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Local competitive environment and male condition influence within-bout calling patterns in túngara frogs

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ABSTRACT

Flexible signalling behaviour is widespread, with adjustments often enhancing gains or reducing costs of signalling based on the current state of the signaller's local communication network. Male túngara frogs call within multispecies communication networks containing conspecifics (both target receivers and rivals) and eavesdropping predators. These diverse players all exert an influence on male calling strategies. We investigated the degree to which patterns of changes in call characteristics across individual túngara frog calling bouts were influenced by callers' social environment, body condition, and ambient temperature. Most call bouts exhibited two distinct phases, an initial steep increase in call amplitude (the rise) followed by a longer period of more gradual amplitude increase (the plateau). Rises were completed more quickly when males called in denser choruses, while call amplitude increases during plateau phases were greater for males in better body condition. Males also produced more complex calls and increased complexity sooner when calling in denser choruses. Our results suggest that the social environment is the main driver of within-bout calling patterns. This could be due to (i) increased call effort required when competing in denser choruses, (ii) dilution effects provided by nearby rivals releasing callers from eavesdropping risk or, likely, (iii) a combination of both.

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Anuran communication; communication networks; flexible signalling; unison-bout callers

Introduction

Males of many species perform elaborate sexual displays to attract females as mates (Andersson 1994; Rosenthal 2017). In many anurans and insects, males signal acoustically to females in dense choruses (Gerhardt and Huber 2002). For each male, calling in an aggregation has consequences for attracting mates. For one, males must avoid having their calls interfered with by the calls of neighbours. This can select for complex signal–timing interactions among neighbouring signallers and high amplitude calls (Greenfield 1994, 2002; Gerhardt and Huber 2002; Wollerman and Wiley 2002; Love and Bee 2010). Signalling aggregations also allow females to compare the calls of multiple males

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simultaneously or in quick succession, meaning a male's attractiveness relative to his neighbours, not simply his absolute attractiveness, can become a critical determinant of his mating success (Callander et al. 2013).

The challenges for males of courting females as members of signalling aggregations select for flexible responses to the social environment. This flexibility can operate over a variety of timescales in anurans. Over long stretches of time, and even entire breeding seasons, males can employ one of a number of different mating strategies, such as defending a territory or intercepting females as they approach territory holders (Arak 1983; Zamudio and Chan 2008). Males can also alter calling strategies throughout a single night, such as painted African reed frog males (*Hyperolius marmoratus*) altering the proportion of aggressive vs. attractive calls they produce as the night progresses (Grafe 1995). Finally, over shorter timescales, males can dynamically respond to the calling strategies of nearby rivals. Males can adjust call timing (Greenfield 2015), duration and spacing of calls (Wagner 1989; Stirman and Pfennig 2019), amplitude and frequency of calls (Halfwerk et al. 2016; Shen and Xu 2016), and the inclusion of different note types (Goutee et al. 2010; Zhu et al. 2017). In unison bout callers, the onset and offset of calling activity by chorus-mates occurs at roughly the same time, producing periods of collective calling punctuated by silent intervals (Greenfield 2015). Chorus-mates must then navigate calling interactions with neighbours anew at the onset of each bout (Jones et al. 2014). Thus, bouts can be considered a discrete temporal unit over which males can exhibit flexible calling strategies in response to rivals (Wagner 1989).

Conspecifics are often not the only players that exert selection for flexible signalling behaviour. In broader multi-species communication networks (McGregor 2005), risks imposed by heterospecific eavesdroppers create a well-studied trade-off for signallers; increasing attractiveness and detectability to mates can increase conspicuousness to these natural enemies (Zuk and Kolluru 1998). Signallers employ a range of strategies to reduce risks from eavesdroppers and, once again, these strategies can operate over a range of timescales (Zuk and Kolluru 1998; Larter 2021). From night to night, signallers can reduce the detection-range of signals when abiotic conditions heighten the risks posed by eavesdroppers (Tuttle and Ryan 1982; Römer et al. 2010). Over the course of a night, changes in the social environment can influence the risks to signallers; risks of calling can vary due to the magnitude of dilution effects garnered by signalling from within aggregations of different sizes (Ryan et al. 1981; Alem et al. 2011), which can influence calling strategies (Jennions and Backwell 1992). Additionally, shorter-term strategies can operate in a single sequence of calls. Many species cease calling when they detect cues associated with eavesdroppers (Jennions and Backwell 1992; Lewkiewicz and Zuk 2004; Remage-Healey et al. 2006) or cues of detection of eavesdroppers by conspecifics (Dapper et al. 2011) or heterospecifics vulnerable to the same predators (Phelps et al. 2007). Males of some species also reduce their risks by synchronising signals with neighbours to exploit cognitive constraints of eavesdroppers (Tuttle and Ryan 1982; Legett et al. 2020, 2021). In unison bout callers, onset of calling after a silent inter-bout interval represents a sudden increase in risk of detection by

eavesdroppers. As such, changes in immediate risk to signallers, or the information signallers have about their immediate risk, may change throughout call bouts, resulting in alterations of calling strategies.

The competitive environment and eavesdropping risks are not the only factors shaping male calling strategies. Calling is an energetically expensive behaviour (Taigen and Wells 1985; Wells 2001), and higher call effort imposes more extreme energetic costs (Wells and Taigen 1989). Thus, often only males in the best physical condition can maintain prolonged high call effort (Voituron et al. 2012). In anurans, intra-specific differences in body mass and condition can be related to call rate (Zimmitti 1999), duration (Ziegler et al. 2016), and amplitude (Gerhardt 1975; James et al. 2021), although evidence for these associations across species is mixed. In addition to internal physiological constraints, aspects of the abiotic environment may constrain calling behaviour. In ectotherms, such as frogs, ambient environmental temperature can be an important constraint on calling behaviour (Navas and Bevier 2001; Ospina et al. 2013).

Thus, male calling strategies can be influenced over a variety of timescales by a multitude of factors, including the competitive environment, trade-offs resulting from eavesdropping enemies, and energetic and abiotic constraints. In the current study, we investigated influences on calling strategies employed over the course of single calling bouts in túngara frogs (*Engystomops* (= *Physalaemus*) *pustulosus*). Túngara frogs are unison bout callers that form choruses of varying size, and males adjust their calling strategies to the social environment (Ryan 1985). Calling in túngara frogs is costly both energetically (Bucher et al. 1982) and in terms of increasing conspicuousness to eavesdroppers (Tuttle and Ryan 1981; Ryan et al. 1982; Bernal et al. 2006). This variability in the social environment that callers experience, flexibility in calling behaviour, and the occurrence of discrete calling bouts make the túngara frog ideal for an investigation into how short-term calling strategies are influenced by social, physiological, and abiotic factors.

