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Multimodal Signal Testing Reveals Gestural Tapping Behavior in Spotted Reed Frogs

Iris Starnberger², Philipp Martin Maier, Walter Hödl, and Doris Preininger¹

Department of Integrative Zoology, University of Vienna, Althanstraße 14, 1090 Wien, Austria

Abstract

In the majority of anuran species, acoustic signals are the dominant mode of inter- and intrasexual communication. Male calls are always accompanied by the movement of a more or less conspicuous vocal sac—a potential visual cue. Reed frogs possess a striking vocal sac with a colorful patch of gland tissue clearly visible once the vocal sac is inflated during acoustic signaling. To investigate the visual signal function of vocal sac and gular gland, we presented male Spotted Reed Frogs (*Hyperolius puncticulatus*) with unimodal and multimodal signal playbacks of conspecific rivals in their natural habitat and recorded their behavioral responses. We found no difference in receiver response to unimodal advertisement call stimuli and to multimodal stimulus presentations of calls combined with visual signals of an artificial vocal sac with or without a gular patch, moving synchronously or asynchronously with the call playback. The inflations of a vocal sac with a colorful gular patch did not alter receiver response and neither increase nor decrease signal salience during male–male communication. Interestingly, males frequently displayed a novel hind and front foot-tapping behavior in response to all playbacks. Comparison of male responses to advertisement and aggressive call playbacks showed that Spotted Reed Frogs approached the sound source less during aggressive call presentations. Tapping behavior was not influenced by either call playback. We suggest that the gestural tapping behavior might act as vibrational signal and discuss its potential signal function in male contests and courtship for females.

Keywords

Communication; *Hyperolius*; Limb movement; Old World anurans; Tanzania; Visual display

Amphibian species are excellent model systems to test unimodal and multimodal communication (Starnberger et al. 2014a). Many species produce acoustic and visual signals during intraspecific communication (reviewed in Hödl and Amézquita 2001). The influence of single and combined signal components can be tested with relatively simple playback experiments in the natural environment of the species (Narins et al. 2003; Sztatecsny et al. 2012; Preininger et al. 2013a).

²Correspondence: iris.starnberger@univie.ac.at.

¹Present Address: Vienna Zoo, Maxingstraße 13b, 1130 Wien, Austria

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Acoustic signals, the dominant signal component in most anurans, provide information about body size and mass (Ryan 1988; Gingras et al. 2013), species identity (Blair 1958; Hödl 1977) and mediate sexual behavior and reproduction (Taylor et al. 2011; but see Gerhardt and Huber 2002; Narins et al. 2007). Additional or alternative visual signals facilitate detection, recognition, and discrimination in dense aggregations and noisy habitats in diurnal species (de Luna et al. 2010; Sztatecsny et al. 2012; Preininger et al. 2013a; Starnberger et al. 2014a,b). The anuran vocal sac inevitably moves as a consequence of sound production and inflates synchronously during calling. In several frog species visual cues of the inflating vocal sac make the acoustic signal more attractive to female receivers (Rosenthal et al. 2004; Cummings et al. 2008; Taylor et al. 2008; Gomez et al. 2011; Taylor and Ryan 2013) or elicit agonistic behavior and facilitate detection of opponent males (Hödl and Amézquita 2001; Narins et al. 2003; Sztatecsny et al. 2012; Preininger et al. 2013a; Starnberger et al. 2014a). In a species of Dancing Frog (*Micrixalus kottigeharensis*) from the Western Ghats of India, vocal sac inflations combined with calls elicit visual signals such as foot flagging and hind limb tapping in rival males. The temporal relationship between the acoustic and visual component can influence signal attractiveness (Taylor et al. 2011; Zeyl and Laberge 2011). In Túngara Frogs (*Engystomops pustulosus*), the visual signal of an inflating vocal sac does not enhance the attractiveness of a call if it is presented 100 ms or longer after the call (Taylor and Ryan 2013).

