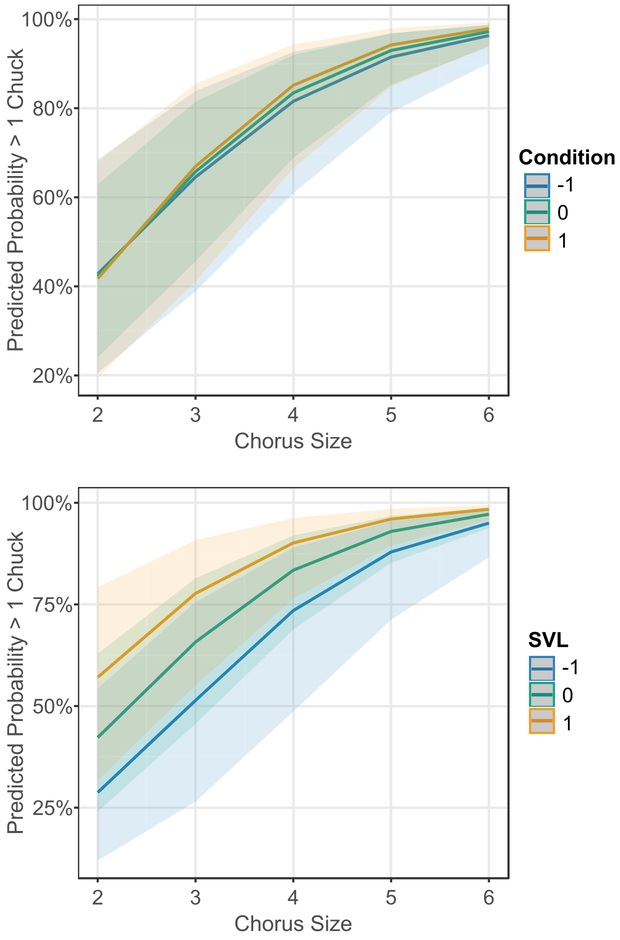

Figure 8: *Top*, marginal effect plot showing the interaction between body condition and chorus size in predicting multichuck calls. *Bottom*, plot showing the effect of snout-vent length (SVL) in predicting multichuck calls across a range of chorus sizes. In both plots, 0 represents the mean value, while 1 and -1 represent 1 SD above and below the mean, respectively.

Male Phenotype and Social Environment as Drivers of Call Elaboration. In agreement with previous results (Bernal et al. 2007), our logistic model (model 2 in table 2) revealed that the probability that males appended more than one chuck to their calls increased as choruses became larger (fig. 8). Previous work found no association between any aspects of male phenotypes and degree of call elaboration (Bernal et al. 2007). However, our model revealed that the probability of adding more than one chuck to a call was predicted by a significant interaction (LRT, $P = .042$) between body condition and chorus size (table 2). As shown in figure 8*A*, high- and low-condition males have similar probabilities of producing multichuck calls in two-male choruses, while higher-condition males have a slightly greater probability of doing so in choruses of three to six males. However, this effect was small; in choruses of three to six males, an increase of 1 SD in condition predicted a 1%–2% increase in the probability of producing multichuck calls. SVL had a nonsignificant (LRT, $P = .08$) positive effect (average probability increase of ~8% with increase of 1 SD across chorus sizes; fig. 8*B*). We retained SVL in our final model as its P value was suggestive and its removal greatly altered the coefficient for condition (~50% change in coefficient value).

Our Poisson model (model 3 in table 2) revealed that chorus size had a significant positive effect on the number of three or more chuck calls produced (LRT, $P < .001$), although a minuscule one; a male of mean SVL was predicted to have an average increase of 0.01–0.02 three or more chuck calls per 100 calls for each male added to the chorus. SVL had a similarly positive but minuscule effect (LRT, $P = .017$); for a male calling in a six-male chorus, an increase of 1 SD in SVL predicted an average increase of ∼0.1 three or more chuck calls per 100 calls. Condition had no effect.

