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ORIGINAL ARTICLE

Condition dependence in the sexual communication system of the túngara frog

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Abstract

Sexual selection can result in the evolution of extreme armaments and ornaments, and the development and maintenance of these traits can come at a considerable cost. These costs have been implicated in enforcing an upper limit on trait divergence and promoting condition-dependent traits, such that only individuals in sufficiently high condition can effectively wield these armaments and advertise these ornaments. Numerous studies demonstrate the condition-dependence of sexually selected traits, especially those used by males to advertise to females. In this study, we investigated condition-dependent mating calls in the túngara frog *Physalaemus* (= *Engystomops*) *pustulosus*. We manipulated male condition in the laboratory over a nine-day period by restricting food availability. We then documented: the relationship between male condition (the relative change in body mass from night 1 to night 9) and acoustic parameters of his mating call; how male condition influenced the male's responses to call playbacks; and finally, how male condition influenced the attractiveness of the male's calls to females. Males who were not fed during this period showed significant changes in call frequency, duration, and amplitude. In response to playbacks, unfed males called less, and made fewer complex calls. Finally, in phonotaxis experiments, females were more attracted to the calls of unfed males on night 1 to the calls of the same males on night 9. Fed males, on the other hand, showed no significant differences between nights 1 and 9 in call parameters, calling effort, and call attractiveness. This study shows the pervasive effects of condition on three aspects of sexual communication: signal parameters, behavioral response to vocal competition, and mating call attractiveness.

Significance statement

This study shows the widespread effects that an animal's condition has on its communication system, including: influences on the acoustic parameters of the male's mating call, their calling effort, and the sexual attractiveness of mating calls to females.

Keywords Túngara frog · Condition-dependent · Sexual communication · *Physalaemus* (= *Engystomops*) *pustulosus*

Introduction

Communication is a critical component of sexual selection as it can involve the expression of sexual displays by courters (usually, but not always, males) to influence their attractiveness to choosers (usually, but not always, females). These sexually selected signals often are extreme in their development, the peacock's "tail" (actually its train) being the canonical example, but such traits exist in all sensory modalities of communication (Ryan and Keddy-Hector 1992; Andersson 1994; Rosenthal 2017; Rosenthal and Ryan 2022). These sexually selected signals offer an advantage in mate attraction but they come at a cost, such as the high metabolic cost of expressing these traits (e.g. frogs,

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Bucher et al. 1982; fish, Cummings and Gelineau-Kattner 2009; crickets, Mowles 2014), and exposure to eavesdropping predators and parasites (e.g. Tuttle and Ryan 1981; Zuk and Kolluru 1998; Bernal and Page 2023). In addition, there could be substantial variation within individuals that could influence their ability to acquire food (see review by Dougherty 2021).

Zahavi (1975; see also Maynard Smith 1956) argued that the state of an animal's sexual signal should correlate with the animal's condition, and a number of reviews substantiate this basic assumption (e.g. Johnstone et al. 2009; Hill 2011; Warren et al. 2013). For example, a recent review of 147 species confirmed the expectation that higher-condition males invest more in sexual signaling, although the effect size was not large and there was substantial variation (Dougherty 2021). Some examples show that higher-condition males in crickets call louder, more often, at lower carrier frequencies (Holzer et al. 2003; Scheuber et al. 2003a; Harrison et al. 2013), higher-condition male birds have brighter plumage (Hill 1990), male frogs in higher condition call more often or longer (Sullivan 1992; Gerhardt et al. 2000), and in sage grouse such males increase the vigor or intensity of their courtship dance (Patricelli and Krakauer 2010). In each of these studies, the greater mating success of higher-condition males has either been demonstrated or assumed based on what is known of female preferences for courtship displays in those species. Less is known about how a male's condition influences his interactions with other males during sexual signaling. However, it is known that some frogs will call less or adopt a satellite non-calling strategy when their condition is low (Leary et al. 2004; Crocker-Buta and Leary 2018).

Many of the factors that can influence condition have been well-studied in frogs. Researchers have shown that body condition can be correlated with single extreme climate events (Cronin et al. unpublished data), developmental temperatures (Drakulić et al. 2016), parasite abundance (Comas et al. 2014), and habitat disturbance (Matías-Ferrer and Escalante 2015), among other examples. In this study, we manipulate male condition to address three questions: Are there changes in the call of a male when he is in low condition versus high condition? Does the male's condition influence how he responds in vocal competition with other males? And, is the male's call more attractive to females when he is in high condition compared to when he is in low condition?

The system

Most male anurans rely primarily on vocalizations to attract potential mates, and studies of male frog calls and female call preferences have made important contributions to our understanding of sexual communication (Gerhardt and Huber 2002). Túngara frogs have been an especially well-studied system (Ryan 1985, 2011; Wilczynski and Ryan 2010; Ryan and Guerra 2014). Males produce a mating call that always contains a whine, a frequency sweep from about 700–400 Hz in about 350 ms, that is both necessary and sufficient to attract females for mating (Wilczynski et al. 1995). Males can also add from 1 to 7 chucks to the whine, these chucks are short bursts of sound, typically 40 ms in duration. Adding a chuck increases the male's attractiveness to females five-fold (Ryan et al. 2003, 2019). Frog-eating bats (Ryan et al. 1982) and blood-sucking midges (Bernal et al. 2006) feed on túngara frogs and locate them by homing in on the advertisement call; both have a strong preference for calls with chucks to those without chucks, as do.

Túngara frogs breed throughout much of the rainy season, which in Panama extends from May to December although the beginning and end of the season can be variable. Ryan (1985) monitored male chorus attendance and mating success for 152 consecutive nights, encompassing most of the breeding season. Six hundred seventeen males were marked, and males were present for an average of seven nights ranging from 1 to 47. A male's mating success was strongly influenced by the number of nights he spent at the breeding site (Ryan 1985); on average males spent around 16% of their nights at the breeding site and successfully attracted a mate 19% of those nights (Ryan 1985). It appeared that males rarely fed while at the breeding site.

The ability to obtain food might influence both the number of nights a male can spend at a breeding site and his proclivity to call when he is there, thus increasing his chance of mating (Ryan 1985). A previous study showed that males deprived of food for a short period (5 days) showed no effects on chorus attendance (Green 1990). Marler and Ryan (1996), however, monitored the amount of calling by males in outdoor mesocosms for 16 days. One mesocosm was supplemented with food (termites) while the other was not. Males without supplemental food were less likely to call than were males in the mesocosm with additional food. Calling males had higher levels of plasma testosterone, and in the laboratory elevation of corticosterone decreased testosterone levels and the likelihood of calling (Marler and Ryan 1996). In sum, these studies of túngara frogs showed a link between food availability and investment in sexual advertisement.

