

Floating frogs sound larger: environmental constraints on signal production drives call frequency changes

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Abstract

In animal communication, receivers benefit from signals providing reliable information on signalers' traits of interest. Individuals involved in conflicts, such as competition between rivals, should pay particular attention to cues that are “unfakeable” by the senders due to the intrinsic properties of the production process. In bioacoustics, the best-known example of such “index signals” is the relationship between a sender's body size and the dominant frequency of their vocalizations. Dominant frequency may, however, not only depend on an animal's morphology but also on the interaction between the sound production system and its immediate environment. Here, we experimentally altered the environment surrounding calling frogs and assessed its impact on the signal produced. Our results show that frogs that are floating are able to inflate their vocal sacs fully and that this change in inflation level is correlated with a decrease of call dominant frequency.

Keywords Acoustics · Sexual signaling · Honest communication · Sound production · Morphological constraints

Introduction

Animal communication signals that are “unfakeable” due to morphological or physiological constraints imposed on their production process (also known as “index signals” (Fitch and Hauser 2003; Smith and Harper 2004; Searcy and Nowicki 2010) ensure reliable (“honest”) communication. Characteristics of these signals thus provide reliable

information on the signaler's particular morphological or physiological traits (Garcia et al. 2017). The most widely used example of “honest” communication is the negative frequency-body size relationship that is often reported in bioacoustics (Fitch and Hauser 2003; Dunn et al. 2015; Garcia et al. 2017).

Vocalization frequency is largely influenced by the size of the sound-producing organs, even in species able to modulate the frequency of their vocalizations, and individuals that differ in size tend to call or sing at different frequencies, providing receivers with a reliable cue about the size of their mate or opponent (Ryan and Brenowitz 1985; Gerhardt and Huber 2002; Fitch and Hauser 2003; Bradbury and Vehrencamp 2011). However, certain characteristics of display sites can also directly influence signal production and transmission. Signalers often exploit the resonance properties of the structures from where they call (Cui et al. 2012; Mhatre et al. 2016). For example, the sounds of signalers that call or chirp from burrows in the ground or hollow tree cavities are amplified (Cui et al. 2012; Márquez et al. 2005; Muñoz and Penna 2016).

Call site properties may influence signal production also more directly through the interaction with signalers' morphology. A recent study on túngara frogs (*Engystomops pustulosus*) revealed, for example, that when the water level

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is experimentally lowered, males are less able to inflate their lungs, and therefore, there is less air available to push from their lungs through their larynx into their vocal sac (Halfwerk et al. 2017). As a result, males in shallower water called at lower amplitudes and with less complexity, which in turn influenced their attractiveness to females. Importantly, the impact of water level treatment appeared to be size-dependent (Smit et al. 2019). Larger males were more influenced by water level treatment compared with smaller males. In fact, a binary choice experiment, playing calls of a large versus small male from opposite directions, revealed that females preferred the calls of the larger male when these were recorded in deep water and calls of the smaller males when recorded from shallow water (Smit et al. 2019).

Here, we reexamine the videos of the experiments described above (Halfwerk et al. 2017) with the specific aim to link call site-induced signaling constraints to variation in dominant frequency. More specifically, we selected the trials in which males were fully floating, allowing for maximum signaling performance, and the trials in which the same males were clearly non-floating. We compared floating and non-floating trials to test whether males were constrained in vocal sac inflation when non-floating and whether this was related to variation in dominant frequencies.

Material and methods

The study was carried out with male túngara frogs, *Engystomops* (= *Physalaemus*) *pustulosus* collected in August 2014 from Soberanía National Park, near Gamboa, Republic of Panama. All individuals were released back to the site from which they were collected on the same night. All experiments with frogs were licensed and approved by STRI (IACUC permit: 2014-0805-2017) and the Autoridad Nacional del Ambiente de Panama (SE/A-82-14).

Male frogs were recorded individually in a semi-anechoic chamber under IR-lighting. At the start of the experiment, a male was placed in a small cage consisting of a ring of evenly spaced nylon monofilament (diameter of 0.05 mm fishing line every 0.5 cm, see Supplementary Video S1). The cage was placed in a pool (diameter of 50 cm) containing a tube that allowed the experimenter to either add (using a funnel) or subtract (using a 50-ml syringe) water, in order to manipulate water depth at the position of the frog. Males were stimulated with a low-amplitude chorus recording until they were readily calling for 1 min. We assessed male calling behavior at four different water depths (0.25, 0.5, 1.0, and 2.0 cm). Each trial lasted for 1 min, followed by a 2-min break during which the water depth was altered (starting 1 min before the next trial).

