



The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback

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Acoustic signals produced by most anurans are accompanied by inflation of a conspicuous vocal sac. We presented female túngara frogs, *Physalaemus pustulosus*, with acoustic playback of the male advertisement call, synchronized with a video playback of vocal sac inflation. Females significantly preferred a stimulus combination including vocal sac inflation over an identical set of stimuli with the vocal sac inflation removed. Neither a moving rectangle bearing the gross contrast and spatiotemporal properties of the vocal sac inflation sequence, nor the image of a noncalling male significantly enhanced the attractiveness of the acoustic stimulus. Both the form and spatiotemporal properties of the vocal sac thus appear to be salient to females. The results indicate that the vocal sac can serve as a visual cue, which may account for the conspicuous pigmentation found on the vocal sacs of males in many species. Gular inflation in synchrony with a call may function to facilitate female localization of individual males in an aggregation.

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Inflation of a vocal sac characterizes advertisement calling behaviour in nearly all male frogs and toads. Several acoustic and nonacoustic functions have been proposed for the vocal sac (Duellman & Trueb 1986; Rand & Dudley 1993). The vocal sac is not an acoustic cavity resonator, although it may serve to direct the call towards the receiver, or as a reservoir of mechanical energy during calling (Rand & Dudley 1993). The vocal sac is pigmented and conspicuous in many species (Greenberg 1942; Duellman & Trueb 1986; Hödl & Amézquita 2001), and in some species can change colour during the course of courtship (Wells 1980), suggesting that it may play a role in visual communication. In one diurnal species, the dendrobatid frog *Colostethus palmatus*, females display a normal behavioural response to a rubber model of a male inflating his throat, but fail to display a complete behavioural sequence to the model if inflation is not simulated (Lüddecke 1999). Males in many bird species also feature a prominent vocal sac as a component of courtship display (Dantzker et al. 1999). Thus, in addition

to any putative function in acoustic signalling, the anuran vocal sac may function as a visual cue.

A visual stimulus produced in concert with an acoustic signal may serve to increase the likelihood that receivers will attend to a male advertisement call. Signals with components in more than one modality can facilitate detection and recognition, as well as aid in discrimination among multiple stimuli (Rowe 1999). Combinations of acoustic and visual cues can also enhance spatial localization (McDonald et al. 2000).

The túngara frog, *Physalaemus pustulosus*, is a small, common species of frog found throughout much of the lowland tropics of Central America and northern South America. Male advertisement calls are accompanied by conspicuous gular sac inflation. Túngara frogs are nocturnal; males call from shallow, stagnant pools of water in groups of varying size. For example, mean group size was 27 (average nearest-neighbour distance 0.22 m) in 1978 in a 1 × 2 m artificial pool on Barro Colorado Island, Panama (Ryan 1985). Females attend to interindividual variation in male call characteristics, preferring more complex and lower-frequency calls (Ryan 1985). On clear nights, the pulsing of a vocal sac is readily detectable to a human observer (Ryan 1985), suggesting that the vocal sac might serve to facilitate a difficult acoustic task, identifying and localizing a preferred male out of a complex acoustic background.

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Female and male *P. pustulosus* display optomotor responses to a rotating black-and-white striped drum, both under low-light conditions in the laboratory and outdoors under a range of natural, nocturnal conditions. *Physalaemus pustulosus* also display optomotor responses under these conditions when the colours of the drum are filtered to mimic the reflectance spectrum of the male's inflated vocal sac (M. Cummings, X. Bernal, C. Jennings, A. S. Rand & M. J. Ryan, unpublished data). These results indicate that the visual system of *P. pustulosus* can detect the vocal sac under the range of conditions in which males call in nature.

Females respond readily to simultaneous playback of synthetic acoustic stimuli in an acoustic chamber (Ryan & Rand 1995). By adding playback of computer-generated video animations to this paradigm, we examined the role of the vocal sac in determining female response to the male's advertisement display. We tested whether vocal sac inflation accompanying a call increased female preference for a male. We also evaluated the specificity of vocal sac inflation in eliciting female response by presenting females with an abstract stimulus bearing only the gross temporal and spatial properties of the vocal sac display.