Neither of these studies compared the acoustic properties of mating calls of low- and high-condition males, how males respond to different stimuli based on condition, and how the attractiveness of their calls to females varies with the male's condition. This study aims to investigate each of these three factors to gain a greater understanding of how body condition influences a male's ability to compete in the sexual marketplace.

Methods

Collection and experimental setup

We collected male and female túngara frogs from several field sites in Gamboa, Panama from June-August 2017–2019 and between the hours of 1900–2030 h. The frogs were immediately transported to our nearby lab at the Smithsonian Tropical Research Institute (STRI). We placed individual males into sound-attenuating boxes for the duration of the experiment (9 nights). Each acoustic box was fitted with small broad-range microphones (fitted inside at the top of the box) and speakers (fitted inside the back of the box) to broadcast and record male vocalizations. Within the sound attenuating box (30.5 × 46 × 30.5 cm), the males resided in acoustically transparent terraria (30 × 20 × 20 cm) equipped with 1" PVC elbow fitting as housing and a water dish, which provided a calling site. They were kept at ambient temperature, ca. 25–27 °C, on a 12/12 light cycle.

Each day we calibrated the speakers and microphones to 82 dB SPL (re. 20 µPa) at 16 cm, the distance of the male to the speaker using a calibrated tone. This allowed us to measure the absolute amplitude of the male's response to playbacks. We randomly partitioned the males into two groups, fed and unfed (food-deprived). We tested twelve fed males and eleven unfed males. The fed males were given termites, a major component of their diet (Ryan 1985), ad libitum at the end of each night of testing while the unfed males received none. At the end of each night of testing, we recorded their mass. Unfed males were monitored closely to ensure they did not lose more than 30% of their original body mass. If an unfed male lost too much mass, we removed him from the experiment, kept him for two nights while feeding him termites ad libitum, and then released him at his home site in the field. At the end of testing, unfed males also were kept two additional nights and fed termites ad libitum to regain their original mass.

We had two control groups of males. These males were individually placed in a sound attenuation box for nine days, one group was fed and the other group was unfed. Both groups were deprived of a water pool; thus they did not call. This allowed us to determine if merely being exposed to calls influenced the male's condition. We used an analysis of variance to compare the mass of males in the various treatment groups.

Females collected in the field were in amplexus with a male. The amplexed pair was transported to our lab and the pair remained in amplexus in a dark container until we removed the male from the female for phonotaxis experiments. After testing on that same night, the female was re-paired with her male partner, and the pair was returned to their home site.

Signal presentation

We tested males from 2030–0200 h each night. We broadcast a natural túngara frog chorus (73 dB SPL at 16 cm) until the test male initiated calling. Upon calling, we ceased the chorus playback, initiated our synthetic playback stimulus (82 dB SPL), and commenced recording. Our playback stimulus was a synthetic male túngara frog call, which on average was as attractive as a natural call (Rand et al. 1992) and has been used in many previous studies (e.g. Ryan et al. 2019). The stimuli included either a whine (W), a whine-chuck (WC), or a whine triple chuck (WCCC), each separately broadcast in a series of 30 calls with a call period of about two seconds. We randomized the order of the three series to control for order effects. If a male left the water where he was calling, we halted the playback and broadcast the chorus until the male returned to the water and called (males only call when they are floating on the water's surface (Ryan 1985)). We recorded the calls of the treatment males in Audacity at a sampling rate of 44.1 kHz.

Condition-dependent acoustic parameters

We determined how changes in condition influenced the acoustic parameters of the males' mating calls. Call analysis was done blind relative to treatment and night. After the completion of testing, we edited the recordings by deleting calls that could not be analyzed due to overlap with the playback stimuli. We then randomly extracted 10 calls from each male for night 1 and night 9. If 10 calls were not available, we used as many as possible. We imported calls into Raven Pro 1.4 (Cornell Lab of Ornithology) and measured a standard set of acoustic parameters (Ryan and Rand 2003; Fig. 1). These 10 calls were then averaged for each parameter. These included the dominant frequencies of the whine and the chuck, the initial and final frequencies of the whine, the durations of the whine and chuck, the rise (duration from the start of the call to its peak/maximum amplitude) and fall times (duration from the peak/maximum amplitude to the end of the call) of the whine, and the absolute amplitudes of the whine and chuck (Fig. 1).

We imported these values into RStudio (R Core Team 2018) and used a linear mixed-effects model and ggplot2 (Bates et al. 2015; Kuznetsova et al. 2017; Wilke 2020) to assess differences between and within treatments across nights 1 and 9. We used the specific acoustic parameters as a response variable, treatment (fed vs. unfed) and night (1 vs.

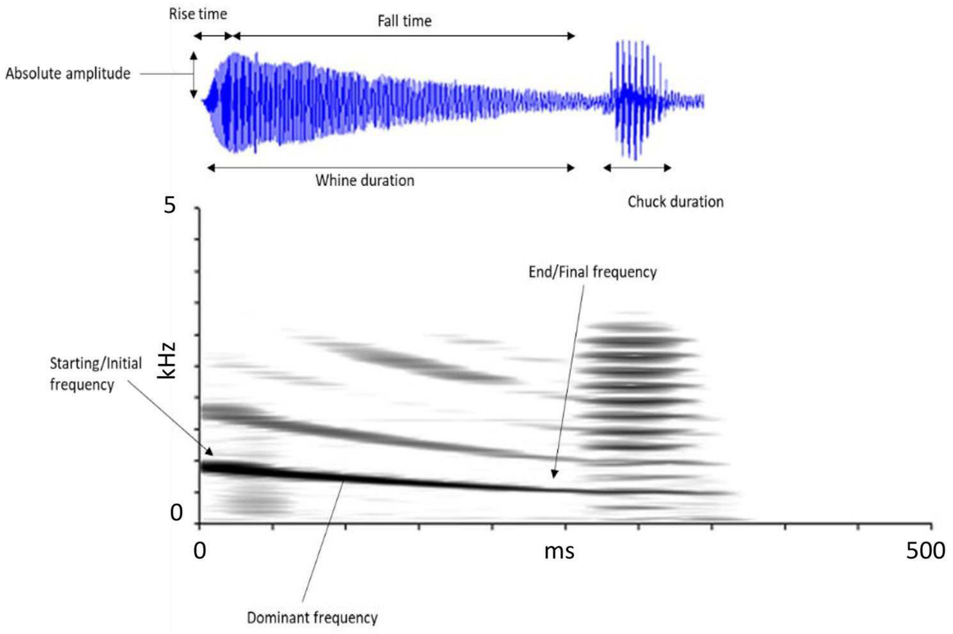


Fig. 1 Call parameters measured illustrated in a whine-chuck mating call. We measured the absolute amplitude, rise and fall times, whine and chuck duration, the dominant frequency, and the starting and end frequencies

9) as fixed effects, and individual (frog) as a random effect. We tested each of the 10 acoustic parameters separately. To account for multiple testing we performed a Bonferroni correction, adjusting the alpha level for significance from 0.05 to 0.005.