There is increasing evidence that the vocal sac might also play a role in chemical signaling. The family of African Reed Frogs (Hyperoliidae) exhibits substantial variation in body coloration, morphology, and reproductive modes, but males of all Reed Frog species share a common feature: a prominent gular patch on the vocal sac (Pickersgill 2007). The conspicuous patch, with high contrast to the surrounding vocal sac skin, is a gular gland complex that produces volatile substances and potentially emits scent signals during calling (Starnberger et al. 2013). Reed Frogs might use a combination of acoustic, visual, and chemical signals to enhance their ability to detect conspecifics within dense multispecies breeding aggregations typical for hyperoliid frogs (Ryan 1988; Gerhardt and Huber 2002; Narins et al. 2007; Taylor et al. 2011; Starnberger et al. 2013).

Spotted Reed Frogs (*Hyperolius puncticulatus*, Pfeffer 1893) are found throughout Eastern Africa, occurring in and around forests (Schjötz 1999; Pickersgill 2007). During the breeding season, males call at night from vegetation around forest pools or in swamps (Schjötz 1967). Their advertisement call consists of a series of short, high-pitched notes (0.2 s) at a dominant frequency of 2.0 kHz (Schjötz 1999). Male *H. puncticulatus* emit a second call type, consisting of low rasping sounds with slow pulses (I.S., P.M.M., and W.H., personal observations). Preliminary observations indicated this call is used in aggression, with a similar function to the aggressive call described in other Reed Frog species (Passmore et al. 1992; Grafe 1995; Sinsch et al. 2011).

Complex environments can degrade signals and impair signal transmission and reception, especially in anurans using a narrow frequency bandwidth important for species-specific recognition and discrimination in sexual communication. Higher frequency calls are more easily attenuated in heterogeneous environments (Ryan and Brenowitz 1985; Kime et al. 2000). Multimodal signal strategies, however, might allow signal transmission in all

environments and thereby enhance the ability to convey the message even in nocturnal species. Some nocturnal species use visual cues and signals for species recognition and mate choice (Rosenthal et al. 2004; Richardson et al. 2010; Sztatecsny et al. 2010; Taylor et al. 2011), prefer colorful vocal sacs (Gomez et al. 2009), and even discriminate colors at night (Yovanovich et al. 2017). For the vast majority of nocturnal anurans, however, vocal sac inflations most likely serve to recycle air while calling for extensive periods, and their visual function in the communication system remains poorly understood or might even be insignificant.

We tested the hypothesis that visual signals of the vocal sac increase signal salience in male–male interactions and influence opponents in close-range male contests of nocturnal *H. puncticulatus*. To investigate the communicative function of acoustic signals and vocal sac displays, we tested receiver responses to unimodal and multimodal playbacks. When presenting multimodal signals, we predicted that asynchrony between the acoustic and visual components should degrade signal salience and reduce receiver response. In addition, we modified the visual stimulus of the gular gland to test its visual importance in the multimodal communication system in male–male interactions. Finally, we used unimodal acoustic playbacks to test male receiver responses to advertisement and aggressive calls.

Materials and Methods

Study Site and Animals

We studied a population of *H. puncticulatus* in and around the Amani Pond, a swampy inundation area of the Zigi River in the Amani Nature Reserve (5.098673°S, 38.629646°E; datum = WGS84) in the East Usambara Mountains of Tanzania during the rainy season in August 2014. Males were abundant in the swamp and usually were spaced at a distance of at least 1 m. Males in the study population had a mean (± 1 SE) snout–urostyle length of 26.1 ± 0.41 mm (range = 24–29 mm) and a mean mass of 1.01 ± 0.04 g (range = 0.85–1.27 g; $n = 12$). The frogs were captured with permission of the Amani Nature Reserve and released immediately after taking body measurements. All behavioral experiments were performed without physical contact to the focal animals.

Experimental Setup

Focal males were located in situ by their species-specific vocalizations. To test their reaction to unimodal and multimodal playbacks, we set up a tripod (Cullman Nanomax 260) 0.5 m in front of the calling male (Fig. 1). A bamboo stick was attached to the tripod (parallel to the ground) that held a speaker (Sony SRS-M 30). In front of the speaker a large *Asplenium* leaf was attached to the bamboo stick to serve as naturalistic platform or calling perch for playback stimuli. The artificial vocal sac was attached via an aquarium air tube (2 m long) to rubber hand bellows (Hama DustEx 00005635) to mimic rhythmic vocal sac inflations of rival males. To investigate the influence of the yellow gular patch as a visual signal on receivers in male–male interactions, we prepared one artificial vocal sac by using a tip of a white latex glove with a central patch painted with yellow nail polish. For the second artificial vocal sac without gular patch, we used the tip of a white latex glove only. The different vocal sacs could be easily exchanged between playback experiments. The speaker

was connected to an mp3 player (Odys pax). The experimenter could inflate and deflate the artificial vocal sac as well as start the acoustic playback from a distance of 3 m.