Based on previous results (Pauly et al. 2006; Halfwerk et al. 2016), we anticipated that individual males' calls would increase in amplitude throughout their calling bouts. Exploratory data analysis (described in *Methods*) confirmed this and revealed a predominant within-bout pattern characterised by two distinct phases: a steep initial rise in amplitude (the rise phase) followed by a lengthier levelling out of amplitude (the plateau phase) (Figure 1). We analysed both phases separately to address how the amplitude and acoustic energy of different call components changed throughout the bout, and how these patterns were influenced by a male's social environment, his body condition, and environmental temperature. We also investigated how these same factors influenced patterns of call complexity across bouts. We present non-mutually exclusive hypotheses and predictions regarding the drivers of within-bout call patterns in Table 1. Higher amplitude and more complex calls are more attractive to females (Akre and Ryan 2010b), so a general assumption underlying our predictions is that, other factors being equal, males should adopt a calling strategy that maximises their attractiveness to females.

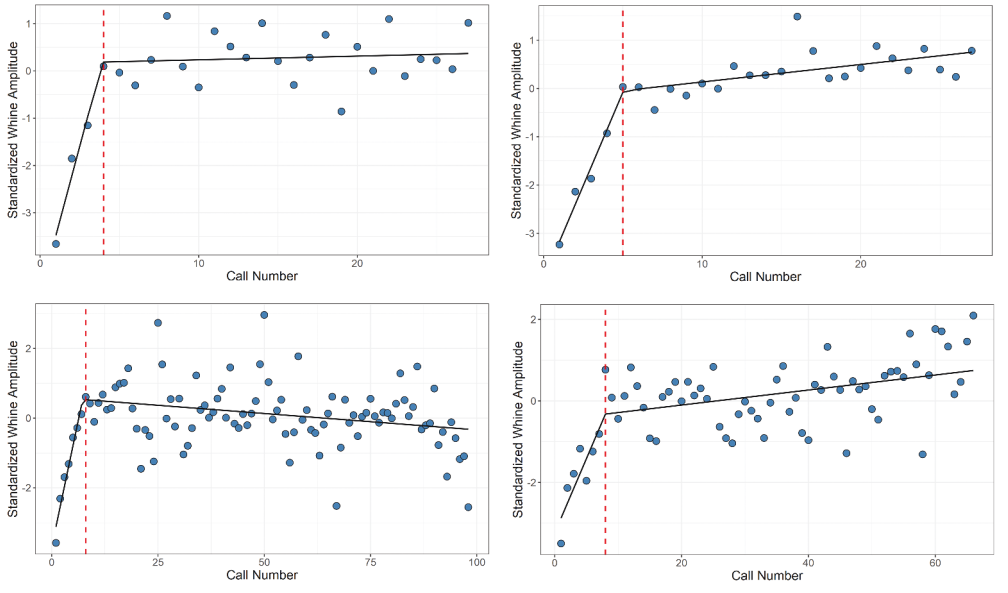

Figure 1. Examples of the typical pattern of whine amplitude change over call bouts, and an illustration of our scheme for dichotomising call bouts into 'rise' and 'plateau' phases. Black lines are the fitted regression line implemented using the 'Segmented' R package. Lines to the left of the dotted red line denote the 'rise' phase, and lines to the right denote the 'plateau' phase.

Table 1. Non-Mutually exclusive hypotheses, the predictions associated with them, and the rationale behind the proposed hypotheses.

Hypothesis	Predictions		Rationale
Within-bout calling patterns are primarily driven by the local competitive environment	Focal males with more rivals calling within 1m will have bouts with...	**i)** A steeper increase in call amplitude and spectral attractiveness during the rise and plateau phases of their bouts **ii)** A higher proportion of complex calls **iii)** Increases in call complexity occurring sooner during their bouts	Males calling in denser choruses will need to increase their relative attractiveness compared to rivals, and may also be released to a degree from the risks of conspicuous calling in the presence of eavesdroppers
Within-bout calling patterns are primarily driven by energetic and abiotic constraints	Males calling in warmer water, and males in better body condition, will have bouts with...	**i)** A steeper increase in call amplitude and spectral attractiveness during the rise and plateau phases of their bouts **ii)** A higher proportion of complex calls **iii)** Increases in call complexity occurring sooner during their bouts	High call effort is energetically expensive and may be constrained by stored energy reserves, and ambient temperature can constrain physical activity in ectotherms

Methods

The study species

At dusk during the Panamanian rainy season (May to December), male túngara frogs gather to call in choruses of variable size (Ryan 1985). Males call while floating in water, and breeding sites are typically shallow, stagnant or slow-moving pools, such as puddles and drainage areas. Inter-male spacing at these sites results from males placing themselves certain distances apart and maintaining these distances by producing aggressive 'mew' calls (Ryan 1985). Males resort to physical altercations when other males approach to within 5–10 cm of them (Ryan 1983). Males do not attend choruses every night. The energetic costs of chorusing require males to intersperse nights of chorus attendance with nights of feeding to replenish their energy reserves (Marler and Ryan 1996). Thus the numbers and identities of males at a breeding site each night vary widely (Ryan 1983). Females attend these breeding sites during the night and move among the calling males, eventually selecting a mate. Túngara frog males produce a two-part advertisement call. All calls begin with a simple whine, a frequency sweep whose fundamental frequency begins at 700 Hz and ends 300 ms later at 400 Hz. The whine is necessary and sufficient to attract females (Ryan 1985), but males can append from 0 to 7 short (~35 ms) harmonically structured chuck notes to whines to produce complex calls (Figure 2). All males can produce both simple and complex calls, and these complex calls are more attractive to females than simple (whine-only) calls (Ryan et al. 2019). Complex calls with greater numbers of chucks are more attractive to females at certain amplitudes (Akre and Ryan