Condition-dependent calling effort

We determined how the males' condition influenced their calling effort in response to three call playbacks: whine (W), whine-chuck (WC), and whine-chuck-chuck-chuck (WCCC).

After the completion of testing, we counted the total number of call responses to each of the presented stimuli. We edited the recordings by deleting calls that were overlapping with the playback stimuli, which prohibited us from accurately identifying the call type. We also recorded the complexity of the male responses to the different stimuli presented to them. Each response of the focal male was noted as a W, WC, WCC, or WCCC. There were too few WCCCs for statistical analysis.

We used an analysis of variance to assess differences between and within treatments across nights 1 and 9 as well as the total number and types of calls produced throughout the experiment.

Condition-dependent call attractiveness

In these experiments, we determined if condition-dependent changes in the male's calls influence the attractiveness of those calls to females. We randomly selected calls from 5 males (3 unfed, 2 fed) to determine if females discriminated against calls of the same male on night 1 versus night 9. Unfed males were in high condition on night 1 and low condition on night 9, while fed males were in high condition on both nights. We randomly chose three calls from night 1 and night 9 from each male and matched them against each other in every combination to produce 9 sets of paired stimuli per male. These combos consisted of one call from night 1 (Table 1, start calls, labeled s) and one call from night 9 (Table 1, end calls, labeled e). We used the same protocol to pair the calls of the two fed males. This resulted in 45 separate phonotaxis experiments. For each pair of calls, we initially tested 20 females in each trial, but as weather changed and more frogs became available, we increased the sample size to 24 females. There was a total of 912 individual female choice tests. (We had planned to add more males in the subsequent years but STRI was closed down during the pandemic and the primary author had moved on by the time STRI was open to visiting researchers again.)

Table 1 Outline of phonotaxis experiments. For each of three unfed males, we randomly selected 3 calls from the first and last night calling and paired them with each other to create 9 pairs of trials for each male. The same protocol was followed for the calls of the two fed males. s = start call; e = end call

Male Calls	Night 1 – call 1 (s1)	Night 1 – call 2 (s2)	Night 1 – call 3 (s3)
Night 9 – call 1 (e1)	s1 vs. e1	s2 vs. e1	s3 vs. e1
Night 9 – call 2 (e2)	s1 vs. e2	s2 vs. e2	s3 vs. e2
Night 9 – call 3 (e3)	s1 vs. e3	s2 vs. e3	s3 vs. e3

We conducted phonotaxis experiments under infrared light in a sound-attenuating chamber (2.7 × 1.8 × 1.78 m, L x W x H; Acoustic Systems, Austin, TX) with two speakers placed opposite one another at each end of the long axis of the chamber. Before each set of trials, we calibrated the speakers to the absolute amplitude of each call at the center of the chamber. Absolute amplitude was previously measured in sound boxes at 16 cm, so we adjusted those amplitudes to a distance of 116 cm, the distance between the front of the speakers and the center of the arena (which resulted in a difference of -16.7 dB). We separated females from their male partners and placed them under an acoustically transparent funnel in our phonotaxis chamber. We played to females two calls antiphonally, one from night 1 and one from night 9, and each call once every two seconds for a two-minute acclimation period, after which we lifted the funnel remotely and allowed her to choose between the calls. We recorded a choice if she approached within 10 cm of one of the speakers. We tested females in different trials until she ceased responding in two consecutive trials; we only used a female for a single night. These were not repeated measures, so a female only recorded one choice per experiment (one pair of calls). On occasion, no response was recorded. This was when a female took longer than 10 min to choose, climbed the walls, or followed the walls to a speaker choice zone. We returned all frogs to their capture location at the end of the night.

We used a two-tailed binomial test to assess the null hypothesis of no preference between the night 1 and the night 9 calls for each trial for each of the 45 experiments. We compared the results (preferences for night 1 call, preferences for night 9 call, no preference) in response to calls of fed and unfed males using a Freeman-Halton extension of the Fisher exact probability test.

Results

Condition-dependent acoustic parameters

We compared the percent change in mass between fed and unfed males in the control groups (no exposure to male calls) and the treatment groups. The control unfed males and the treatment unfed males lost significantly more mass than their fed counterparts over the course of the nine days of the experiment (ANOVA, $F=15.54$, $df=21$, $p<0.001$; Fig. 2). The percent change in mass of the control fed males and the treatment fed males was not significantly different (ANOVA, $F=0.029$, $df=21$, $p=0.86$; Fig. 2). Thus, being exposed to mating calls over the nine-day testing by itself did not influence male condition.

The Bonferroni corrected alpha level for comparisons within each acoustic parameter was adjusted to 0.005. We found no differences in any of the call parameters between nights 1 and 9 for the fed males ($-0.98<t<1.26$, all $p>0.233$; Figs. 3 and 4). In the unfed treatment, however, most call parameters were significantly different within individual males between nights 1 and 9, these included:

