VISUAL SIGNALING IN PHYRNOBATRACHUS KREFFTII
BOULENGER, 1909 (ANURA: RANIDAE)

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Abstract: During intraspecific interactions involving males, females and subadults, acoustic as well as visual signals were observed in the diurnal frog Phrynobatrachus krefftii. Strikingly, interactions between adult males are highly dominated by inflations of the bright yellow subgular vocal sac without sound production. We studied the signaling behavior in males of P. krefftii at the Amani Nature Reserve, Usambara mountains, Tanzania, from November 2001 to March 2002. Under nonmanipulated conditions, we registered 641 male-male interactions involving 31 individuals during 323 hours of observation. Most (496 or 77%) inflations of the vocal sac were purely visual; the remainder (145 or 23%) of the signals were bimodal, and accompanied by sound production. To test whether exclusive visual signaling can be evoked under experimental conditions, we introduced tethered males into the visual field of 28 residents, registering the elicited responses over 10 minutes after the introduction. Unimodal (i.e., exclusive visual) signals (825 out of 1106 responses) dominated over vocal-sac inflations accompanied with sound production. In seven selected focal males, the rate of exclusive visual signaling (0.57 signals/min), however, was more frequent than under nonmanipulated conditions (0.13 signals/min). Phrynobatrachus krefftii is the first species of anuran amphibians reported to perform nonaudible vocal-sac inflations during intraspecific male-male signaling behavior.

Key words: Acoustic communication; Agonistic behavior; Amphibia; Anura; Phrynobatrachus krefftii; Visual signaling; Vocal sac

The most prominent mode of communication in anuran amphibians is, by far, the use of sound production. Compared to acoustic cues, visual signals play a subordinate role in amphibian communication. Some anuran species, however, perform remarkable visual displays in variable social contexts (Amézquita and Hödl, 2004; Davison, 1984; Haddad and Giaretta, 1999; Harding, 1982; Hödl et al., 1997; Lindquist and Hetherington, 1996, 1998; Richards and James, 1992). All presently described species performing visual displays emit acoustic signals as well (Hödl and Amézquita, 2001), which makes it difficult to separate the functional role of both communication modalities (but see Narins et al., 2003). Diurnal species of anurans commonly produce visual displays (Durant and Doyle, 1975; Wells, 1980) with limb signals being the most distinctive behavior described (e.g., Davison, 1984; Harding, 1982; Heyer et al., 1990; Hödl,...
et al., 1997; Pombal et al., 1994). In addition to its acoustic function, the vocal sac also may act as a conspicuous visual signal. Despite of the variety of diverse vocal-sac types, their visual relevance has not yet been explored thoroughly (Hödl, 1996).

Visual signaling so far has been recorded only in tropical frogs, with many species occurring along streams and creeks (Hödl and Amézquita, 2001; Lindquist and Hetherington, 1998). We searched for other visually communicating species along East African mountain streams, and found that the diurnally active Phrynobatrachus krefftii appeared to be a good candidate. Males of this ranid species are dull brown in color, but possess a strikingly bright-yellow vocal sac. Thus, the aim of this study was to investigate the possible role in communication of the conspicuously colored gular region during intraspecific encounters.

The exclusive African frog genus Phrynobatrachus currently contains 66 species, all of which occur in sub-saharan Africa (Poynton, 1999). The endemic P. krefftii is diurnal and inhabits localities of seepage water, rivulets and streams in submontane and montane rainforest within the East and West Usambara Mountains in Tanzania (Barbour and Loveridge, 1928). Within the Amani Nature Reserve (ANR) of the East Usambara mountains, P. krefftii occurs at elevations above 800 m in a submontane evergreen forest.

A remarkable color dimorphism is found between males and females: Males reach up to 50 mm in snout–vent length (SVL) and possess a brightly yellow subgular region. Females reach 40 mm in SVL (Barbour and Loveridge, 1928), and their throat region is light brown colored (Fig. 1). Inflatable vocal sacs are present only in sexually mature males. Two more species of the genus Phrynobatrachus, P. alleni and P. accraensis (= P. latifrons), also possess yellow throat coloration in breeding males (Rödel, 2000, 2003; Rödel and Agyei, 2003).