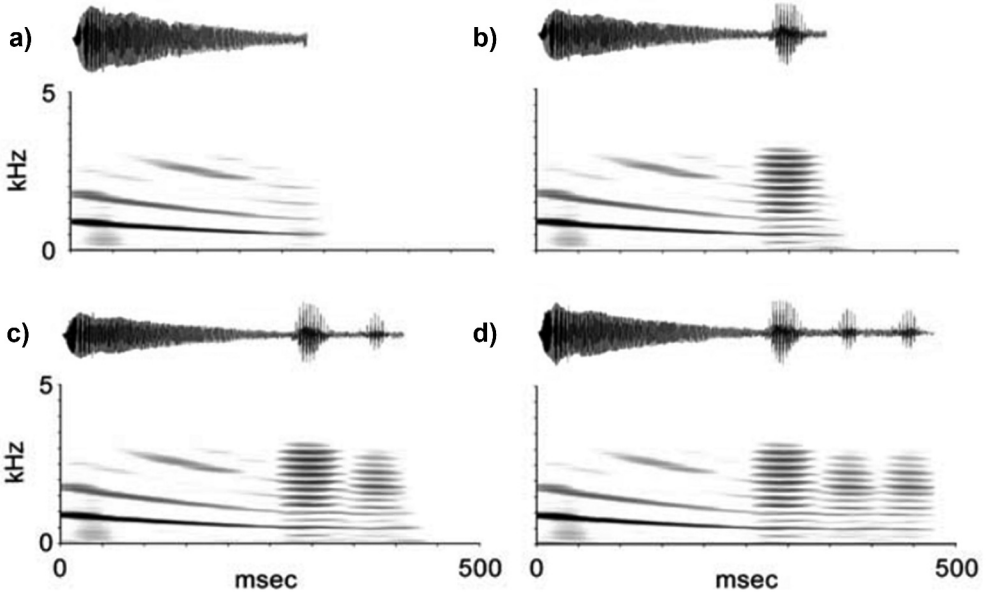

Figure 2. Waveforms and spectrograms of variation in call complexity in túngara frogs. (a) a simple whine-only call, (b) a whine plus chuck (complex call), (c,d) a whine plus two and three chucks, respectively (highly complex calls). Highly complex calls can contain up to 7 chucks.

2010b). This preference for chucks is due to the structure of the female auditory system. Túngara frogs, like other anurans, have two inner ear organs; the amphibian papilla (AP) and basilar papilla (BP). The BP is most sensitive to frequencies >1500 Hz, and ~90% of the acoustic energy of chucks exceeds this threshold (Ryan et al. 1990). Stimulation of the BP by chucks results in the heightened attractiveness of complex calls to females (Ryan et al. 1990; Wilczynski et al. 1995).

Túngara frogs are unison bout callers, and call bouts can last several minutes and consist of up to hundreds of calls (mean = 32.63 calls) (Bernal et al. 2009). Bouts typically begin with one or two males calling. Other males in the chorus then join in calling until the chorus reaches a peak of calling activity which persists for a variable amount of time. Bouts tend to end more abruptly than they begin, with all males ceasing to call at around the same time. Bouts are punctuated by silence, with the mean duration of silent inter-bout intervals being 25 s (Akre and Ryan 2010a). Male calling strategies are sensitive to the calls of neighbours. Males time their calls to avoid call overlap with nearby rivals (Greenfield and Rand 2000), and increase call rate (Green 1990; Halfwerk et al. 2014), call complexity (Rand and Ryan 1981; Bernal et al. 2007; Goutee et al. 2010), and call amplitude (Halfwerk et al. 2016) in response to rival calls and chorus noise.

Two costs to male calling have been well documented in túngara frogs. One is imposed by eavesdroppers: frog-eating bats and frog-biting midges are attracted to male calls, especially complex calls, meaning callers increase their risk of attack as they increase their attractiveness to females (Tuttle and Ryan 1981). The other cost is metabolic; in túngara frogs, as in other anurans (Wells 2001; Bevier and de Andrade 2017), calling is metabolically expensive (Bucher et al. 1982). Energetic costs are driven by the production of whines, not chucks (Bucher et al. 1982).

Field recordings

Male túngara frogs were recorded in June and July 2004 from a variety of breeding sites in Gamboa, Panama (9° 070”N, 79° 419”W), near the facilities of the Smithsonian Tropical Research Institute, as part of a study on the dynamics of calling behaviour (Bernal et al. 2007). We recorded a single complete call bout from 85 individual focal males using a WM-D6C Sony tape recorder and a Sennheiser ME-66 shotgun microphone placed 1 m from the caller. Call bouts were defined as a series of calls with inter-call gaps of less than 10 s (Pauly et al. 2006). In this study, we only included bouts for which we could be sure that all calls, including the very first call, were recorded. During these recordings, we visually counted the number of calling males within 1 m of the focal male; this ranged from 0 to 4 (Table 2). After recordings, we measured water and air temperature at the calling site. We then captured and weighed focal males to the nearest 0.001 g using a digital balance and used calipers to measure their snout-vent length (SVL) to the nearest 0.01 mm. We used SVL and mass to determine the relative body condition of frogs by dividing the residuals of a linear regression of the cube root of body mass on SVL by SVL to provide an index of condition relative to body length (Dyson et al. 1998). To allow our condition score to be on a similar scale to other variables, we used standardised condition score for all analyses. To avoid duplicate recordings, we toe-clipped frogs for individual identification. We returned males to capture sites on the same night. After recording a focal male at a certain breeding site, we waited at least seven nights before

Table 2. Summary statistics for independent and dependent variables used in whine rise and plateau slope analysis.

Variable	N	Mean	SD	Min	Max
Body condition score	17	0.0002	0.002	−0.003	0.005
Number of males within 1 m	17	1.35	1.17	0	4
Bout length	17	45.65	29.66	14	114
Water temperature	17	26.22	0.78	24.9	27
Rise slope	17	0.87	0.68	0.05	2.31
Plateau slope	17	0.01	0.03	−0.04	0.09

returning to that same breeding site to sample a new focal male. Chorus membership has a high turnover (Ryan 1983), so it is unlikely that focal males recorded from the same breeding site at different times were recorded as members of choruses containing the same individuals. All animals were handled according to ASIH guidelines (Accessible at https://asih.org/animal-care-guidelines). All research was licenced and approved by the University of Texas at Austin Institutional Animal Care and Use Committee (IACUC 6,041,701) and the government of Panama (ANAM permit SE/A-37-05).