MATERIALS AND METHODS

We synchronously audio- and video-recorded a male *P. pustulosus* calling in the field at Gamboa, Panama, and constructed a 2-s base stimulus (inflated, Fig. 1a, b). The audio track consisted of 1.57 s of silence and the 0.43-s whine-chuck call (Fig. 1d). We used the SIGNAL sound analysis program and hardware (Engineering Design, Belmont, Massachusetts, U.S.A.) to digitize the audio track and filter low-frequency noise. Video was digitized using a Targa 1000 video board (Pinnacle Systems) on a Dell Optiplex computer. We then used Adobe Premiere to synchronize the audio and video tracks, and create two novel stimuli. For 'not inflated' (Fig. 1a), the portion of the video sequence containing vocal sac inflation was excised and replaced with the preceding sequence of the male sitting still but in reverse order to avoid loss of continuity; the male effectively appeared motionless, like a silent male in nature rather than a frozen frame. 'Rectangle' (Fig. 1c) was a synthetic animation (created using 3D Studio Max, Discreet) of a moving rectangle with the same mean colour value, and amplitude and frequency of horizontal motion as the vocal sac of the exemplar male, and with the same total area as the vocal sac at its maximum extent. Stimuli were scaled so that the male would be life-sized during playback. An additional stimulus, blank, consisted of the audio track with no video signal, so that the screen displayed a black background comparable to the calling background in nature.

We conducted three pairs of experiments. The first, inflated versus not inflated, tested whether vocal sac inflation enhances a male's attractiveness. The second, not inflated versus blank, assessed whether the visual cue of a noncalling male increases the probability of phonotaxis. The third, rectangle versus blank, tested whether the

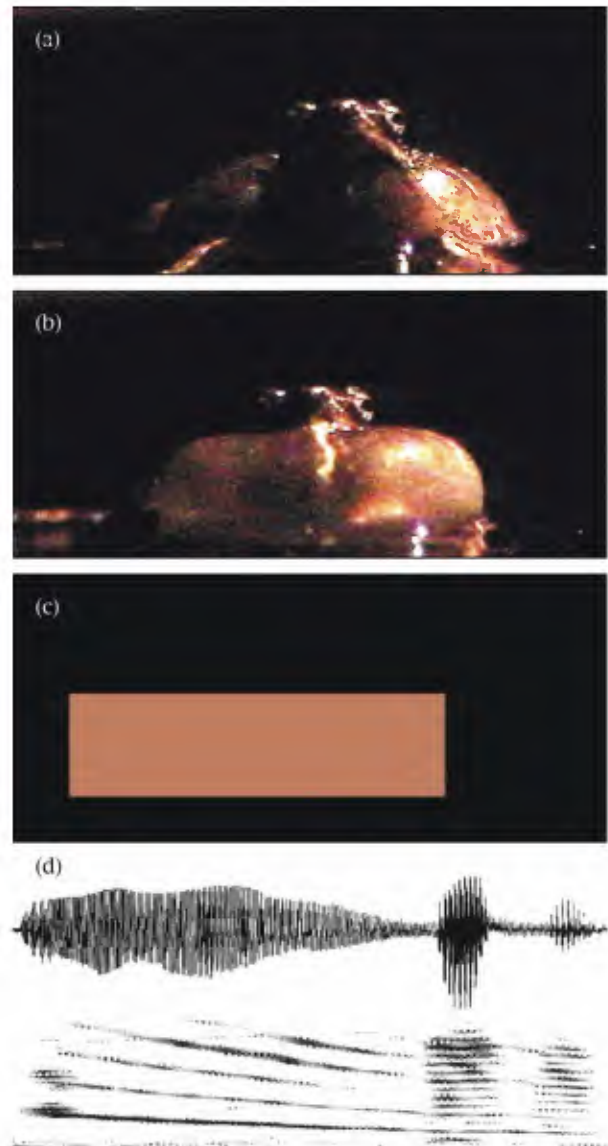


Figure 1. Experimental stimuli. The base stimulus, 'inflated', consisted of a normal inflation sequence (uninflated, a; maximum inflation, b). The 'not inflated' stimulus had the maximum inflation portion of the sequence excised, and the 'rectangle' stimulus (c) bore the same gross spatiotemporal properties as the normal inflation sequence. Stimuli were synchronized to the advertisement call (power and frequency spectra, d) of the exemplar male.

motion and gross spatial properties of vocal sac inflation are sufficient to elicit a response.