![Figure 1](image_url)

**Fig. 1.**—*Phrynobatrachus krefftii*. Female (above left) and male without (above right), partly (below right) and fully expanded vocal sac (below left). Note the bright yellow subgular region of the male. Photographs taken by W. Hödl at Zigi River (Amani Nature Reserve), Tanzania.
Due to the regular occurrence of egg clutches and tadpoles throughout a nine month observation period (July 2001–March 2002), *P. krefftii* is considered a “prolonged breeder” (sensu Wells, 1977). Males occupy breeding territories containing rocks and fallen branches in shallow water. Territories are sites for mate attraction and oviposition, as well as areas for feeding and shelter. Male residents readily scrutinize conspecific intruders in their visual field by leaving their calling sites and approaching the offenders. At night, adult as well as juvenile frogs are found at resting sites on leaves within the vegetation along the banks of the stream at approximately one meter above the ground. Out of 158 recaptures, 59 (30%) toe-clipped individuals were found less than 20 cm from the initially marked site on leaves each night over a period of seven days. Calling activities of male frogs were observed throughout the day (W. Hirschmann and M. Franzén, unpublished data).

**Materials and Methods**

The study was conducted near the village of Amani (5°6′ S and 38°38′ E, 900 m), Tanga-District, Tanzania within a submontane forest of the East Usambara Mountains belonging to the Zanzibar-Inhambane transitional rainforest type (White, 1983). Mean annual rainfall (1902–1970) is 1910 mm (Rodgers and Home-wood, 1982) with peak precipitation in March, April and May. Two study areas were selected within the boundaries of the Amani Nature Reserve. A 400-m transect along the margin of the Zigi river (5°09′ S and 38°63′ E) and a 112-m stretch along the border of a rivulet (5°10′ S and 38°63′ E) were marked by colored tapes. The stream was bordered by gallery forest and secondary growth, with the soil surface continuously being soaked by seepage water. The rivulet was partly covered with dense vegetation. Because of unusual dryness during the study period the water level in both stream and rivulet was exceptionally low.

The behavior of *P. krefftii* was studied from 19 November 2001 to 09 March 2002, totaling 77 days and 323 h of observation. For orientation within the study sites and to observe the patterns of activity and site fidelity of *P. krefftii*, a preliminary study was carried out in July 2001, with observations taken during both day and night hours. The frogs were located on their nocturnal resting sites in the dense vegetation using a head lamp between 2000 h–0300 h every night between 09 June 2001 and 15 July 2001. All individuals larger than 20 mm SVL were toe clipped (according to Hero, 1989). The nonmanipulated observations started at 0900 h and ended at 1700 h, and corresponded to the typical period of diurnal activity of *P. krefftii*. In total, 14 sites (seven each along a stream and a rivulet) with good visibility and access were selected, each containing at least four adult males. The order of observations within the pool of individuals was chosen randomly. Nonmanipulated observations were obtained from a distance of approximately 2 m from the focal individual. Each individual observation lasted 30 min. For vocal recording, a microphone was placed 0.75 m in front of the focal male. Recordings were made with a Sony Professional Walkman WMDC6 using a directional microphone (AKG C568 EB). To measure the background noise and to test the possible acoustic energy produced during nonaudible vocal sac inflation, a sound-pressure level meter (Volticraft No. 33-2050; “A” weighted, fast response) was positioned 0.75 m in front of the signaling individual. Sonagrams were produced with a MAC computer using Canary (1.2.4) software.

To test the importance of visual encounters and to confirm that the visual signal is directed towards an introduced male frog during territorial interactions, we performed experiments with tethered frogs. Individual *P. krefftii* males (SVL \( \bar{x} \pm SD = 36.4 \pm 3.6 \text{ mm}, n = 15 \)) were caught and used at the onset of the rainy season between 28 February 2002 and 15 March 2002 starting each day at 0900 h and ending at 1700 h. Experimental manipulations were restricted to the manual introduction of a tethered male *P. krefftii* hanging on a fishing rod, which was tied to a waist band. Because preliminary observations showed that soundless vocal-sac expulsions occur mainly during close-range encounters, the tethered males were positioned within the visual field of a resident male at a 30 cm distance (cf. Amézquita and Hödl, 2004). We observed the behavior for 10 min immediately following the insertion of the tethered male. Focal individual and all-occurrence samplings (con-
tinuous recording) were used for behavioral records (Martin and Bateson, 1986). Descriptions of the behavior were made of toe-clipped individuals (according to Hero, 1989) observed and videotaped in the field. With the exception of two adult specimens (female and male) deposited at the Institute of Zoology at the University of Dar Es Salaam USDM 1697 (male), USDM 1698 (female), all tested individuals were released after the experiments. Chi-square tests for significance were two tailed with $\alpha = 0.05$.