To ensure accurate measures of acoustic parameters such as amplitude, we focused on a subset of these call bouts in which there was minimum overlap of the focal males' calls by the calls of other males. This yielded 24 call bouts (912 calls in total), each from a different male, with a mean bout length of 38 calls per bout (range = 7–114 calls; SE = 5.743). Calls were digitised at 44.1 kHz and analysed with SIGNAL (Engineering Design, Belmont, MA, USA: http://www.engdes.com/). From the waveform of each call, we measured the peak amplitude of all whines and chucks. We also calculated a fast Fourier transform (FFT) for the whine and for the chuck. Since the higher harmonic frequencies present in chucks (above 1500 Hz) are primarily responsible for the attractiveness of chucks to females (Wilczynski et al. 1995), we compared the relative amount of chuck acoustic energy in the FFT for frequencies below 1500 Hz and above 1500 Hz.

Statistical analyses

All analyses were performed in RStudio (RStudio Team 2018). Regression models were built using the 'lme4' package (Bates et al. 2007). For all models, we performed iterative model selection, beginning with a full model and removing the explanatory variable with the highest non-significant *p*-value each time until only significant variables remained. Successive models were compared using likelihood ratio tests, and assumptions specific to each model type were checked at each iteration. Outliers were identified with the 'car' package (Fox et al. 2012). When outliers were detected (at a threshold of 3*(mean Cook's distance)) (Cook 1979), we ran models with and without these outliers; in all cases, our results did not change, so outliers were retained. Specific statistical analyses for each research question are described below.

Changes in whine amplitude throughout call bouts

As the whine is the standard call, we first plotted whine amplitude throughout call bouts to visually assess which type of analysis would be appropriate. Most bouts could be described as consisting of two phases: first, a sharp increase in call amplitude in the initial

part of the bout which we refer to as the 'rise' phase, followed by a longer section in which call amplitude levels out and amplitude change occurs more gradually: the 'plateau' phase (Figure 1). For our subsequent analysis, we needed sufficient data points (calls) to precisely estimate the slopes of both bout phases, thus we only included males with bouts at least 10 calls in length. This excluded two males, leaving us with n = 22. Seventeen of these 22 males showed the typical rise/plateau pattern, so we focused on these males' bouts for analysis concerning the slopes of these phases. Males not fitting this typical rise/plateau pattern had no obvious similarities among them in within-bout amplitude change patterns.

Based on this rise/plateau pattern, we opted to use segmented regression analysis using the 'Segmented' package (Muggeo 2008). First, to make call bouts comparable, we standardised the amplitude of each male's calls within his call bout. Then, to model relative amplitude change per call, we used the 'Segmented' function to model the relationship between a male's standardised whine amplitude and call number (with bouts beginning with call number 1 and ending at, e.g. 100 for a 100 call bout). This function uses a bootstrap-restarting optimisation algorithm (Wood 2001) to insert an optimal breakpoint, to model this relationship as a regression with two slopes. This approach estimates a slope for each male's rise phase and another for his plateau. Thus, breakpoints were chosen objectively, but agreed with our subjective perception of where bout phases transitioned (Figure 1).

With rise and plateau phase slopes in hand, we then used linear regression to investigate the influence that our variables of interest had on these slopes. We built a linear model with rise slope as the response variable, and the number of males calling within 1 m of the focal male, male body condition, water temperature, and overall bout length as explanatory variables. We built a similar model with the cube-root (to satisfy model assumptions) of plateau slope as the response variable, and the same explanatory variables, but additionally included slope of the preceding rise phase as an explanatory variable to assess the relationship between the two slopes. Normality, linearity and homoscedasticity assumptions were verified for all models visually, and via Shapiro-Wilk and Breusch–Pagan tests using the 'lmtest' package (Hothorn et al. 2015). Steeper rise slopes could be driven by similar amplitude gains occurring over fewer calls or greater amplitude gains occurring over a similar number of calls. Thus, post-hoc, we investigated which of these was driving differences in slope steepness. We built a linear model with rise slope as the response variable, and the number of calls comprising the rise phase and the standardised amplitude gain occurring over the rise phase as explanatory variables.

Changes in chuck amplitude and energy throughout call bouts

Males vary in their latency to produce chucks during call bouts, meaning the rise phase of some males' bouts had insufficient numbers of chucks to model in the same way as we modelled whine amplitude patterns. Thus, for chucks, we only modelled the influences on the slope of the plateau phase of bouts. We used breakpoints found during the segmented regression analysis of whine amplitude to denote the start of the chuck plateau phases and analysed characteristics of chucks past this point. We obtained a slope for the chuck plateau phase for each male via a linear regression of standardised chuck amplitude on call number. We then built a linear model with the slope of the plateau phase as

the response variable, and the number of males calling within 1 m of the focal male, male body condition, water temperature, and overall bout length as explanatory variables. We performed an identical analysis to investigate how the proportion of chuck acoustic energy in the BP auditory range (>1500 Hz) changed throughout bouts. For both models, we cube root transformed the response variable, to satisfy assumptions.

Latency to produce complex and highly complex calls

Because all males produced complex calls during their bouts regardless of bout length or whether they showed the typical rise/plateau pattern of whine amplitude, we included all call bouts in the following analyses (n = 24). To examine the influences on the latency for males to produce calls of differing complexity, we modelled call bouts using Cox proportional hazard (coxph) models using tools in the 'survival' and 'survminer' packages (Therneau and Lumley 2015; Kassambara et al. 2017). For all models, we verified that proportional hazards assumptions were met and verified the goodness of model fit. To investigate the influences on males' latency (number of calls) to produce calls with at least one chuck appended (complex calls), we built a coxph model with the call number of the first complex call as the response variable, and the number of males calling within 1 m of the focal male, male body condition, water temperature, and overall bout length as explanatory variables. Males can produce calls of varying complexity (adding 0 to 7 chucks), so we built a similar model to that mentioned above to model latency to produce a call with more than one chuck appended (a highly complex call).

Call bout length and proportion of complex calls

To investigate the factors influencing the length of a male's calling bout, as well as the proportion of calls of differing complexity within a bout, we built Poisson regression models. All models were checked for over-dispersion using the 'lmtest' package. When we detected over-dispersion, we opted to use negative binomial models.