We recorded male calling behavior with a camera that was mounted on top of the cage (mini 1/4" CCTV camera; 2.8 mm lens; connected to a desktop PC). We recorded male calls with

a microphone setup (G.R.A.S. 40 BF microphone amplified by 20 dB by G.R.A.S. 26 AC amplifier connected to an Avisoft 116Hm Ultrasound gate, G.R.A.S. Sound & Vibration A/S, Holte, Denmark) onto a desktop PC, using a sampling rate of 50 kHz. The microphone was placed at a 45° angle and at a distance of 50 cm from the frog. The microphone was calibrated prior to each experiment using a tone generator (G.R.A.S. 42 AB, 114 dB at 1 kHz).

We analyzed the videos of male calling in our setup and scored whether males were completely floating with all four limbs suspended, or not. We selected for each trial three video stills from the beginning, middle, and end of a call bout. For each call, we selected the video with the maximum inflation of the lungs as well as the maximum inflation of the vocal sac. We used the program ImageJ (Schneider et al. 2012) to measure from the width of the vocal sac at maximum inflation (in mm). Sound recordings were analyzed in SASLab Pro (Avisoft Bioacoustics, Berlin, Germany; 44.1 kHz sample rate, window size of 512 samples, 90% overlap, resulting in 50 Hz spectral resolution). We selected three calls from the start, middle, and end of a trial and measured the dominant frequency of the whine of each call corresponding to the video stills used in the video analyses. For each male, lung and vocal sac size and call frequency values obtained from these three calls were averaged per trial.

Overall, 531 calls of 20 males were analyzed. We assessed whether floating affected vocal sac inflation and dominant frequency during calling and tested the correlation between vocal sac inflation and call dominant frequency in R (v.3.2.2; R Core Team 2020). We constructed linear mixed models using the package *lme4* with a Gaussian distribution with identity link function and male ID as random intercept. Significance of fixed effect (floating or non-floating, vocal sac inflation corrected for body size, and the ratio between vocal sac and lung inflation) was assessed using the likelihood ratio test. All models were tested for normality, overdispersion, and heteroscedasticity by examining the quantile-quantile plot (QQ plot) and conducting a Shapiro test on the residuals. See Halfwerk et al. (2017) for a more detailed description of the setup, experimental procedures, and analyses.

Results

All twenty males we tested floated in the trials in which the water level was raised above 2 cm, whereas none of these males were able to float when we lowered the water level to below 0.25 cm. During intermediate water level (0.5 and 1 cm) trials, males would often have their front or hind legs touching the base of the setup. When the water level was raised, we could clearly see males actively pumping air into their lungs until they had enough buoyancy to allow them to float freely on the water surface.

We compared trials in which males were fully floating with trials in which they were not floating (with either their front or hind legs touching the base). Males were able to inflate their vocal sacs and their lungs to a greater extent during trials in which they could float (GLMM; $n = 20$ males; $\beta = 2.42$; $SE = 0.3$; $\chi^2 = 60.00$; $d.f. = 1$; $P < 0.001$; Fig. 1). The increase in vocal sac inflation was accompanied by a decrease in dominant frequency (from 995 ± 307 Hz to 797 ± 63 Hz; Fig. 1.) as we found floating male calls had a lower dominant frequency compared with non-floating males ($\beta = -217.23$; $SE = 27.24$; $\chi^2 = 59.91$; $d.f. = 1$; $P < 0.001$). Vocal sac inflation (corrected for body size) was significantly correlated with call dominant frequency ($\beta = -188.55$; $SE = 96.15$; $\chi^2 = 3.91$; $d.f. = 1$; $P = 0.048$). Additionally, the difference in inflation between the vocal sac and the lungs was significantly smaller in floating males ($\beta = 0.04$; $SE = 0.01$; $\chi^2 = 26.20$; $d.f. = 1$; $P < 0.001$) and inversely correlated with call frequency ($\beta = -345.34$; $SE = 166.12$; $\chi^2 = 4.32$; $d.f. = 1$; $P = 0.038$).

Discussion

The dominant frequency of vocalizations is generally assumed to be a reliable indicator of a signaler’s body size (Ryan 1980, 1985; Gillooly and Ophir 2010), and the negative association between these variables is widespread in frogs (Tonini et al.