**RESULTS**

Males of *P. krefftii* were acoustically and visually active during the daytime and throughout the study period. After rainfall and during the rainy periods in December 2001 and February 2002, signaling activity increased. Males displayed four conspicuous inter-individual behaviors: orientation shift to face an intruder, assuming an upright posture displaying their throat, vocalization, and expulsion of the vocal sac without audible sound production. Soundless expulsions of the vocal sac were observed in 49 out of 51 studied individuals under nonmanipulated conditions. Visual communication of *P. krefftii* consists of unimodal (visual) and bimodal (acoustic and visual) signals as described below.

**Visual Signaling Performed by Males**

Purely visual signals were associated with the exposure of the highly contrasting gular region. In contrast to the cryptic dorsal and pectoral coloration, the singular subgular vocal sac is brightly yellow-colored, and thus highly conspicuous. When the male is observed at ground level from the front, as is the case during encounters with conspecifics, the visibility of the male in alerted position is greatly enhanced (Figs. 1, 2). We quantified the visual displays of 49 signaling males and observed that 77% of vocal-sac expulsions were performed without (audible) sound production under nonmanipulated conditions (Table 1). During nonaudible vocal-sac expulsions no excess energy beyond the background noise was detectable within the frequency range of the A-weighted sound-pressure level (SPL) meter positioned 0.75 m in front of the signaling males. In contrast, SPL of the two recorded call-types clearly exceeded the SPL of the environment (SPL $\bar{x} \pm SD = 51.5 \pm 2.7$ dB, $n = 15$) at 0.75 distance (Fig. 3).

![Fig. 2.—Territorial male of *Phrynobatrachus krefftii*, in upright position and without (left) or with vocal-sac display (right). Drawn by H. C. Grillitsch after a photograph taken by W. Hölzl at Zigi River (Amani Nature Reserve), Tanzania. (Graphics courtesy of H. C. Grillitsch).](image)
Agonistic Behavior by Males

During the day, males signaled in shallow water on rocks and from fallen branches within their territory. Individual males \((n = 51)\) used almost the same calling sites within their territory in successive days throughout the study period and returned to the marked nocturnal resting site over a period of seven nights (W. Hirschmann and M. Franzen, unpublished data). No evidence of female territorial behavior was observed. However, females \((n = 30)\) returned to the same nocturnal shelter over seven successive nights (W. Hirschmann and M. Franzen, unpublished data). Males were observed attracting females and actively hunting prey in the vicinity of their calling sites. Males of \(P. \text{krefftii}\) appear to have an acute visual sense of movements. We observed males moving quickly and highly directionally towards potential prey, such as small insects, three meters away. When a territorial male perceived an intruder, it turned to the intruder, assumed an upright position, and started or increased vocal sac inflations (Fig. 2) towards the conspecific male. A maximum of nine visual signals was performed consecutively without sound production. If the intruder had not retreated after these signals, he was leaped upon and chased away.

Over the course of 70 observed male-male encounters, we registered 641 behavioral acts performed by 31 individuals: 469 of these acts \((77\%)\) were exclusively visual displays, 93 \((15\%)\) were of call-type 1, and 52 \((8\%)\) were of call-type 2. The mean duration of the soundless vocal-sac expulsions was 2.52 s \((SD = 1.49\ s; range 1.44-6.64\ s; n = 11)\). Other behavioral acts observed during agonistic interactions included turning, approaching or jumping away. During 21 observed male-female encounters, involving 19 males, acoustic displays

![Figure 3](https://example.com/fig3.png)