For acoustic stimulus presentations, we first recorded male calls with a directional microphone (Sennheiser Me 66, AKG D 190 E), and a digital recorder (Zoom HN4; settings = 44.1 kHz, 16-bit resolution). We measured peak sound pressure levels (SPLs) from a distance of 1 m with a Voltcraft sound pressure level meter (SL-100; settings = fast/max, C weighted). The recorded subjects ($n = 4$) produced calls comprised of 1–17 notes, but 2-note calls were by far the most common (median = 2, interquartile range = 2). The acoustic signals of amphibians are typically time invariant and stereotyped, showing little variation within or among individuals. We selected a two-note vocalization for playbacks with average spectral and temporal properties representing the population mean. The average species-specific advertisement call selected for playback presentations consisted of two notes with a call length of 0.4 s (note length = 0.05 s) and a SPL of 78.5 dB at 1-m distance. The notes were noise reduced; sound in the narrow frequency bandwidth of notes was filtered; and to avoid interference from background noise, the internote interval was generated by adding a silent period of 0.3 s between notes by using sound analysis software (Raven Pro). Similarly, we recorded and analyzed aggressive calls of four frogs and selected an aggressive vocalization with mean parameters of the recorded individuals for the playback (call length = 0.2 s, SPL = 80 dB at 1 m).

Acoustic playback—To investigate the function of the two call types in *H. puncticulatus*, we presented advertisement and aggressive calls and recorded male response frequencies. The first part of the acoustic stimuli consisted of 10 advertisement calls (0.4-s call time, 5.5-s intercall interval, 53.5-s total call presentation, SPL = 80 dB at 1 m), followed by a 150-s control phase (no sound) and was continued with further 10 advertisement calls and another 150-s control phase (Fig. 1B). The same advertisement call stimuli were also used for audio playback presentations in unimodal and multimodal playbacks. To test responses to advertisement and aggressive calls, the first part of the stimulus presentation was followed by 10 aggressive calls (0.2-s call time, 2.3-s intercall interval, 23-s total call presentation, SPL = 80 dB at 1 m) and a control phase (77 s; Fig. 1B). The end of the playback, and therefore of the trial, was marked by 1 s of white noise, which was clearly audible in the frog chorus.

Unimodal and multimodal playback—Responses of a focal male were tested with one of the following randomly chosen unimodal or multimodal stimuli (total $n = 39$): (1) audio presentations ($n = 10$) consisted of unimodal advertisement stimuli without the artificial vocal sac; (2) “W-sync” presentations ($n = 11$) included multimodal stimuli of the artificial vocal sac in synchronous movement with the playback calls (inflations were not randomized, but followed the rhythm of the call playback); (3) “W-asynch” presentations ($n = 8$) consisted of playback calls and rhythmic inflation of the artificial vocal sac, but the inflations occurred asynchronously with the call (in the intercall interval of the audio playback); (4) “W-patch-sync” presentations ($n = 10$) were identical to W-sync except for the inclusion of an artificial vocal sac with a yellow patch.

As soon as the setup was positioned and the focal male stopped producing advertisement calls for more than 5 s, we started the stimulus presentation. The respective stimuli were presented twice for 53.5 s, each time followed by a 150-s control interval of silence (Fig. 1C). The behavior of the focal male was recorded throughout the trial with a video camera (Sanyo Xacti WH1) for later analysis. It was not possible to record data blind because our study involved focal animals in the field. However, blinded methods were used for video analysis to minimize observer bias. After each trial, a photograph of the focal frog's dorsal pattern was taken for individual identification to avoid retesting of males. Frequencies of all responses by focal males during playback presentations and control phases were analyzed with the behavioral coding software Solomon Coder beta v.1.1.01.22 (Péter 2011). The number of responses to advertisement calls were coded according to note number (e.g., one-note, two-note call). Calls with more than six notes were coded as multinote calls. The term "advertisement call" pools the sum of all call responses to playback trials independent of note number.