Overall then, increasingly competitive social environments strongly increased the propensity for males to increase call elaboration, as would be expected if additional chucks served to protect calls against increasing detrimental overlap risk in larger choruses. However, aspects of phenotypes predicted to constrain a males' ability to append additional chucks had negligible or uncertain effects (see also SI8 in the supplemental PDF). In fact, in both models most of the variation in call elaboration was explained by the random effect of frog ID nested within chorus ID (SD: 3.58 in model 2, 3.12 in model 3; see conditional and marginal R^2 values in table 2). This suggests that males' baseline propensities for appending different numbers of chucks are primarily driven by individual differences that remain to be identified.

Musings on Modal Degree of Call Elaboration

Detrimental effects of call overlap for leading calls have been demonstrated (Wells and Schwartz 1984; Grafe 1999) or suggested (Schwartz and Wells 1984) in other anurans. Males of some *Dendropsophus* species have evolved defenses involving extending call durations, similar to appending additional chucks in túngara frogs. However, in contrast to túngara frogs, their defenses are more active. *Dendropsophus phlebodes* males add more secondary notes to interrupted calls (Schwartz and Wells 1984, 1985), as do *D. microcephala* males, while also increasing internote intervals of interrupted calls (Schwartz and Wells 1985). Similarly, *D. ebraccatus* males employ lengthier aggressive calls when interrupted, allowing terminal secondary notes to remain unmasked (Reichert 2011). Túngara frog defenses are more passive; males do not respond to interruption on a per-call basis and show infrequent changes in call elaboration over long stretches of calling (Bernal et al. 2009). Rather, they append more chucks to calls as choruses grow larger and overlap risks increase, decreasing the probability that their calls suffer complete detrimental chuck overlap. This logic is similar to that of the interference risk hypothesis proposed for *Dryophytes versicolor* (Schwartz et al. 2008).

With such passive defenses, it is surprising that calls with more than two chucks are rare. This rarity has been demonstrated in the field (Bernal et al. 2007) and the laboratory (Bernal et al. 2009), in competitive social environments (Bernal et al. 2007; this study), and in the presence of mate-searching females (Akre and Ryan 2011). In six-male choruses in this study, only 5% of calls had more than two chucks (table 1), only 12 of 36 males produced even a single call with more than two chucks, and 67% of all such calls were produced by only 2 of 36 males (see SI8 in the supplemental PDF). This is despite three-chuck calls being, on average, 12% better than two-chuck calls in preventing complete detrimental chuck overlap in six-male choruses (fig. 7). This increased protection suggests that males that consistently produced calls with three or more chucks in the crowded choruses common in this species might gain significant attractiveness benefits. Energetic or anatomical constraints can limit signal elaboration beneath its theoretical optimum (Ryan 1988; Reichert and Gerhardt 2012). However, in túngara frogs, appending chucks to whines does not appear to increase energetic costs (Bucher et al. 1982), and our results revealed that phenotypic traits that often limit energetically constrained call elaboration (body condition and size) had negligible or uncertain effects (figs. 8*A*, 8*B*, S8). Furthermore, aberrant wild males producing up to seven-chuck calls have been described (Bernal et al. 2007), suggesting that the construction of a vocal apparatus capable of appending more chucks is feasible.

An intriguing possible explanation for the rarity of calls with more than two chucks invokes potential constraints imposed by the specific way that the túngara frog vocal apparatus operates. In many anurans that can flexibly alter numbers of notes in multinote calls (e.g., *Dendropsophus* and *Dendrophytes* species mentioned above), introductory and secondary notes are acoustically similar and appear to be produced by the same vocal structures via separate small exhalations. Conversely, in túngara frogs, whines and chucks are acoustically distinct, and anatomical and modeling investigations of the vocal apparatus have revealed that the harmonic chuck notes arise from impact oscillations caused by vibration of a fibrous mass at the termination of the whine (Gridi-Papp et al. 2006; Baugh et al. 2018; Kime et al. 2018). Furthermore, activation of the fibrous mass to produce complex calls appears to occur passively with sufficient laryngeal airflow, without requiring active neural control (Kime et al. 2018). Whine amplitude is positively correlated with laryngeal airflow rate (Dudley and Rand 1991), and within males the number of chucks appended to a whine is positively correlated with the amplitude of that whine (Halfwerk et al. 2016; Larter et al. 2022). This suggests that higher rates of laryngeal airflow resulting in higher-amplitude whines may also passively result in greater numbers of chucks.