**Fig. 3.**—Sonagram (above) and oscillogram (below) of call-type 1 (A) and call-type 2 (B) of *Phrynobatrachus krefftii*. Air temperature 20°C, water temperature 19°C.
dominated significantly ($\chi^2 = 157.4$, df = 1, $P < 0.05$). Call-type 2 (78%, $n = 147$) dominated over call-type 1 (18%, $n = 33$). With only 8 out of 188 responses, visual signals did not play a significant role in male-female encounters (Table 1). In 37 male-subadult encounters with 24 individual males involved, we registered a total of 190 signals. Call-type 1 (23%, $n = 43$) dominated over call-type 2 (20%, $n = 38$). With 109 responses (57%), visual signals were frequently used in male-subadult encounters (Table 1). Additionally, we observed 51 males signaling without a visible conspecific individual within the vicinity. Out of 1856 signals given without another individual of *P. krefftii* obviously present, 353 (19%) purely visual signals (i.e., soundless vocal-sac expulsions) were observed. We recorded 985 acoustic signals of call-type 1 (52%) and 88 displays of call-type 2 (29%) in this context.

During our experimental introduction of tethered males ($n = 15$), all focal males ($n = 28$) tested performed signals with vocal-sac expulsions. Out of these 1106 signals, 825 (75%) were purely visual. Bimodal signals were given at a rate of 25%, including 17% of call-type 1 and 8% of call-type 2. Within these staged male-male interactions, purely visual signaling significantly dominated over the bimodal signals ($\chi^2 = 267.6$, df = 1, $P < 0.05$).

In male-male encounters, unimodal (i.e., purely visual) signaling under nonmanipulated conditions (77%) as well as in experimental manipulations (75%), significantly exceeded bimodal signaling (Table 1, Fig. 4). Calls (type 1 and 2 summarized) made up 23% of the signals under nonmanipulated conditions and 25% in experimental manipulations, respectively. In seven selected focal males, the rate of exclusive visual signaling (0.57 signals/min), however, was four times more frequent during the experimental manipulations than under nonmanipulated conditions (0.13 signals/min).

**Acoustic and Visual (Bimodal) Signaling**

Due to the process of vocal-sac expulsion during calling, each acoustic signal has a visual component. Male *P. krefftii* produces two call-types: Call-type 1 (1127 out of 1909 calls; Fig. 3) was most frequently recorded. It consists of a single note, with a duration of 177–374 ms...
(\(\bar{x} \pm SD = 268.0 \pm 66.3\) ms; \(n = 9\)). The dominate frequency is 1.97–2.60 kHz (\(\bar{x} \pm SD = 2.20 \pm 0.20\) kHz; \(n = 9\)). When other individuals were absent or at least not visible to us, 52% of the acoustic signals given consisted of call-type 1. The mean SPL of call-type 1, recorded at 0.75 m distance of vocalizing individuals, measured 61.5 dB (SD = 3.3 dB; \(n = 9\)) and thus exceeded the ambient SPL by 10 dB.

Call-type 2 (Fig. 4) consisted of 4 to 6 notes. The mean duration of call-type 2 was 950 ms (SD = 100.6 ms; range 842.2–1180.4 ms; \(n = 7\)). The first note of call-type 2 lasted from 243–335 ms (\(\bar{x} \pm SD = 278.7 \pm 31.8\) ms; \(n = 7\)) and possessed a dominant frequency at 2.075–2.52 kHz (\(\bar{x} \pm SD = 2.31 \pm 0.14\) kHz; \(n = 7\)) and thus resembles call-type 1. The duration of the following notes is much shorter: 34–47 ms (\(\bar{x} \pm SD = 39.3 \pm 4.4\) ms; \(n = 28\)), with a dominant-frequency range between 1.92–2.575 kHz (\(\bar{x} \pm SD = 2.20 \pm 0.24\) kHz; \(n = 28\)). Each note was separated by an interval of 108.7–146 ms (\(\bar{x} \pm SD = 123.8 \pm 9.6\) ms; \(n = 27\)). The SPL of call-type 2 (\(\bar{x} \pm SD = 62.9 \pm 1.8\) dB; \(n = 7\)) exceeded the ambient SPL by 11.4 dB.