To model the factors influencing the length of the call bout, we built a negative binomial model with the count of the number of calls for that bout as the response variable, and the number of males calling within 1 m of the focal male, male body condition, water temperature, and overall bout length as explanatory variables. To model the factors influencing the proportion of calls that were complex, we built a Poisson regression with a log link, with the count of complex calls per bout as the response variable, the same explanatory variables mentioned above, and with log(number of calls per bout) included as an offset to model this as a proportion. We built a negative binomial model to model the factors influencing the proportion of complex calls that were highly complex, with the count of highly complex calls per bout as the response variable, the same explanatory variables, and log(count of complex calls per bout) included as an offset.

Comparisons of call characteristics of different call types

As call amplitudes and complexity may be related to one another, we investigated whether different call types and call components differed in amplitude. As our results indicated that both whine amplitude and the likelihood of calls of varying complexity change throughout bouts, we needed to control for these confounding effects in our models. To see whether whine amplitude predicted call type while controlling for placement within the bout, we built a mixed effects logistic regression model with

simple/complex call as a dichotomous response variable, whine amplitude, call number, and their interaction as fixed effects, and male ID as a random effect. We built a similar model with complex/highly complex call as the dichotomous response variable. To assess whether whine and chuck amplitude were correlated within the same call, we built a linear mixed-effects model with chuck amplitude (chucks from complex calls and initial chucks from highly complex calls) as the response variable, the preceding whine amplitude as an explanatory variable, and male ID as a random effect. Finally, to see whether chuck acoustic energy >1500 Hz was correlated with chuck amplitude, we built another LMM with the cube root of proportion of chuck energy >1500 Hz (for chucks from complex calls and initial chucks from highly complex calls) as the response variable, chuck amplitude as an explanatory variable, and male ID as a random effect.

Results

Changes in whine amplitude throughout call bouts

There was moderate evidence that a greater number of calling males within 1 m of the focal male was associated with a steeper increase in whine amplitude during the rise phase (LM β estimate = 0.314 ± 0.126, t = 2.498, p = 0.025) (Figure 3), while there was no evidence for an effect of other explanatory variables ($p > 0.16$ for all other variables). Steeper rise slopes were driven by completion of the rise in fewer calls (LM β estimate = −0.07 ± 0.017, t = −4.155, $p < 0.001$), rather than by a greater relative amplitude gain. There was strong evidence that the slope of the preceding rise (LM β estimate = 0.248 ± 0.063, t = 3.922, p = 0.002) (Figure 4), and standardised male body condition (LM β estimate = 0.137 ± 0.043, t = 3.202, p = 0.006) (Figure 5), had positive effects on the slope of the whine plateau phase (cube root transformed). There was no evidence for an effect of the other variables ($p > 0.13$ for all). Table 2 provides summary statistics for these analyses.

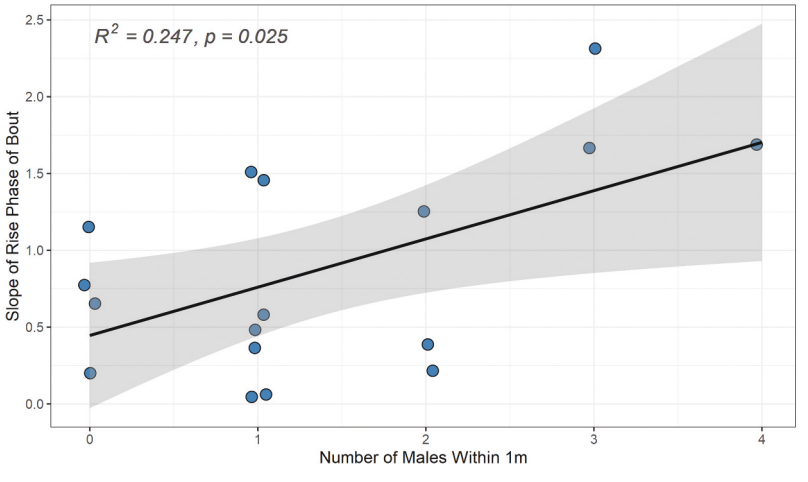

Figure 3. Rise slope vs. number of calling males within 1 m. Shaded area is the 95% CI for the regression line.

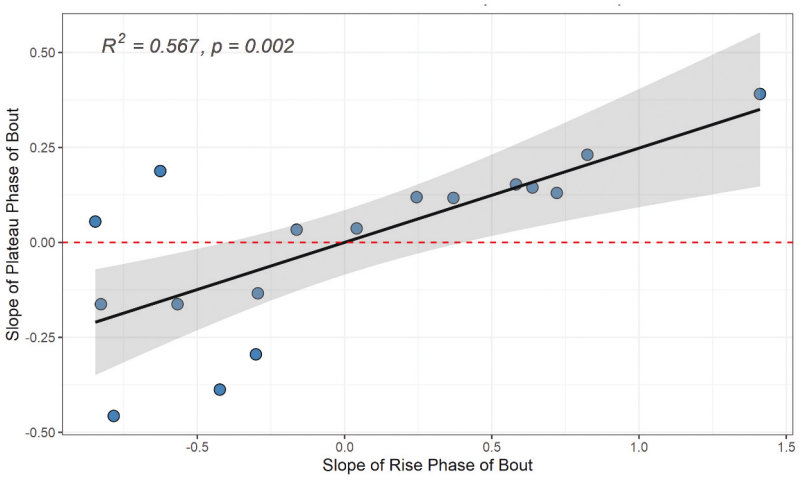

Figure 4. Partial effect plot showing Plateau Slope vs. the preceding Rise slope.

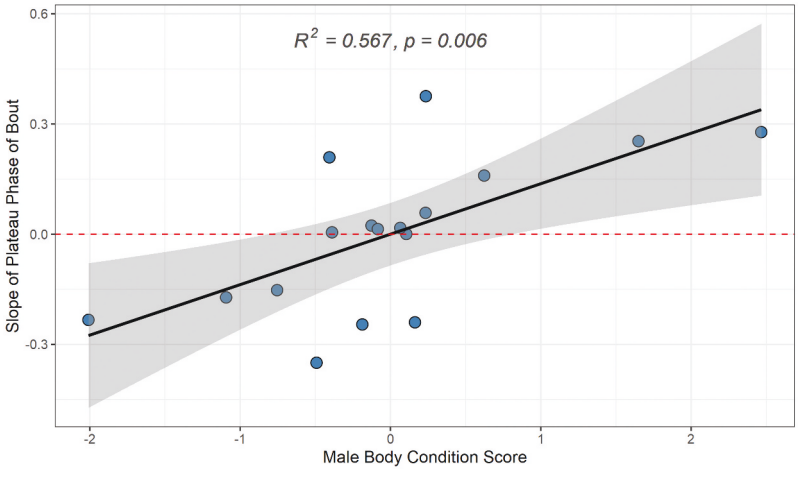

Figure 5. Partial effect plot showing Plateau Slope vs. Male Body Condition Score. Plateau slope was cube root transformed to meet assumptions for analyses, and body condition score was standardised. the R2 shown is the R2 for the full model.