Such passive linkage between call elaboration and whine amplitude could impose interesting constraints on situation-dependent flexibility in male calling behavior. Túngara frogs alter the types of calls they produce in different social environments to minimize trade-offs arising due to the behavior of eavesdropping enemies. Complex calls more effectively attract females, making them beneficial when calling near competitors (Ryan 1985), but they put isolated callers at heightened risk of attack from eavesdropping bats and midges that also favor complex calls (Tuttle and Ryan 1981; Bernal et al. 2006). When calling alone, males produce simple whine-only calls to reduce these risks (Ryan 1985). Similarly, males produce simple calls at the start of call bouts, seemingly to probe the environment for danger and elicit calling by neighbors before calling conspicuously (Larter et al. 2022). Thus, males need to be able to produce both simple and complex calls in different circumstances, using a vocal apparatus that appears to passively append increasing numbers of chucks as laryngeal airflow at the end of the whine exceeds successive thresholds. In this scenario, opposing selective forces could generate stabilizing selection on the "sensitivity" of the fibrous mass (i.e., the shape of the relationship between laryngeal airflow rate and the number of chucks; see fig. 8 in Kime et al. 2018), with intermediate fibrous mass sensitivities representing a compromise; most males, when calling at maximum exertion, are unable to produce calls with more than two chucks, even when doing so might be beneficial in noisy choruses. Conversely, when forced to call in isolation, they can produce whine-only calls (which are sufficient to attract females; Ryan 1985) at moderately high amplitudes without invariably appending chucks and suffering high risk of attack by eavesdroppers. This hypothesis is currently speculative, however. There remain many unknowns about the precise functioning of the túngara frog vocal apparatus (Kime et al. 2018), meaning that other unidentified constraints or trade-offs could make more elaborate complex calls suboptimal.

General Conclusions

Our investigation into túngara frog calling interactions across a range of chorus densities, as well as their effects on female choice, revealed the following. First, highly stereotyped call overlap, with leading calls' chucks being overlapped by the high amplitude beginning of following calls' whines, became common in any chorus larger than three males. Second, such overlap caused females to discriminate against leading calls, even when the overlapping following call was perceived as coming from up to 3 m beyond the leading call. This suggests that risks of overlap to call attractiveness are ubiquitous. Third, overlap-induced attractiveness costs to leading calls were mitigated by increasing call elaboration (appending additional chucks to calls), and males increased call elaboration as chorus size and overlap risk increased. Fourth, male body condition and size had negligible effects on call elaboration, suggesting that other factors are at play in promoting or constraining elaboration in the face of competition.

Our results demonstrate that intercaller interaction patterns in crowded choruses are difficult to predict on the basis of observing only a few interacting callers or callers responding to playback. Furthermore, they emphasize that precisely characterizing the nature of a species' signaling interactions and interference patterns across an ecologically relevant array of social environments is essential for understanding how conspecific interference has shaped the evolution of communication. Had we tested the hypothesis that additional chucks safeguard call attractiveness in noisy choruses by observing female preferences under high levels of unmodulated chorus-shaped noise, we likely would have found no protective effect. Rather, the protection afforded by additional chucks appears to be due to the precise way that the species-specific amplitude- and frequency-modulation patterns of leading and following calls interact within female sensory systems when staggered as they are during the stereotyped call overlap prevalent in larger choruses. Similar investigations into the effects of specific density-dependent interference patterns on the behavior and preferences of eavesdropping enemies would likely also be fruitful and might better inform our understanding of the type and magnitude of protection gained by signaling amid conspecifics. Overall, this study highlights that accurately contextualizing signaler and receiver behavior within the complexities of the social milieu can yield novel insights into the communication of even thoroughly studied species.

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Statement of Authorship

L.C.L. and M.J.R. conceptualized aspects of the project and contributed to experimental design. L.C.L. collected, analyzed, and visualized the data. L.C.L. wrote the manuscript, and M.J.R. provided constructive feedback. L.C.L. and M.J.R. secured funding.

Data and Code Availability

Data and code are available on Figshare (https://doi.org/10.6084/m9.figshare.23681118.v1; Larter and Ryan 2023).

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