We tested females in a standard phonotaxis chamber at the Smithsonian Tropical Research Institute, Gamboa, Panama (Ryan & Rand 1995). Mated pairs were collected at breeding sites near the laboratory. Females were separated from males and were tested between 2000 and 0100 hours on the same night. They were returned to the breeding site after testing. Females were confined to a 50-cm-wide corridor constructed with 10-cm foam walls. At each end was a loudspeaker with a 9-cm (diagonal) LCD monitor (Citizen Electronics, Tokyo, Japan) directly below it. The chamber was illuminated with infrared light, to

which túngara frogs are spectrally insensitive (X. Bernal, unpublished data). Frogs were observed via a Fieldcam television system with a 36 IR LED annular array (Fuhrman Diversified, Seabrook, Texas, U.S.A.). Calls were played back at a sound pressure level of 70 dB (re. 20 μ Pa), near auditory threshold (Marsh et al. 2000), to increase the likelihood of females attending to both modalities (Rowe 1999; McDonald et al. 2000). Females were allowed to choose between pairs of alternating stimuli (Ryan & Rand 1995).

We tested each female twice on the same stimulus pair, alternating the side of presentation of each stimulus; only data from females that consistently went to the same stimulus were retained for analysis. We were thus comparing explicitly the females that chose one stimulus versus the other, independent of location. We conducted a total of 82 trials for inflated versus not inflated, 71 for not inflated versus blank and 107 for rectangle versus blank. We computed the exact binomial probability to evaluate the a priori hypotheses that a vocal sac increased male attractiveness and that the not inflated and moving rectangle stimuli were more attractive than a blank control. Since we had clear predictions of the expected outcome, based on previous studies suggesting a visual role for the vocal sac (Wells 1980; Lüddecke 1999), we used one-tailed tests for all comparisons (Sokal & Rohlf 1995).

RESULTS

Females significantly preferred calls accompanied by vocal sac inflation (Fig. 1a, b) over those with no inflation (Fig. 1a; 14 females responded to inflated versus 6 to not inflated; one-tailed exact binomial test: $P < 0.05$) and failed to prefer the not inflated stimulus (Fig. 1a) to the blank control (9 females responded to not inflated versus 13 to blank, NS). The rectangle stimulus (Fig. 1c) failed to increase call attractiveness (10 females responded to rectangle versus 9 to not inflated, NS).

DISCUSSION

The results indicate that females' attraction to an acoustic cue, the advertisement call, is modulated by the presence of a specific visual cue, the vocal sac. Prey localization in adult anurans is visually guided, and frogs show motor responses to broad ranges of moving stimuli (Ewert 1980). The response of females in the context of mating, however, appears to be highly specific. Females failed to show an increased response to a moving rectangle bearing the same frequency and amplitude of apparent motion, mean contrast properties, and total area as the vocal sac. A visual stimulus of a male without vocal sac inflation also failed to increase female response. The full vocal sac sequence, however, elicited a more than two-fold increase in preference over the uninflated vocal sac.

The duty cycle of vocal sac inflation is closely tied to call duration and onset (Gans 1973), and temporal visual cues may be redundant with acoustic cues in localizing and evaluating a call (Rowe 1999). Conspicuous vocal sac inflation may thus facilitate localization of males in a chorus, where females may have difficulty pinpointing

males using only acoustic cues. Male anurans call against an acoustic background of conspecific and heterospecific calls and ambient noise. The repetitive pulsing of a vocal sac contrasting against a dark background may provide receivers with a readily detectable source of information in addition to the call. Further studies should address how these two modalities could interact over their respective dynamic ranges.

The specificity of female response to vocal sacs, and the great diversity of form, colour and pattern in these structures (Duellman & Trueb 1986; Hödl & Amézquita 2001), suggest that these cues may play a role in conspecific mate recognition and mate preference. *Physalaemus pustulosus*, like most anurans (Dickerson 1906), call at night, and females respond to vocal sac inflation even when there is minimal incident light. Females in diurnal species should be even more likely to use the vocal sac in mate selection, and may attend to variation in the spatiotemporal and spectral characteristics of male vocal sacs within conspecifics. In dendrobatids, a diurnal family including the brightly coloured poison-arrow frogs, females select males on the basis of visual traits (Summers et al. 1999). Vocal sac inflation appears to be necessary to elicit a complete mating response from females in one diurnal, cryptically coloured dendrobatid species, *C. palmatus* (Lüddecke 1999).

Experimental studies have ruled out at least one important acoustic function for the vocal sac, that of a cavity resonator (Rand & Dudley 1993). The vocal sac's function as a visual cue is not incompatible with any of several other acoustic and nonacoustic functions that have been proposed (Rand & Dudley 1993). This study shows that the vocal sac can serve as a visual cue and is a salient part of the advertisement signal in at least some circumstances. The vocal sac's visual role may partially account for the diversity of vocal sac colours and morphologies found in anurans.

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