Changes in chuck amplitude and spectral Energy throughout call bouts

There was no evidence for an effect of any explanatory variables on the slope of the plateau phase when examining chuck amplitude or the amount of chuck acoustic energy in the BP range (>1500 Hz) ($p > 0.15$ for all variables).

Latency to produce complex and highly complex calls

All males produced complex calls at some point within their calling bout. There was weak evidence that males with more rivals calling within 1 m produced their first complex calls earlier (in terms of number of calls) during their call bouts (coxph; $df = 2$, $\beta = 0.397$, $\exp(\beta) = 1.488$, $z = 1.813$, $p = 0.07$) and that males calling in warmer water produced their first complex calls later during their call bouts (coxph; $df = 2$, $\beta = -0.507$, $\exp(\beta) = 0.603$, $z = -1.846$, $p = 0.065$), but there was no evidence of an effect of body condition ($p > 0.2$). There was very strong evidence that males with more rivals calling within 1 m produced their first highly complex calls earlier during their call bouts (coxph; $df = 1$, $\beta = 1.028$, $\exp(\beta) = 2.794$, $z = 3.821$, $p < 0.001$), but no evidence for an effect of body condition or water temperature ($p > 0.3$ for both). Seven out of eight males with ≥2 rivals produced highly complex calls at some point in their bout (Figure 6).

Call bout length and proportion of complex calls

There was strong evidence that warmer water temperatures were associated with longer call bouts (GLM β estimate = 0.404 ± 0.148, $z = 2.738$, $p = 0.006$), with no evidence of an effect of other variables ($p > 0.17$ for all). There was strong evidence that the number of males calling within 1 m of the focal male had a positive effect on the proportion of calls in a bout that were complex (GLM β estimate = 0.105 ± 0.024, $z = 3.197$, $p = 0.001$) (Figure 7), and very strong evidence that the number of

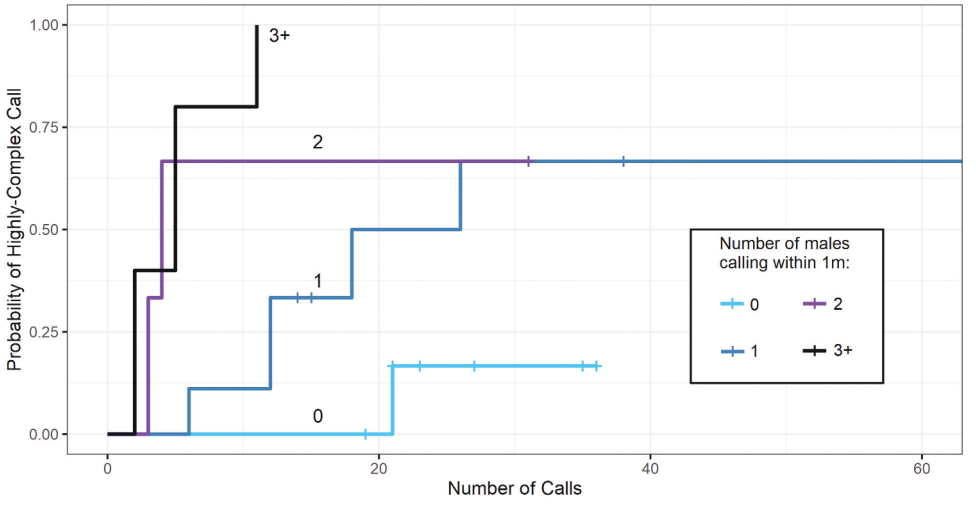

Figure 6. A Kaplan–Meier curve showing that having a social environment with more calling males nearby increases the probability of producing a highly complex call. Due to low numbers, males with 3 and 4 rivals have been binned into a single category (3+) for this visualisation. Not all males produced highly complex calls during their bout, and were considered censored (represented by '+'s). For viewing in black and white, lines in the figure have also been labelled with the strata they represent.

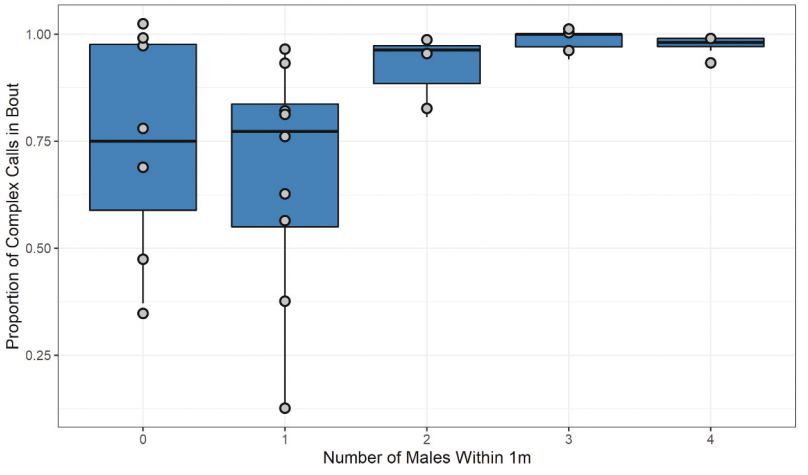

Figure 7. Box plot showing the proportion of complex calls vs. number of calling rivals within 1 m. a small amount of vertical jitter has been added to reveal overlapping points.