Data Analysis

To test for differences in behavioral response to playback presentations of aggressive and advertisement calls, we compared response frequencies during 100 s of the first advertisement call playback and the consecutive control period to the responses displayed during 100 s of aggressive call playback presentations and the following control period. The response frequencies of "tap" (see Results), "advertisement call," "aggressive call," "approach," and "move away" were analyzed using linear mixed models (LMMs). The statistical assumptions for LMM analysis were met. The LMMs allow for repeated measurements of the same individual to be fitted in the model as random variable. The respective response frequencies were entered as dependent variables, with playback presentation as predictor variable. The identities of individuals were entered as a random variable.

To test the influence of unimodal and multimodal playback presentations on receivers, we pooled the response frequencies displayed during the complete playback (two multimodal stimulus presentations and two control periods; Fig. 1C) according to the behavioral categories of tap, move away, approach, advertisement call, and aggressive call. Response frequencies were compared using Kruskal–Wallis tests. To understand whether the frequency of advertisement calls (i.e., the number of calls elicited) with a specific note number increases or decreases with respect to playbacks, we compared one-note, two-note, three-note, four-note, five-note, and multinote calls with six or more notes between stimulus presentations by using Kruskal–Wallis tests. All analyses were done in SPSS (v.19, SPSS Inc., Chicago, IL). In addition, we analyzed recordings of advertisement calls and aggressive calls in Raven Pro software (v.1.3, Build 32, Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, NY). Unless stated otherwise, response values are reported as means $\pm$ 1 SE.

Results

Call Descriptions

The advertisement call of *H. puncticulatus* in the study population consisted of 1 to 16 notes with 2-note calls (0.51 ± 0.01 s, range = 0.46–0.60 s; $n=12$) being the most common. A single note had a duration of 0.055 ± 0.002 s (range = 0.043–0.066 s; $n=12$) and a dominant frequency of 2982 ± 33 Hz (range = 2832–3182 Hz; $n=12$). The aggressive call is pulsatile with a call duration of 0.22 ± 0.019 s (range = 0.11–0.36 s; $n=12$) and a dominant frequency of 2975 ± 36 Hz (range = 2815–3167 Hz; $n=12$; Fig. 2). If a male approached another calling male, the calling male likely switched from producing advertisement calls to aggressive calls. If the intruding male did not move away, the resident male often engaged in agonistic behavior such as fighting.

Behavioral Responses to Playback Presentations

Of the 3482 behavioral events recorded in response to unimodal and multimodal playback presentations, 49% of the displayed behaviors of male Spotted Reed Frogs were advertisement calls, 10% aggressive calls, 6% approaches to the sound source, and 3% movement away from it. In addition, males displayed a previously undescribed behavior for this species, which we termed “tapping,” at a relatively high frequency (32%). Tapping denotes the brief lifting of a limb (i.e., one arm or leg) from the substrate without stretching or waving. Tapping behavior could involve either the right or left front or hind limb, with alternation between the two sides especially apparent when the hind limbs were used. Tapping was only observed in male subjects and was mostly displayed in addition to advertisement calls (see Video S-1 in the Supplemental Material available online).

Acoustic playback—*Hyperolius puncticulatus* approached advertisement call playbacks more readily than aggressive call playbacks ($F_{1,18} = 5.00$, $P = 0.04$; Fig. 3). Although aggressive call playbacks tended to be answered less often with advertisement calls than advertisement call playbacks, this difference was not significant ($F_{1,18} = 3.47$, $P = 0.08$). For each type of stimulus presentation, we found no differences in the frequencies of tap ($F_{1,18} = 0.53$, $P = 0.48$), aggressive call ($F_{1,18} = 1.03$, $P = 0.32$), or move away responses ($F_{1,18} = 0.58$, $P = 0.46$) to advertisement and aggressive call playback presentations ($F_{1,18} = 4.23$, $P = 0.06$; $n = 10$).