**DISCUSSION**

Our results demonstrate evidence of unimodal visual signaling in *P. krefftii* and this complements other studies that have demonstrated visual signaling behavior in several frog genera (reviewed in Hödl and Amézquita, 2001). Consecutive expulsions of the vocal sac without sound production significantly dominated male-male encounters. These results indicate that visual signals have a distinctive function in *P. krefftii* male-male agonistic interactions.

During male-male agonistic interactions, exclusive visual signals are more frequently used than bimodal signals, both in the number of interactions in which they occur and in the number of behavioral acts within a single interaction. The extensive use of purely visual signals suggests that they are functional components of the communication system; this raises the question about their role in communication and the evolutionary scenario under which they evolved.

From various studies of anuran acoustic communication (Bogert, 1960; Duellman and Trueb, 1986; Kluge, 1981; Rand, 1988), it becomes apparent that social interactions have favored the evolution of cross-species categories of signals. In addition to advertisement calls, used both to attract females and to keep competitors at a distance, at least two other types of calls are known: aggressive/territorial calls, and—in some species—courtship calls. Aggressive calls are produced by frogs prior to or during agonistic interactions. Unlike the advertisement call, aggressive vocalization often exhibits structural gradation that may reflect the level of aggression (Wells, 1988). We believe, however, that exclusive visual signaling in *P. krefftii* does not support non-agonistic purposes, for we rarely observed visual displays performed by individuals that were not approached by a second adult male. Visual signaling usually requires the presence of another male. The possibility still remains that purely visual vocal-sac displays function as courtship signals that allow, for example, sex recognition between males and females (Rand, 1988; Wells, 1977; Zimmerman and Zimmerman, 1988). For that purpose, further experiments including females of *P. krefftii* are needed.

Advertisement calls often fulfill the dual function of mate-attraction and territorial defense. The advertisement call thus communicates not only the male’s reproductive state, but also the readiness to defend the calling site or mating territory. When the minimally acceptable threshold distance is exceeded, the resident enters into physical conflict with the intruder.

Females of *P. krefftii* did not show aggressive behavior. However, females of *Colostethus collaris*, *C. hermine*, and *C. marchesianus* (*Dendrobatidae*) exhibit a yellow slowly pulsating throat during aggressive bouts (Duellman and Trueb, 1987; Johnston, 1998; Sexton, 1960).

Hödl (1991) discussed visual components of the vocal sac and demonstrated in a film that males of *Epipedobates pictus* at the Panguana field site in Peru are easily detected at a large distance due to conspicuous white dots on their pulsating dark vocal sac. Bright yellow gular markings which become visible during vocal-sac expulsions in calling individuals of members of the diurnal *Dendrobates ventri-
maculatus group, as well as in nocturnally active Hyperolius species (f.e. Hyperolius puncticulatus), also may serve to increase localization by conspecific individuals (W. Hödl, unpublished).

According to Wells (1977), prolonged breeding favors indirect competition for females. Males engage in signaling competition for the attention of females and may defend territories around calling sites, courtship areas, or oviposition sites. A recent playback study showed evidence that female Túngara frogs, Physalaemus pustulosus, significantly preferred advertisement calls accompanied by video playback of vocal-sac inflation over calls presented with male frogs without vocal-sac expulsion, and therefore bimodal cues may play a key role in amphibian mate choice (Rosenthal et al., 2004). Thus, in that species, the female’s attraction to an acoustic cue is modulated by the presence of the inflated vocal sac.

The functional role of purely visual signals in P. kreffitii can be compared to the role of aggressive calls. Since focal males performed visual displays during all intrusion experiments, we interpret the soundless expulsion of the vocal sac as a behavioral act that signals an aggressive motivational state. Furthermore, inflation of the vocal sac elicited visual displays by interacting males. Calls of type 1 also were produced after the intrusion of a male, but, interestingly, we did not find any evidence of a distinctive aggressive call in P. kreffitii. Instead, most males respond to intruders by combining type-1 calls and soundless vocal-sac expulsions, suggesting that visual displays may functionally replace aggressive calls as a spacing mechanism (Amézquita and Hödl, 2004).