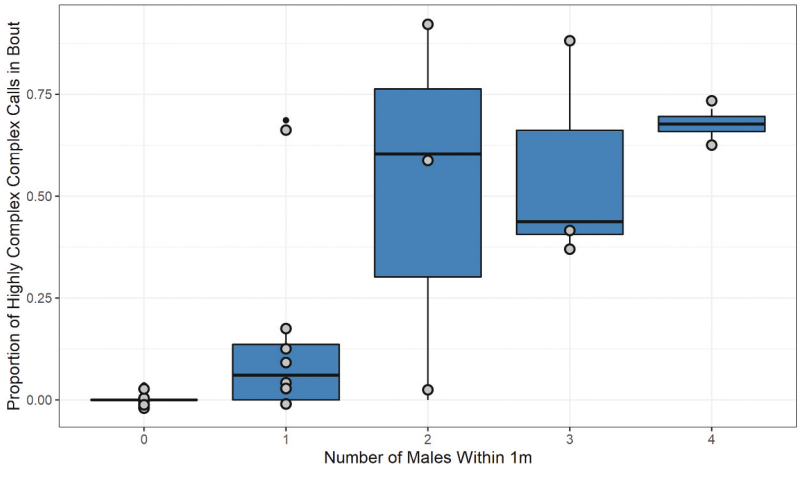

Figure 8. Box plot showing the proportion of complex calls that were highly complex vs. number of calling rivals within 1 m.

males calling within 1 m had a positive effect on the proportion of complex calls that were highly complex (>1 chuck appended) (GLM β estimate = 1.001 ± 0.23, $z = 4.344$, $p < 0.001$) (Figure 8). There was no evidence of an effect of other explanatory variables on the proportion of complex or highly complex calls ($p \geq 0.18$ for all variables in both models).

Comparisons of call characteristics of different call Types

When controlling for placement of the call within the bout (in terms of call number) and the interaction between whine amplitude and placement, there was very strong evidence that higher whine amplitudes had a positive effect on the probability that a call was complex (Logistic Regression β estimate = 4.379 ± 0.427, $z = 10.25$, $p < 0.001$) and the probability that a complex call was highly complex (Logistic Regression β estimate = 2.416 ± 0.52, $z = 4.644$, $p < 0.001$). There was strong evidence that chuck amplitude was associated with the amplitude of the preceding whine (LMM β estimate = 0.79 ± 0.074, $t = 10.621$, $p < 0.001$) and that the proportion of chuck spectral energy above 1500 Hz (cube root transformed) was associated with chuck amplitude (LMM β estimate = 0.253 ± 0.004, $t = 69.97$, $p < 0.001$).

Discussion

To assess the factors influencing and constraining short-term calling strategies in túngara frogs, we investigated the influences of the social environment, male condition, and ambient temperature on the trajectory of call characteristics, and the use of different call types, within and across individual calling bouts. Our results suggest that male calling patterns at the level of bouts are primarily influenced by the local social environment experienced by males. In terms of call amplitude, focal males with more rivals calling within 1 m showed a quicker increase in whine amplitude in the initial rise phase of the calling bout. Males with more calling rivals nearby also had bouts with a greater proportion of complex calls (calls containing chucks), and a greater proportion of these complex calls were highly complex (containing more than one chuck). They also produced these complex and highly complex calls sooner during their bouts.

Physiological constraints were also important in influencing certain features of calling strategies; whine amplitude increased more steeply during the plateau phase of call bouts in males in higher body condition, and call bouts consisted of more calls when males called in warmer water. Finally, there was a link between the characteristics of the two phases of calling bouts, with steeper rise phases leading to steeper plateau phases. Thus, males seem to adjust their calling behaviour during bouts in response to their social environment, but also seem to be constrained by features of their abiotic environment and internal condition.

Influences on call amplitude during the rise phase, and call complexity

Most males showed a steep rise in whine amplitude at the beginning of their call bout. This agrees with previous studies on túngara frogs showing a similar pattern (Pauly et al. 2006; Halfwerk et al. 2016). Why should such a rise phase occur? Calling at higher amplitudes can have costs related to energetics (Ryan 1988) and eavesdropper risk (Tuttle and Ryan 1981; Bernal et al. 2006) but, after settling upon a maximum call amplitude for a bout, a caller who produced all calls at this maximum amplitude could maximise his attractiveness to females (Akre and Ryan 2010b). The occurrence of a rise could be due to the well-studied trade-off callers in this system face: between increasing attractiveness to potential mates and increasing conspicuousness to heterospecific eavesdroppers.

Gradually increasing call amplitude at the beginning of call bouts could be a means for males to probe their local environment for signs of predators within earshot, and to induce any neighbouring males present to start calling, before beginning to call more conspicuously. This explanation can account for the socially mediated flexibility we see in the slope of the rise phase. In contrast to lone males, males calling in aggregations enjoy reduced risks from eavesdropping predators due to dilution effects provided by nearby calling rivals (Hamilton 1971; Ryan et al. 1981), as well as advanced warning of predators due to perceiving predator-detection cues of neighbours (Phelps et al. 2007; Dapper et al. 2011). This diminishment of risk should scale with number of rivals calling nearby and so could promote the quicker rise to near-maximum call amplitude (steeper rise slopes) we saw when males called in denser choruses. Such a socially mediated reduction in caution parallels findings that males calling in larger choruses resume calling more quickly after being presented with a predation stimulus (Jennions and Backwell 1992).

In addition to safety from dilution effects potentially underpinning steeper rise slopes in males with more nearby rivals, it is also likely that there is an effect of local competition. Signalling in aggregations means that a male's attractiveness relative to nearby rivals may become more important than his absolute attractiveness and, especially in acoustically signalling species, males will have to signal at high amplitude to be heard above the din of the crowd (Ryan and Cummings 2005; Love and Bee 2010; Halfwerk et al. 2016). In response to the calls of rivals or the noise of nearby choruses, túngara frog males flexibly adjust many aspects of their calling behaviour in a way that increases their attractiveness to females (Ryan 1985; Green 1990; Greenfield and Rand 2000; Bernal et al. 2007; Halfwerk et al. 2014). This includes increasing amplitude in response to chorus noise (Akre and Ryan 2010b; Halfwerk et al. 2016). Thus, increasing amplitude more quickly when in denser choruses could provide a competitive edge; quickly reaching high whine amplitude could increase the chances that a larger proportion of a male's calls for a given bout will be conspicuous to nearby females over the noise of the chorus.