Unimodal and multimodal playback—Behavioral reactions of male *H. puncticulatus* were similar in response to unimodal and multimodal playbacks (Fig. 4; in all cases, we report results from Kruskal–Wallis tests). Focal males displayed tapping behavior ($H = 0.32$, $df = 3$, $P = 0.96$), produced advertisement calls ($H = 3.15$, $df = 3$, $P = 0.37$) and aggressive calls ($H = 1.76$; $df = 3$, $P = 0.62$), and approached the stimulus ($H = 0.18$, $df = 3$, $P = 0.98$) or moved away from it ($H = 1.76$, $df = 3$, $P = 0.62$) in similar frequencies in response to all four playback types. We found no difference in number of one-note calls, two-note calls, or multinote calls between stimulus presentations; hence, males did not change their call duration by emitting a greater or lesser number of notes per advertisement call in response to a playback presentation ($df = 3$, $P > 0.05$ for all tested notes per call).

Field Observations on Courtship Behavior in *H. puncticulatus*

We observed the interaction between a male and a gravid female (eggs were partly visible through the translucent skin on the ventrum) in the study population. The female moved through the vegetation approaching the advertising male and then perched on a leaf in the vicinity of the calling male. The female remained motionless for several minutes, while the male emitted advertisement calls and frequently tapped using front and hind limbs. The female then jumped toward the calling male and, after landing, oriented away from him. The male approached the female while continuously emitting advertisement calls and then jumped onto her back into amplexus. The amplexant pair then left the calling site. This interaction was ~7 min in duration (see Video S-2 in the Supplemental Material available online).

Discussion

Regardless of the presence or absence of the gular patch on the simulated vocal sac, movement of this structure did not act as a visual display that increases agonistic response behaviors (e.g., approaches, attacks, or aggressive calls) toward conspecific males in *H. puncticulatus*. Receiver responses were not reduced when calls and vocal sac inflations were presented in asynchrony, even though this fixed composite signal (sensu Partan and Marler 2005) occurs synchronously as a biomechanical consequence of call production. When temporally offset, acoustic and visual signal components could be still recognized and detected by receivers, because signal saliency relies on the relative relationship of signal components (Rubi and Stephens 2016; Stange et al. 2016).

Several studies have demonstrated that multimodal signals enhance detection and recognition of the signaler (Preininger et al. 2013a; Taylor and Ryan 2013; reviewed in Starnberger et al. 2014a) and that components act as backup or alert receivers in fluctuating noisy environments (e.g., Partan and Marler 2005). If receivers rely on the comparison of multimodal components in mate choice, however, fluctuating environmental conditions can cause signal interference (Taylor et al. 2011; Zeyl and Laberge 2011; Halfwerk and Slabbekoorn 2015; Halfwerk et al. 2016). Unlike females, male reed frogs might not depend on precisely locating another male but rather use an estimated distance to a caller to detect the presence of a nearby rival. When nearby male *H. puncticulatus* move too close to a male's calling perch, resident males switch from advertisement to aggressive calls. In response to unimodal aggressive call playbacks, the focal male usually stopped approaching. Our observations of pulsatile aggressive calls and variable multinote advertisement calls correspond to those reported for other *Hyperolius* calls (Grafe 1995, 1996; Schiøtz 1999). The average call duration of a two-note advertisement call in the study population was however twice as long as the overall call duration reported by Schiøtz (1999). A consistent stimulus presentation of two advertisement calls followed by one aggressive call was used during playback presentations to mimic a natural call sequence of a conspecific male in close proximity. We chose this call sequence to minimize the influence of aggressive calls on advertisement call responses. We acknowledge, however, that testing males with only aggressive calls vs. only advertisement call presentations, a greater sample size, or both might have provided differing responses or response frequencies. Therefore, our

interpretations reflect the pattern of successive call presentations and provide only initial information for future investigations. Nevertheless, we suggest *H. puncticulatus* switches to aggressive calls to defend perching sites and that conspecific males stop their approach to avoid attacks from the resident individual.