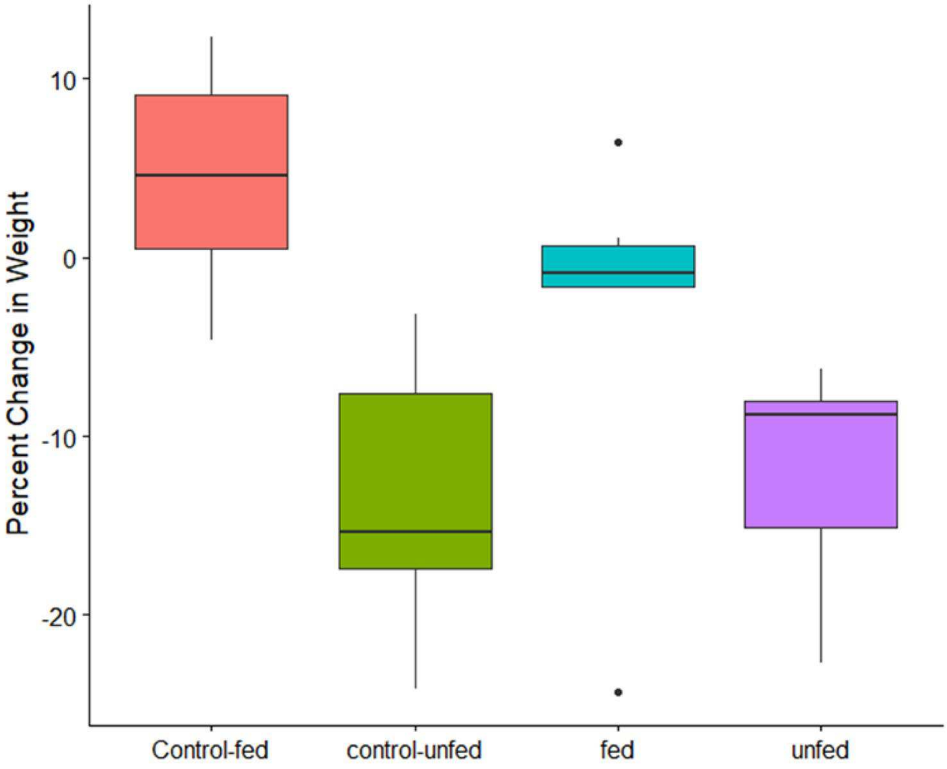


Fig. 2 Percent weight loss over nine days in 4 experimental treatments. The thick black bar in the middle is the median and "whiskers" show the range of all males in that group, and the box shows the interquartile range. Control treatments were not exposed to acoustic playbacks. Unfed males in both aspects lost significantly more mass than fed groups and were presumed to be in lower condition than the other treatments. ANOVA, $p<0.001$, $N=34$

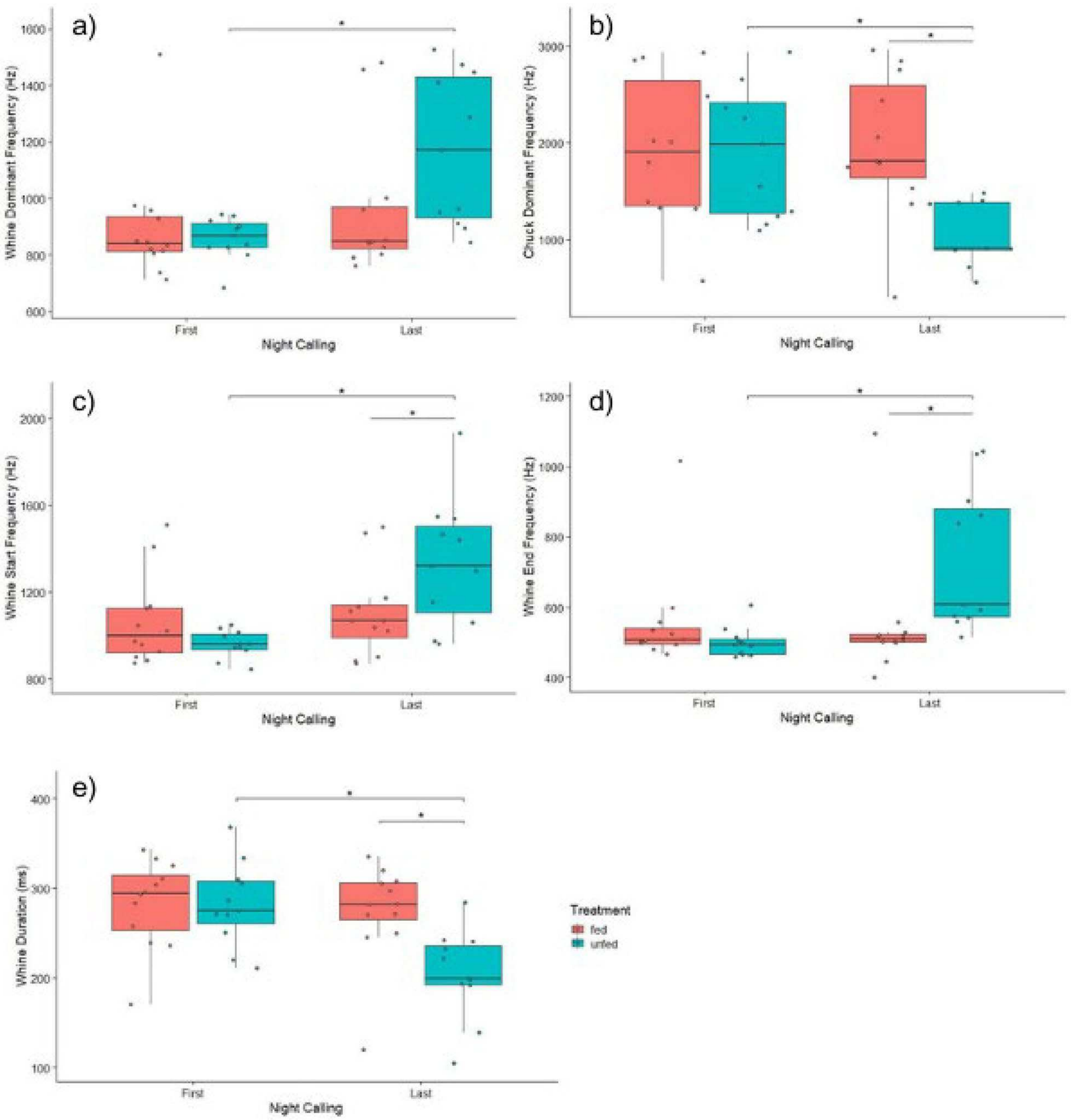


Fig. 3 Box plot comparisons of frequency and whine duration on night 1 and night 9 of fed and unfed treatments. The thick black bar in the middle is the median and "whiskers" show the range of all males in that group, and the box shows the interquartile range. The "*" indicates significant differences (Bonferroni corrected alpha level = 0.005) between groups connected by the ends of the line below the "*". See Fig. 1 for illustration of call parameters

dominant frequencies of the whine (t = 3.74, df = 20, $p = 0.001$; Fig. 3A) and chuck (t=-3.431, df = 18, $p = 0.003$, Fig. 3B); initial frequency of the whine (t = 4.188, df = 20, $p = 0.0004$; Fig. 3C); final frequency of the whine (t = 3.792, df = 20, $p = 0.001$; Fig. 3D); duration of the whine (t=-4.139, df = 10, $p = 0.002$; Fig. 3E); absolute amplitude of the whine (t=-3.811, df = 20, $p = 0.001$; Fig. 4A), and whine fall time (t= -4.63, df = 10, $p = 0.001$; Fig. 4C). Given the Bonferroni corrected alpha level, we did not detect significant differences in the absolute amplitude of the chuck (t=-2.928, df = 18, $p = 0.009$; Fig. 4B), rise time (t=-1.267, df = 21, $p = 0.21$; Fig. 4D) or chuck duration (t=-1.548, df = 17, $p = 0.14$; Fig. 4E). In summary, on their last night of calling compared to their calls on night 1, calls of unfed