The use of tethered males in our experiments was initiated because we rarely observed agonistic displays during our field study. All observed visual displays were given at inter-individual distances below 30 cm. Resident males performed both soundless vocal-sac inflations and clearly audible calls. Introduced males rarely performed vocalizations or purely visual signals, possibly because we tied the frogs to the fishing-line with elastic rubber bands around their waist. Inter-male encounters are highly dominated by unimodal visual displays, and soundless vocal-sac inflations are the prominently given signals at a short distance. The Brazilian Torrent frogs, Hylodes asper and H. dactylocinus will call even when no rivals are present, while flagging signals (with or without accompanying calls) are given only once the presence of a rival is detected, indicated by the defendant’s rapid turn towards the intruder (Hödl et al., 1997).

The evolution of visual communication in anuran species apparently has been favored by the availability of ambient light, noisy environments, and highly structured habitats (Hödl and Amézquita, 2001; Lindquist and Hetherington, 1996). Most frog species exhibiting visual displays are diurnal. In P. kreffitii, the dorsal coloration does not contrast against natural backgrounds. Amézquita and Hödl (2004) discuss the possible importance of visual signaling for communication in environments where acoustic localization of the sender is hindered. Calling males were found on the ground or on low perches between the boulders of the creek-bed and the vegetation. Under such conditions, sound propagation may be reduced (Penna and Solis, 1998; Richards and Wiley, 1980). Concealing coloration and highly structured environments may reduce localization of senders to predators. During this study we observed several vertebrates (e.g., mongoose, heron) as well as large crabs preying upon P. kreffitii. In spite of the potential benefits as an anti-predator strategy, calling from relatively hidden perches and dull coloration reduces the localization of males to potential conspecific receivers, which could, in turn, lessen mating opportunities. Within this scenario, the simultaneous production of auditory and visual signals may increase the sender’s localization at short distances, when a conspecific receiver is detected.

River margins with shallow water, the preferred habitat of P. kreffitii, provide constant humidity, reducing the risk of desiccation which supports diurnal activity. The combination of all these factors probably favored the evolution of contrasting colors and dynamic visual communication in P. kreffitii. Due to several global climatic changes in geological history (Lovett, 1993), the annual amounts of precipitation in the study area varies. It is reasonable to believe that visual signaling with the brightly colored vocal sac originally evolved within a much noisier environment. Highly eroded riverbeds near Amani spectac-
ularly demonstrate higher amounts of rainfall over the course of geological history.

The present study shows for the first time that the anuran vocal sac can be used for repeated signaling in males without accompanying audible sound production.

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PHENOTYPIC EFFECTS OF THERMAL MEANS AND VARIANCES ON SMOOTH SOFTSHELL TURTLE (APALONE MUTICA) EMBRYOS AND HATCHLINGS

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ABSTRACT: Temperature is a crucial factor in the development of oviparous organisms. Under natural conditions, the eggs of many species are subjected to changing thermal environments, but most laboratory studies have incubated eggs at constant temperatures. To evaluate the phenotypic effects of different thermal means and variances and to separate temperature effects from maternal effects, eggs from 10 clutches of smooth softshell turtles (Apalone mutica) were equally distributed among six temperature treatments that reflect thermal conditions observed in natural nests: two eggs each at a mean of 28.5 or 32.5 °C, with ranges of 0, 2, and 4 °C. In addition to embryonic traits (change in egg mass, hatching success, and incubation length), we measured and evaluated body size, swimming performance, and righting time of the hatchlings. The interaction between mean temperature and temperature fluctuation exerted a significant influence on eight of the ten traits we measured, indicating that fluctuating temperatures do not have equivalent phenotypic effects at different mean temperatures. Clutch of origin also was responsible for explaining a large fraction of the variation for nearly all of the traits. Altogether, these results suggest that clutch effects are pervasive and that thermal effects during embryonic development are complex and deserve further investigation.

Key words: Apalone mutica; Clutch effects; Eggs; Incubation temperature; Offspring; Turtle

PHENOTYPE is the end result of an amalgam of genetic, maternal, and environmental effects experienced by an embryo during its development (Ackerman, 1991). Due to the combination of effects involved in the natural development of a phenotype, describing a physical trait in terms of a single causative factor is difficult. In a laboratory setting,