Aggressive calls have generally been suggested to play a role in male–male-spacing and in female assessment of a male’s quality (Taylor and Ryan 2013; Toledo et al. 2015). Compared to advertisement calls, however, aggressive calls are likely unattractive to females (Wells 1988; Grafe 1995; Pickersgill 2007; but see Marshall et al. 2003). Therefore, eliciting aggressive calls might come at the cost of being unable to produce the advertisement calls that attract females and signal the male’s readiness to defend a calling site (Passmore et al. 1992; Narins et al. 2007; Starnberger et al. 2013).

Our results indicate that visual cues of the vocal sac and gular patch have little effect in the male–male communication system of *H. puncticulatus*. Even though responses of only 8–11 males were tested, our results demonstrate no differences in response frequencies to unimodal or multimodal playback presentations. An increased sample size seems unlikely to alter the results. It is worth noting, however, that the vocal sac was presented in absence of a model frog. Future investigations should test visual signal response to inflations in the context of a frog model. Furthermore, it is still unclear whether the movement of the vocal sac increases the detectability of a male eliciting aggressive calls, or could be used as agonistic signal at a close range. We should also consider the potential role of visual cues in female choice. The contrast between the vocal sac and the gular patch could facilitate detection of a calling male in dense vegetation (Starnberger et al. 2014b). Conversely, the visual properties of the vocal sac might provide information about a male’s health, overall condition, or both. In the nocturnal Treefrog *Hyla arborea*, females prefer more intensely colored vocal sacs, indicating that carotenoid-based yellow-to-orange coloration might be a condition-dependent cue (Gomez et al. 2009, 2011). Gravid reed frog females have a high risk of predation attributable to their increased activity during mate search. Preference for conspicuous males that are therefore easy to locate might be beneficial (Grafe 1996, 1997; Schiøtz 1999; Pickersgill 2007). In anurans, conspicuousness is often achieved with energetically expensive high call rates and SPLs (Schiøtz 1967; Klump and Gerhardt 1992). For example, female *Hyperolius marmoratus* prefer nearby males eliciting advertisement calls at a high rate (Grafe 1997; Schiøtz 1999). Female Reed Frogs might potentially use concomitant visual vocal sac cues to assess mates similar to *Engystomops pustulosus*, where gravid females are most sensitive to the visual cue (Cummings et al. 2008).

Sexual selection could favor a close-range signal in a different signal domain with slower, but more constant, transmission such as a chemical cue. In male Reed Frogs, the gular patch is a conspicuous gland situated on the vocal sac with ducts that exit at the skin surface. Volatile chemicals produced in the gland might be radiated during calling (Starnberger et al. 2013). Species-specific scents might act as courtship signals comparable to pheromones distributed by Newts in a self-generated current (Ryan and Brenowitz 1985; Kime et al. 2000; Woodley 2010; Bradbury and Vehrencamp 2011). During the nocturnal courtship of *H. puncticulatus*, we observed a vocal sac remaining inflated for more than 6 min before amplexus was initiated (see Video S-2 in the Supplemental Material available online).

Evidence supporting the function of the gular gland remains anecdotal, but it seems likely that the transmission and perception of volatile chemicals could be more effective than visual signals under low light conditions (Grafe 1995, 1996; Schiøtz 1999; Waldman and Bishop 2004; Byrne and Keogh 2007).

In response to playbacks, Spotted Reed Frogs performed a previously undescribed gestural display—a tapping behavior consisting of a series of front and hind limb lifts. Even though no differences in the frequency of tapping were recorded in regard to playback presentations, tapping and advertisement calls were the most frequently displayed behaviors of males. Visual signals of hind foot and arm lifting or waving have been thus far described as agonistic behaviors in the repertoire of anurans (Hödl and Amézquita 2001). Even more conspicuous gestural displays such as foot-flagging behavior were only recorded in an agonistic context during male–male interactions to defend perch sites (Amézquita and Hödl 2004; Preininger et al. 2009; Grafe et al. 2012) and are suggested to originate from an aggressive kicking behavior used during physical attacks (Preininger et al. 2013b). Kicking bouts are also displayed by male *H. marmoratus* in defense of conspecific intruders (Grafe 1997), and males in several Reed Frog species use physical combat to defend their position in the lek (Telford 1985; Dyson et al. 1992). Male spacing increases mating success because females prefer males with larger sound fields and cannot detect overlapping calls (Grafe 1995, 1996). Limb displays in *H. puncticulatus* could be an agonistic visual signal perceived by potential rivals. We also observed tapping behavior in the presence of a female, and the display was not regarded aversive; on the contrary, the signaling male mated with the female (see Video S-2 in the Supplemental Material available online). Therefore, we suggest that tapping behavior might attract females and repel males in a manner similar to the use of advertisement calls.