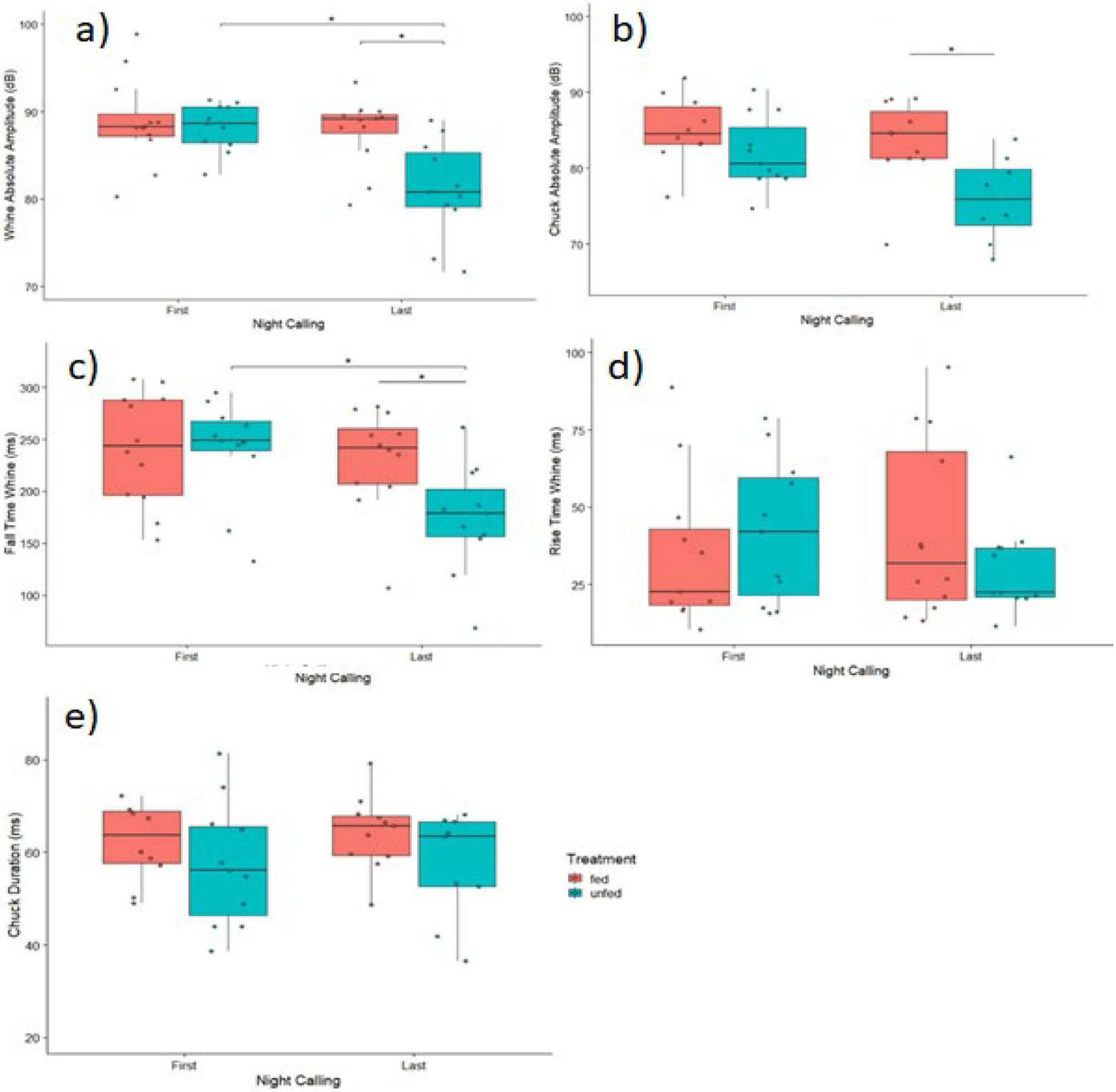
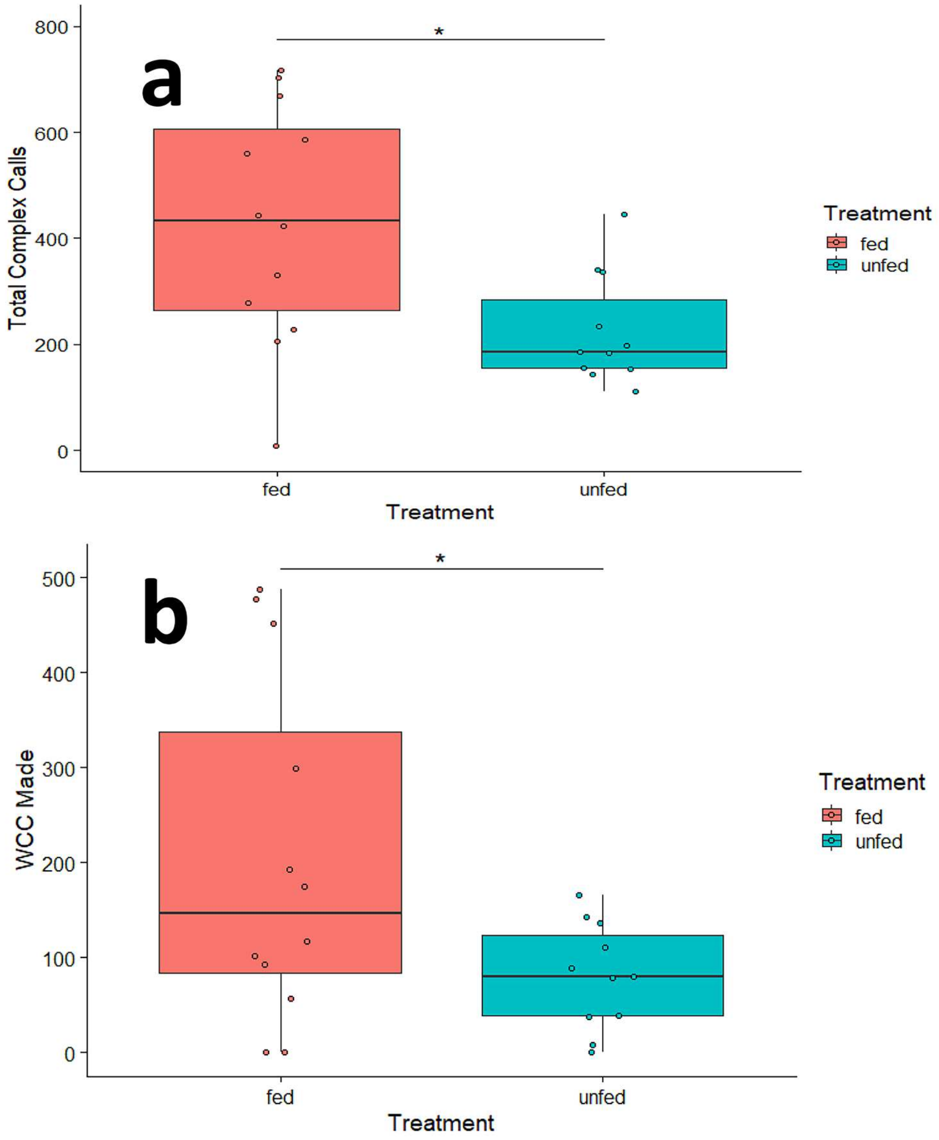


