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Large odorous frogs (*Odorrana graminea*) produce ultrasonic calls

Jun-Xian Shen · Zhi-Min Xu · Albert S. Feng ·
Peter M. Narins

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Abstract We present the first data on the vocalizations of large odorous frogs (*Odorrana graminea*, previously *Odorrana livida*), from southern China. The males produce diverse broadband signals most of which contain ultrasonic harmonics. Six basic call-types were identified based on the number of call notes, fundamental frequency, call/note duration, frequency modulation patterns and spectral composition. *O. graminea* is one of only a few non-mammalian vertebrates able to detect ultrasound, but its tympanic membranes are not recessed. These results should stimulate further studies to provide new insights into the mechanisms underlying high-frequency communication in anurans.

Keywords Ultrasonic detection · Tympanum · Anuran · *O. livida* · Hearing

Introduction

Acoustic communication plays a vital role in anuran reproduction. Frogs and toads generally produce a limited number of repetitive, highly stereotyped communication calls

that exhibit little spectral and temporal variation and contain frequencies between approximately 100 Hz and 5–6 kHz (Capranica 1965; Narins and Capranica 1977; Brenowitz et al. 1984; Ryan 1985; Schwartz and Wells 1986; Glaw and Vences 1994). However, some Old World frogs can produce quite diverse vocalizations. The treefrog *Polypedetes leucomystax* in Southeast Asia may produce up to nine distinct calls (Narins et al. 1998; Christensen-Dalsgaard et al. 2002). The endemic Madagascar treefrog *Boophis madagascariensis* has a large vocal repertoire with 28 distinct call types that differ in temporal pattern and spectral bandwidth (Narins et al. 2000).

Recent research has revealed that males of the concave-eared torrent frog *Odorrana tormota* (previously *Rana tormotus*; Wu 1977), which possess deeply sunken tympana, have an unusually large call repertoire with calls containing spectral energy in the ultrasonic range, i.e., above 20 kHz (Narins et al. 2004) and are able to detect frequencies up to ~34 kHz (Feng et al. 2006). Another frog known to possess recessed tympanic membranes is the Bornean endemic, *Huia cavitympanum*, which emits extraordinarily high-frequency calls, some of which contain spectral energy in the ultrasonic range only (Arch et al. 2008). Ultrasonic communication in both *O. tormota* and *H. cavitympanum* may have evolved to avoid masking by the intense, predominantly low-frequency ambient noise generated by swift-flowing mountain streams in their habitat. These species do not overlap in geographical distribution and are unrelated at the generic level (Cai et al. 2007; Stuart 2008). Whether ultrasonic communication is limited to frogs with recessed tympana or is more widespread among anurans is unclear.

The large odorous frog (*Odorrana graminea*, previously *O. livida*) is an arboreal, nocturnal frog living near noisy streams and waterfalls in mountainous areas (elevation > 450 m) of southern China. *O. graminea* does

J.-X. Shen (✉) · Z.-M. Xu
State Key Laboratory of Brain and Cognitive Science,
Institute of Biophysics, Chinese Academy of Sciences,
Beijing 100101, China
e-mail: shenjx@ibp.ac.cn

A. S. Feng
Department of Molecular and Integrative Physiology,
University of Illinois at Urbana-Champaign, Urbana,
IL 61801, USA

P. M. Narins
Department of Integrative Biology and Physiology,
Department of Ecology and Evolutionary Biology,
University of California, Los Angeles, CA 90095-1606, USA

not possess recessed tympana, yet it has the ability to detect ultrasound up to 22 kHz (Feng et al. 2006). Thus, data from this species do not support the hypothesis that recessed tympana are required for the reception of ultrasound. For a better understanding of its acoustic communication, we recorded the vocalizations of the males of *O. graminea* and provide the first descriptive report of this species' call repertoire.

Methods

Field recordings were carried out between 1930 and 2130 hours along the Tau Hua Creek (30°06'N, 118°10'E) in Huangshan Hot Springs, China on 30 July–8 August 2010, with an ambient temperature and humidity of ~22°C and ~60%, respectively. Frog vocalizations were recorded using a 1/4" wideband omni-directional microphone and a preamplifier (models 40BE and 26CB, respectively, G.R.A.S. Sound & Vibration, Holte, Denmark; Frequency response, 4 Hz–100 kHz, ±3 dB) and a digital audio recorder (Sound Devices model 722, Sound Devices, Reedsburg, WI, USA) with a sampling rate of 96 kHz and 16-bit resolution. Several vocalization bouts were also recorded with a high-resolution digital video camera (Sony HDR-SR7) for visual identification of an individual male's calling. Data were saved as wav files. Later in the laboratory, files were transferred to a laptop computer and analyzed (FFT, 512 points) and displayed using SELENA, a custom-designed program (S. Andrzhhevski, St. Petersburg) (Narins et al. 2004; Feng et al. 2006; Shen et al. 2008), and PRAAT (Boersma and Weenick 2008; Feng et al. 2009b).