It is possible that tapping might not stimulate the visual system of conspecifics but instead influence a different sensory modality via substrate vibrations in a limited active signal space. Seismic or vibrational signals could act as a close-range communication channel in arboreal anurans, when the efficacy of visual signal is obscured by physical elements in the environment. Similar to our study species, arboreal and nocturnal Red-Eyed Treefrogs (*Agalychnis callidryas*) form breeding aggregations in vegetation surrounding water bodies. Males produce tremulations to create substrate vibrations used as aggressive signals carrying information about the motivation and size of the signaler (Caldwell et al. 2010). Anurans also use vibrational signals to attract mates. Females of the Common Treefrog (*Polypedates leucomystax*) tap their toes to initiate mating (Narins et al. 1998). Males of ground-dwelling Gunther's White-Lipped Frogs (*Leptodactylus albilabris*) generate seismic signals with their vocal sac while calling (Narins 1990; Lewis et al. 2001; Gridi-Papp and Narins 2010), and male *L. syphax* respond to aggressive calls with a combination of aggressive calls and audible foot taps (Cardoso and Heyer 1995). We suggest vibrational signals, visual tapping signals, or a combination could facilitate and promote detection of the signaler in complex or heterogeneous environments.

Research concerning anuran systems in the past decade has provided valuable information on perception of, and behavioral response to, multimodal signals with components, either always produced together (i.e., fixed components) or independently. Furthermore, a

considerable amount of effort has been made to describe environmental influences and the selection on multimodal signal design in several species. However, not all species respond to or use the available modalities for communication. Admittedly, this study focused on responses to multimodal stimuli during male–male interactions, but we still suggest that vocal sacs, however conspicuous, could sometimes just be a by-product of sound production and selection might not promote visual signal components in nocturnal species. Nevertheless, vocal sac pulsations could facilitate a less explored modality in anurans and might be used to radiate chemical signals from the gular gland. Future investigations should focus on chemical cues emitted by the gular patch and test receiver responses of females to visual and chemical signals. The novel gestural tapping display also raises several questions in regard to its function in male–male interaction and female mate choice. Spotted Reed Frogs provide the opportunity to test the effectivity and complexity of signals in several sensory modalities in a nocturnal anuran species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experimental design for unimodal and multimodal stimulus presentation. (A) Experimental setup for playback presentations. (B) Schematic of stimulus presentations for acoustic playbacks; black line at the end represents 1 s of white noise. (C) Schematic of stimulus presentation for unimodal and multimodal presentations; the same randomly selected unimodal (acoustic) or multimodal (acoustic and visual) stimuli were presented twice during the period termed “multimodal” to a focal individual.

Fig. 2.

Calls of male *Hyperolius puncticulatus* recorded at 20°C. Waveform and corresponding spectrogram below of two average advertisement calls, with one and two notes and an average aggressive call (sound pressure level = 80 dB at a 1-m distance). Intercall intervals were shortened to present three calls in one graph.

Fig. 3. Mean response frequencies (± 1 SE) of male *Hyperolius puncticulatus* to advertisement call and aggressive call playback. Statistically significant differences between responses based on linear mixed model analysis are denoted by asterisk (* $P < 0.05$; $n = 10$ for all playback presentations).

Fig. 4.

Response frequencies of male *Hyperolius puncticulatus* to unimodal and multimodal stimulus presentations. Stimulus presentations include advertisement call playbacks (audio, $n = 10$), with an artificial vocal sac moving either synchronously (W-sync; $n = 11$) or asynchronously (W-async; $n = 8$) with the presented call, and with an artificial vocal sac having a yellow patch that moved in synchrony with the presented call (W-patch-sync; $n = 10$). See text for details on the timing of call presentation.