Fig. 4 Box plot comparisons of absolute amplitude, rise and fall time, and chuck duration on night 1and night 9 of fed and unfed treatments. The thick black bar in the middle is the median and "whiskers" show the range of all males in that group, and the box shows the interquartile range. The "*" indicates significant differences (Bonferroni corrected alpha level = 0.005) between groups connected by the ends of the line below the "*". See Fig. 1 for illustration of call parameters

males had higher dominant frequency whines, lower dominant frequency chucks, higher initial and final whine frequencies, shorter duration whines, lower amplitude whines, and a shorter fall time.

There were no differences in the call parameters between fed versus unfed treatments on night 1 ($-1.456 < t < 0.089$, all $p > 0.119$; Figs. 3 and 4). However, we did find differences between fed and unfed males on night 9 for: whine duration ($t=-3.161$, $df=20.98$, $p=0.004$; Fig. 3E), absolute amplitude of the whine ($t=-3.43$, $df=20.9$, $p=0.003$; Fig. 4A) and chuck ($t=-3.278$, $df=17.7$, $p=0.004$; Fig. 4B), dominant frequency of the chuck ($t=-3.37$, $df=17.5$, $p=0.003$; Fig. 3B), initial frequency of the whine ($t=-2.242$, $df=20.99$, $p=0.003$; Fig. 3C), final frequency of the whine ($t=2.382$, $df=20.96$, $p=0.027$; Fig. 3D), and whine fall time ($t=-2.722$, $df=20.97$, $p=0.013$; Fig. 4C).

Fig. 5 (**a**) The total number of complex calls produced by fed and unfed males over all experiments. (**b**) The number of WCC calls produced by fed and unfed males over all experiments. The thick black bar in the middle is the median and “whiskers” show the range of all males in that group, and the box shows the interquartile range. The “*” indicates significance ($p < 0.05$) between groups connected by the ends of the line below the “*” “*”. See Fig. 1 for illustration of call parameters



Condition-dependent calling effort

We compared the calling effort of unfed versus fed males across all nine nights of the experiments (see below). Summing all the experiments, there were no differences detected in the number of Ws (F = 0.0121, df = 21, $p = 0.91$) or WCs (F = 1.53, df = 21, $p = 0.22$) produced between the two treatments, fed versus unfed. However, we found that unfed males called significantly less than fed males (ANOVA, F = 7.67, df = 21, $p = 0.01$), made fewer total complex calls (F = 7.45, df = 21, $p = 0.012$; Fig. 5a), and fewer WCCs (F = 7.67, df = 21, $p = 0.011$; Fig. 5b). Unfed males also showed a decrease in the number of complex calls made on night 9 compared to night 1 summed across all experiments (F = 6.01, df = 21, $p = 0.023$).

Table 2 Comparisons of the evoked vocal responses (columns) of fed versus unfed males on night 9 to playbacks of three different mating calls (rows). Significant differences are in bold, trends are in italics. When there is a significant difference or trend, the fed males produced more calls

Playback treatment	W	WC	WCC
W	0.172	0.984	0.390
WC	0.807	*0.058*	**0.023**
WCCC	0.339	0.877	**0.014**

We asked if males responded differently to the different playback calls. For fed versus unfed males (Table 2) and for unfed males on night 1 versus night 9 (Table 3), we compared the number of W, WC, and WCC calls produced in response to playback treatments of W, WC, and WCCC. Over all of the comparisons, fed males always produced more calls than unfed males; this general pattern is

Table 3 Comparisons of the evoked vocal responses (columns) of unfed males on night 1 versus night 9 to playbacks of three different mating calls (rows). Significant differences are in bold, trends are in italics. When there is a significant difference or trend the unfed males on night 1 produced more calls

Playback treatment	W	WC	WCC
W	0.518	0.518	0.365
WC	0.254	0.457	**0.035**
WCCC	*0.071*	0.398	*0.051*

statistically significantly different from random (exact binomial test = 0.002), but the same is not true for individual experiments, as we describe below.

In response to the W playback, there was no difference in the number of Ws, WCs, or WCCs produced when comparing fed to unfed males (Table 2) or unfed males on night 1 versus night 9 ($1.98 < F < 3.57$, and all $p > 0.078$; Table 3).

In response to the WC stimulus, we found no differences in the number of Ws or WCs produced when comparing fed to unfed males (Table 2) or unfed males on night 1 versus night 9 (all $1.36 < F < 3.96$, and all $p > 0.058$; Table 3). We did find that on night 9, fed males produced more WCCs than unfed males ($F = 5.01$, $df = 21$, $p = 0.023$; Table 2), and unfed males produced more WCCs on night 1 than they did on night 9 ($F = 6.015$, $df = 21$, $p = 0.035$; Table 3).

When presented with a WCCC stimulus, we found no difference in the number of Ws or WCs produced between fed and unfed males (Table 2) or between unfed males on night 1 and night 9 ($3.61 < F < 7.02$, and all $p > 0.051$). Fed males produced significantly more WCCs than unfed males on night 9 ($F = 7.01$, $df = 21$, $p = 0.014$; Table 2). Two comparisons suggested a trend. Both were within the unfed treatment comparing the first and last night's calling. These were the number of Ws produced ($p = 0.071$) and the number of WCCs produced ($p = 0.051$).

Summarizing these results, when males are deprived of food there is a reduction in their propensity to vocally respond and they are less likely to produce complex calls.

Condition-dependent call attractiveness

We tested the response of females to calls of males, three unfed males and two fed males, on night 1 versus night 9. In response to calls of the three unfed males, females significantly preferred the calls from night 1 compared to night 9 in 25 of the 27 experiments (binomial test, all $p < 0.04$, Fig. 6). In two trials, there was a trend towards preferring the night 1 call (binomial test, both $p = 0.07$). As for the calls of the two fed males, in 13 of the 18 experiments, females showed no preference between the night 1 and night 9 calls (binomial test, all trials, $p > 0.07$; Fig. 5). In four of those experiments, females significantly preferred the night 9 calls (binomial test, all $p < 0.04$), and in one trial females preferred the night 1 call (binomial test, $p = 0.015$). The difference in the responses of females to the night 1 versus night 9 calls of unfed versus fed males (preferences for night 1 calls, preferences for night 9 calls, no preference) was statistically significant (Freeman-Halton extension of the Fisher exact probability test; $p < 0.0001$). These results show that a male's condition influences the attractiveness of his mating calls.