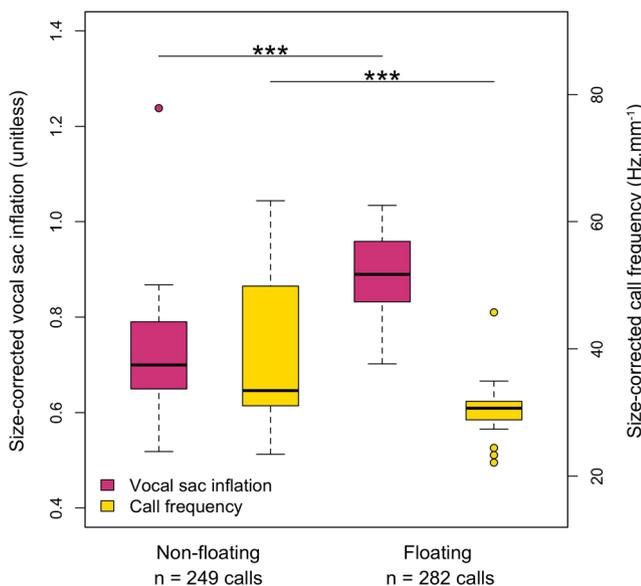


Fig. 1 Floating affects vocal sac inflation and call dominant frequency in túngara frogs (*Engystomops pustulosus*). Size-corrected values of vocal sac inflation (pink; unitless) and call dominant frequency (yellow; Hz mm^{-1}) are shown ($N = 20$ males). Thick horizontal lines represent the medians; box lower and higher bounds represent the first and third quartile, respectively; and whiskers represent 1.5*interquartile range on each bound. Outliers are represented as full circles. Asterisks represent P values of “floating” as fixed effect in mixed model analysis (models including body size and floating as fixed effect and male ID as random effect; *** $P < 0.001$)

2020). In this study, we show that the environment can directly influence signal production, thereby affecting dominant frequency, and hence alter signal reliability in reference to body size. We found that frogs vocalizing while floating on the water produced calls with lower frequencies compared with trials during which they did not float. Furthermore, call frequency was correlated with the inflation of the vocal sac.

Mechanistically, this could be explained by a decrease in differential pressure across the larynx. Indeed, the inflation level of structures such as the vocal sac or of the lungs is directly correlated to their internal pressure (Merritt and Weinhaus 1978). Therefore, an increased pressure in the vocal sac, and thus in the buccal cavity (Dudley and Rand 1991), would lower the differential pressure across the larynx, resulting in a lower call frequency (Dudley and Rand 1991; Pauly et al. 2006; Gridi-Papp 2014). In túngara frogs, the amount of airflow going from the lungs into the vocal sac is proportional to the size of the vocal sac (Dudley and Rand 1991). Increasing the internal pressure of the vocal sac, relative to the pressure in the lungs, could thus be the strategy that males use to decrease their call frequency while maximizing its amplitude (Smit et al. 2019).

Environment-based modulation of call frequency may have important consequences on individual fitness. Multiple studies have shown that lower frequency calls are preferred by gravid females in frogs (Davies and Halliday 1978; Arak 1983) in general and specifically in túngara frogs (Ryan 1980, 1983; Wilczynski et al. 1995; Bosch et al. 2000), for which a difference of 231 Hz is sufficient to elicit a preference (Bosch et al. 2000). Individual males can thus increase the reach and attractiveness of their calls by signaling from deeper puddles where they are able to float on the surface and inflate their lungs and vocal sacs to the fullest. In natural conditions, however, male túngara frogs are found vocalizing in both conditions (while floating and not floating), perhaps due to additional balancing selection pressures such as predation or reflecting the availability of ideal calling sites.

Our results suggest that the properties of anuran call sites can have a strong influence on sexual selection. For example, in shallow water, females may choose, and rivals may respond stronger to smaller floating males over larger, non-floating ones as sound production is only constrained in larger males (Smit et al. 2019). Display site properties may, therefore, drive signal evolution through biomechanical constraints on signal production.

Authors’ contributions W.H. conceived and designed the study and coordinated the data collection and analysis. S.G. analyzed the data and wrote the paper. All authors contributed to the interpretation of the data and subsequent manuscript writing and revisions. All authors agreed to be held accountable for the content therein and approve the final version.

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Data availability Data is available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.jq2bvq86b> and <https://datadryad.org/stash/share/PQkNyeZKg3s0oU21Ssy-x0jeB6d3fBXdapngzpVwTw>.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval All experiments with frogs were licensed and approved by STRI (IACUC permit: 2014-0805-2017) and the Autoridad Nacional del Ambiente de Panama (SE/A-82-14).

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability R code available as supplementary material

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