These two potential explanations for the observed socially mediated increase in rise slope, being able to quickly maximise call amplitude due to enjoying dilution benefits from nearby males vs. needing to quickly maximise call attractiveness to compete with nearby rivals, are not mutually exclusive and could operate synergistically. Similarly, mechanical constraints on calling could be operating in parallel with these effects of the social environment to contribute to the character of the rise phase. A 'warming up' phenomenon in which signalling muscles improve in efficiency with use after a period of rest has been demonstrated in other vertebrates (Dinh et al. 2020; Déaux et al. 2020), but this effect typically operates over timescales of hours, rather than the seconds or minutes that túngara frog call bouts take to unfold (Bernal et al. 2009). It is also possible that males may take several calls to establish an efficient rhythmic interaction between their lungs and vocal sac during the beginning of bouts (Pauly et al. 2006), contributing to the rise phase. However, neither of these possible mechanisms explain why the slope of the rise is modulated by a calling male's social environment, as revealed by our analysis. Thus, our results suggest that the social environment is the primary driver of amplitude patterns at the start of call bouts, but mechanical constraints cannot be ruled out.

Similar arguments regarding the effects of the social environment on calling behaviour likely also underpin our findings that a greater number of rivals calling nearby induces males to produce a higher proportion of complex and highly complex calls, and for these

to occur sooner during their bouts. Calls of greater complexity are more attractive to females (Akre and Ryan 2010a, 2010b; Ryan et al. 2019) and eavesdroppers (Tuttle and Ryan 1981; Bernal et al. 2006). Thus, a similarly synergistic effect of socially mediated reduction of risks via dilution effects, and increased competition, could also explain these patterns.

Influences on call amplitude during the plateau phase

Calling is energetically costly in túngara frogs, and these energetic costs are primarily influenced by whine rate, with no detectable influence of chuck production (Bucher et al. 1982). This fact may underpin our finding that males with higher body condition maintained more positive slopes for whine amplitude during the plateau phase of their bouts. Plateau slopes were predominantly positive for males analysed (12 out of 17 were positive), however, these positive slopes were not much greater than zero (mean ± sd: .01 ± .03). This shows that most of the amplitude gain during a bout is accomplished during the initial rise, which quickly gets males to near-peak amplitude. Vocalising at higher amplitudes has been shown to be more energetically costly for many species (Ryan 1988; Russell et al. 1998; Oberweger and Goller 2001). For example, it is estimated that a 3 dB increase in call amplitude requires a doubling of energetic costs in spring peepers (Parris 2002). In túngara frog bouts, the plateau phase makes up the majority of the bout and begins with a call already at near-maximum amplitude. Thus, maintenance or increase in amplitude during the plateau may require a high level of endurance and may only be attainable by males in the best physical condition. From our field recordings, we were only able to extract relative amplitudes for males' calls within their bouts, rather than absolute amplitudes, so we cannot tell the range of absolute amplitude modulation occurring over plateau phases.

Although whine plateau slopes were influenced by body condition, rise slopes were not. Conversely, rise slopes were influenced by the social environment, while plateau slopes were not. Thus, it appears the two well-known trade-offs facing calling túngara frogs, eavesdropping risk and energetic costs, may be influencing call amplitude patterns differently during the rise and plateau phases of call bouts. Despite these contrasting influences, rise slope and plateau slope were correlated, with steeper rise slopes preceding steeper plateau slopes. It is possible that some difficult-to-measure trait such as a male's motivation could link these patterns; a highly motivated male may be less cautious when increasing amplitude during the rise, while also being willing to expend more energy during the plateau to sustain or increase amplitude. Our current dataset did not allow us to explore motivation or the factors underpinning it (e.g. female presence or behaviour: Kime et al. 2007; Akre and Ryan 2011; or male hormonal state) as possible influences on calling behaviour.

Intriguingly, in contrast to whine plateau slopes, no variables influenced the plateau slopes of chuck amplitude or the chuck sound energy within the frequency range most stimulatory to females. Complex calls are more attractive to females when they contain more energy in harmonic frequencies above 1500 Hz (Wilczynski et al. 1995), so we had expected that chuck amplitude and the amount of energy in this harmonic range might increase more steeply during bouts when males called in more competitive situations, or when they were in better condition. Whines are caused by airflow vibrating the vocal

cords, and changes in the frequency and amplitude of the whine seem to be determined by the rate of airflow (Dudley and Rand 1991). Conversely, chucks are caused by the vibration of a fibrous mass attached to the vocal cords, which does not seem to require active neural control; chucks likely arise passively due to airflow changes (Kime et al. 2019). Thus, there may be less scope for directly flexibly altering chuck amplitude relative to whine amplitude. However, we did find that aspects of whines and chucks were linked. In addition to higher amplitude whines being associated with complex rather than simple calls, and with calls of higher complexity (i.e. more chucks), we found that whine amplitude was positively correlated with chuck amplitude within complex calls, and that the proportion of chuck spectral energy above 1500 Hz was correlated with chuck amplitude. However, the relationship between whine and chuck amplitude was not 1:1 ($\beta = 0.79$). Thus, the factors influencing whine plateau slope may not influence plateau slopes for chuck characteristics in as direct a manner as they do for whines.

Suggestions for future studies of similar phenomena

Future studies addressing similar questions in similar or disparate taxa could expand our understanding of dynamic calling strategies across bouts by incorporating additional variables not accounted for in our dataset. Acoustically signalling insects and anurans are often highly responsive to the calling strategies of males around them (Gerhardt and Huber 2002; Greenfield 2015). As such, including information regarding whether, and how, changes in calling patterns of neighbours throughout bouts influence amplitude modulation patterns of focal males could give us a better idea of how feedback between rivals influences calling patterns. Further, experimental manipulations investigating how repeatable male within-bout calling patterns are, and how much they vary across a range of social, energetic, and environmental conditions, would allow more precise tests of the influence that these conditions have on male calling patterns throughout signalling bouts. Finally, our study modelled the trajectory of call characteristics across bouts by looking at calls sequentially. As call rates exhibit variation and flexibility in many acoustically signalling species and can have major effects on the energetics of calling (Prestwich 1994), future studies would benefit from including information regarding inter-call intervals.

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Data availability statement

The data used for this study are available at DOI: 10.6084/m9.figshare.19395023.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical statement

All animals used as subjects in this study were handled according to ASIH guidelines (Accessible at https://asih.org/animal-care-guidelines). All research was licenced and approved by the University of Texas at Austin Institutional Animal Care and Use Committee (IACUC 6,041,701) and the government of Panama (ANAM permit SE/A-37-05).

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