Discussion

The physical condition of an animal can vary drastically both among and within individuals, and an animal's condition can facilitate or constrain the type of behaviors in which it engages. Studies of condition-dependent signaling have made important contributions to animal communication in general, but especially to our understanding of sexual signaling. Sexual displays are energetically costly, and these costs limit how males can display to females and respond to other males. In some cases, these energetic costs can influence sexual displays in ways that can be apparent to females and influence their mating decisions. In this study, we examined the relationship between energy intake, body condition, quality of sexual signals, and the attractiveness of these signals to females.

We first asked if changes in condition, quantified by relative loss of body mass, influence the qualities of the male's call. A previous study of túngara frogs, conducted in outdoor mesocosms at the level of the population, showed that after 16 days without supplemental food male túngara frogs were less likely to call and had reduced levels of testosterone (Marler and Ryan 1996). In this study, we examine how changes in condition within individuals influenced parameters of the mating call. We showed that on night 9, unfed males produced calls with lower amplitude, shorter duration, shorter fall time, and higher dominant, initial and end frequencies of whines, and lower frequency chucks than their calls on night 1.

These results are similar to an investigation of the effect of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which causes the infectious disease chytridiomycosis, on male calling. Rodriguez Brenes (2016) showed that males infected with chytrid also produced calls of lower amplitude and higher dominant frequency compared to the calls of those same males after they were cleared of chytrid. Interestingly in another study, administration of argentine vasotocin (AVT) by Kime et al. (2010) also showed that calls of treated males had higher frequencies, though perhaps for different reasons. Thus, when males are displaced from their physiological equilibrium in different ways there are similar effects on properties of the male's advertisement

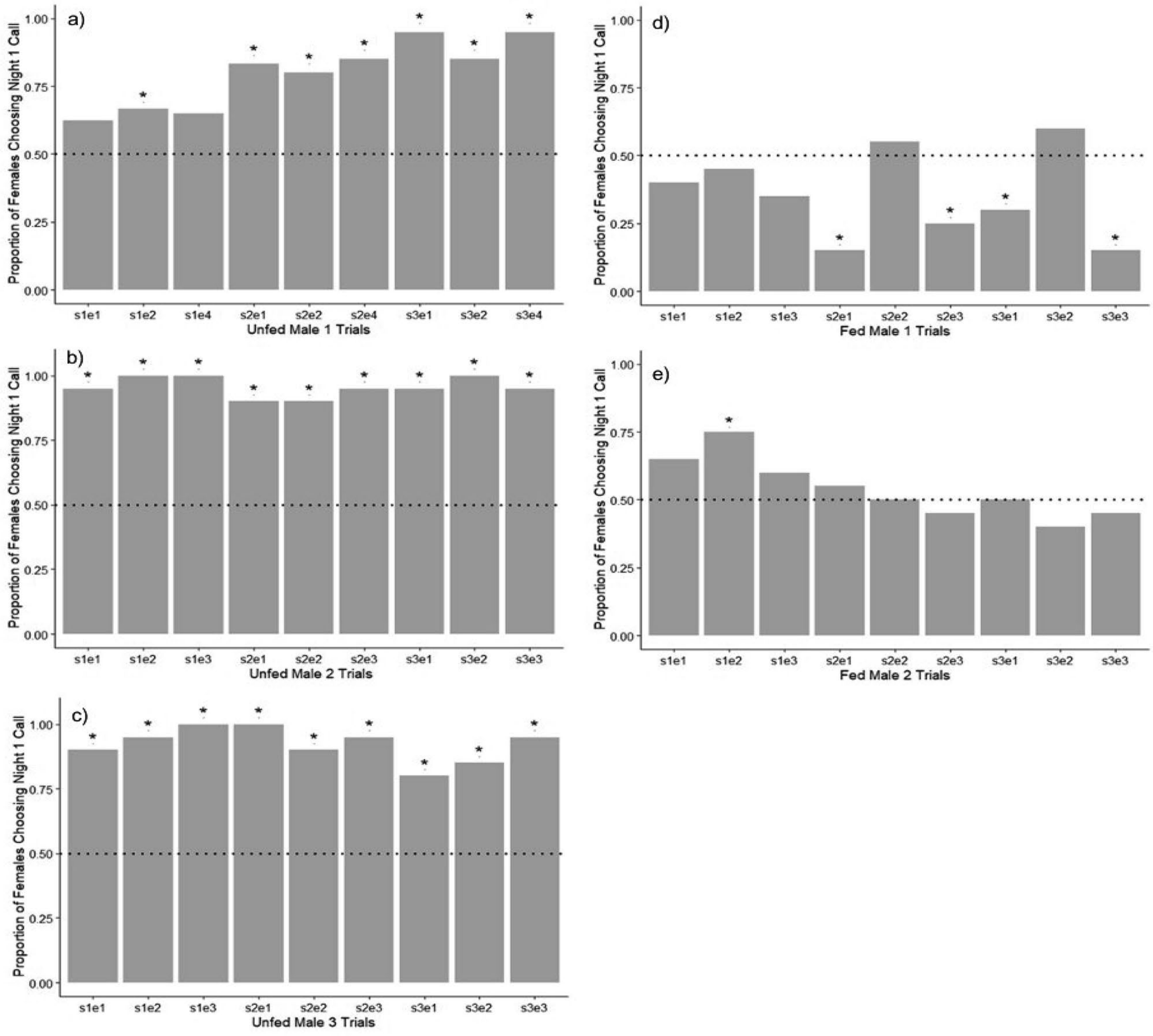


Fig. 6 Results of phonotaxis experiments. Each graph shows female phonotaxis preferences for nine pairs of calls of the same male. We chose three calls that were produced on night 1 (s "start") versus three calls that were produced on night 9 (e "end") to constitute the nine pairs; the number assigned to each call is arbitrary. The pairs of calls tested are on the x-axis; for example, s2e3 indicates that call number two produced on night one and call number three produced on night nine were both presented to the female antiphonally. (In (a) there is a call s4 because one of the three calls randomly chosen from night one was distorted.) See Table 1 for more details. The "*" indicates a significant preference for the night 1 call over the night 9 call

call. In these studies, females preferred the calls of males that were free of chytrid (Rodriguez Brenes 2016) and who were administered a saline control (Kime et al. 2010), respectively.

Our general results of condition-dependent signaling are consistent with most of the previously published literature. Although some studies examined condition only among individuals while others examined condition within individuals, both types of studies are consistent with the fact that changes in condition can influence the quality of mating signals (e.g. Hill 1990; Docherty et al. 1995; Hill et al. 2004; Brepson et al. 2013; Kuczynski et al. 2016).

Although the focus of most studies of sexual signaling is on the male as the signaler and the female as the receiver of the signal, males are often signaling to one another simultaneously. This is certainly true of anuran advertisement calls as many studies have documented vocal interactions among males (Ryan 2001; Gerhardt and Huber 2002). Our study shows that unfed males at the end of the treatment period are less likely to respond vocally and less likely to respond with complex calls compared to their behavior at the beginning of the treatment period, a similar pattern holds when comparing unfed males to fed males.

These results are consistent with the study by Marler and Ryan (1996) showing that males are less likely to call in an environment without supplemental food compared to those males in an environment where food is supplemented. They are also consistent with the study by Larter et al. (2023) that showed that call amplitude increases within a call bout were greater for males in better body condition. It also raises the possibility that chorus dynamics could change in more stressful environments.

These results are not surprising as calling is an energetically expensive behavior in túngara frogs (Bucher et al. 1982), as well as in many frogs and other animals that vocalize (Prestwich 1994). Interestingly, the study by Bucher et al. showed that there was no additional metabolic expense to adding chucks to the whine; our modeling of the biomechanics of chuck production is consistent with that result (Kime et al. 2019).

Although not common, male túngara frogs fight with one another and their wrestling can lead to death (Ryan 1985). Males escalate call complexity during vocal competition, and vocal escalation can precede actual fights; it is not known if the vocalizations per se or the proximity of males to one another cause the fight. A male in lower condition might want to avoid physical exertion especially when it concerns agonistic interactions with another male. If true, then males in low condition might reduce the use of complex calls or not respond to a complex call to avoid a fight they cannot win.

But not all costs are metabolic. Frog-eating bats and bloodsucking midges are more attracted to complex calls (Ryan et al. 1982; Bernal et al. 2006; Akre et al. 2011). In response to threats from the frog-eating bat, males engage in a suite of escape behaviors (Tuttle et al. 1982; Jennions and Backwell 1992). It is possible that males in lower condition would be less able to engage in these escape behaviors. Similarly, when attracting bloodsucking midges male túngara frogs spend a substantial amount of time swatting at the midges with their front legs (Bernal and Page 2023), another activity that might further strain males in low condition.

These results are consistent with other studies that have documented how condition influences the response of males in vocal competition with other males. For example, grey tree-frogs have greater calling plasticity when in higher condition (Kuczynski et al. 2016), and when given extra food male crickets call more (Holzer et al. 2003; see also Crocker and Day 1987; Bertram et al. 2013; Brepson et al. 2013). There is one intriguing exception to this general pattern of low condition compromising calling effort. An and Waldman (2016) documented the calling behavior of male *Hyla japonica* who were and were not infected by *Bd*. They showed that infected males called more rapidly and produced longer calls than uninfected males. The researchers suggested this might be evidence for pathogen manipulation of host behavior.

In our first set of experiments, we demonstrated how certain acoustic parameters, specifically amplitude and dominant frequency, vary with the male's condition. An important question asks do these changes in the call influence the male's reproductive fitness? An important selective force on the calls results from call-based mate preferences of females. Our results show that females preferred the calls of unfed males on night 1 of our experiment to calls of those same males after nine days of food deprivation. We did not deconstruct the importance of individual parameters of the call, but previous studies have also shown that túngara frog females prefer calls with higher amplitudes and lower frequencies (Ryan 1980, 1983; Ryan and Rand 1990; Bosch et al. 2000).

As noted above, infection status of *Bd* (Rodriguez Brenes 2016) and exogenous treatment with AVT (Kime et al. 2010) generate some similar changes in call parameters that we showed in this study: infected males produce lower amplitude and higher frequency calls, and those treated with AVT produce higher frequency calls. Both studies show that these call changes in response to *Bd* and AVT also make these calls unattractive to females. Thus, there seems to be a general syndrome of the mating calls of low-condition (and elevated AVT) males that decreases their attractiveness to females in this species of frog. Although condition-dependent sexual attractiveness is often cited as evidence for good genes selection (Zahavi and Zahavi 1997), it should be noted that we are examining variation in condition and calls within males. Although there is still possibly a gene-by-environment effect, these results should not be interpreted as either rejecting or supporting the hypothesis of good-genes mate choice (more generally, see Achorn and Rosenthal 2020; Rosenthal and Ryan 2022).

A number of other studies have shown female preferences for signals that are correlated with male condition. In green tree frogs, for example, high-condition males show more plasticity in lengthening their calls (Kuczynski et al. 2016), and females significantly prefer longer calls in this species, even when only separated by a few extra pulses (Gerhardt et al. 2000). In two species of tortoise, females prefer high-condition males and can assess this by the number of times they are bumped by males (Pellitteri-Rosa et al. 2011). Female field crickets prefer the calls of high-condition males versus low-condition males based on differences in carrier frequency and chirp rate (Scheuber et al. 2003b). And in guppies, females prefer males who haven't been recently infected with parasites (Houde and Torio

1992). As a male guppy heals from an infection, his coloration dulls and female attention wanes. Also, house finch females prefer to mate with males sporting brighter plumage, a well-known condition-dependent trait (Hill 1991). In a final example, satin bowerbird females also prefer bright plumage as well, and in this case brighter males have lower parasite loads and researchers suggested that females use this as a means to avoid mating with infected males (Borgia and Collis 1989, 1990).

In summary, the studies we present here have shown the widespread effects that an animal's condition has on the sexual communication system in túngara frogs. This includes influences on the acoustic parameters of the mating call, the calling effort of the males, and the sexual attractiveness of the mating calls to females. In all components, males in lower condition perform more poorly than do those same males in high condition and they seem likely to encounter a fitness deficit due to reduced attractiveness to females. These results provide a unique analysis of the pervasive effect of condition on sexual communication.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Dryad depository, https://doi.org/10.5061/dryad.2ngf1vhw8.

Declarations

Ethical approval Handling and toe clip procedures were conducted in accordance with The American Society of Ichthyologists and Herpetologists' Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. This research was permitted by the Government of Panama (SE/AO-1-17, SE/A-44-18, and SE/A-40-19) and all protocols were approved by the STRI-IACUC (STRI IACUC 2018-0411-2021) and The University of Texas, Austin (AUP-2017-00051 and AUP-2020-00034).

Conflict of interest The authors declare that they have no conflict of interest.

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