Results

We recorded and analyzed spontaneous vocalizations from 16 positively identified males of *O. graminea*. Background noise at the calling sites was primarily produced by running water in nearby creeks and was broadband with a peak intensity of ~64 dB SPL around 100 Hz, decaying by 18 and 45 dB at 2 and 20 kHz, respectively.

Males of *O. graminea* produce a wide variety of vocalizations. Unlike most frog species, but similar to the vocal signals of males of *O. tormota* (Feng et al. 2002, 2009b), spectrotemporal features of their calls are highly variable both within and between individual males, indicating that their sound communication is likely more advanced. Six basic call-types were observed (Table 1): short (Fig. 1a) and long (Fig. 1b) tonal calls that contain shallow or no frequency modulation (FM), multi-note calls with 2–4 clustered notes (Fig. 1c), FM calls with overt frequency modulation throughout the duration of the call (Fig. 1d), narrow-band calls (Fig. 1e), and staccato calls (Fig. 1f).

Short tonal calls were the dominant call-type in the field (56/93 recorded, 60.2%). These are characterized by a constant fundamental frequency (F_0) or contain shallow FM over a portion (usually toward the end) of the call; the F_0 can be either low (3.1 ± 0.7 kHz) (mean \pm S.D.), or high (7.4 ± 1.1 kHz). Short tonal calls exhibit pronounced harmonics over the frequency range of the recording system (0.1–48 kHz). FM calls were the second-most common call-type in the field (25/93, 26.9%). These calls generally contain clear FM components with F_0 sweeping down from 5.4 ± 1.4 to 2.3 ± 0.5 kHz; the call harmonics extend to 48 kHz, or beyond. One-note long tonal calls typically have a lower F_0 (3.1 ± 0.7 kHz); the call harmonics reach

Fig. 1 Example spectrograms (top panel) and waveforms (bottom panel) of vocal signals of males of the large odorous frog, *Odorrana graminea* in Anhui Province, China. **a** Short tonal call. **b** Long tonal call. **c** Multi-note calls. **d** FM call. **e** Narrow-band call. **f** Staccato call. In **a–f** the background noise (up to 18 kHz) is due to the rushing water in the Tau Hua Creek. Scale bar 25 ms (**e**), 50 ms (**a, b, d**), 150 ms (**c, f**). For all plots, dynamic range: 80 dB; FFT: 512 points; temperature during recordings: $22 \pm 3^\circ\text{C}$

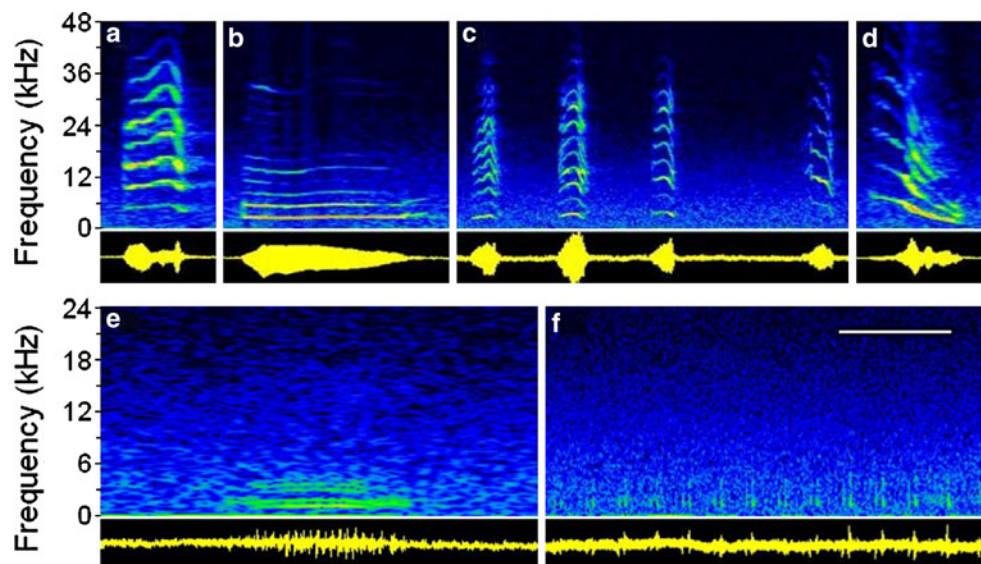


Table 1 Spectrotemporal characteristics of vocal signals of males of *O. graminea*

Call-type	Duration (ms)	F_0 (kHz)	F_{max} (kHz)	Occurrence
Short tonal (LF)	27.3 ± 10.1	3.1 ± 0.7	29.3	37/93
Short tonal (HF)	36.4 ± 13.9	7.4 ± 1.1	44.1	19/93
Long tonal	72.3 ± 9.6	3.1 ± 0.7	34.5	5/93
Multi-note	35.4 ± 12.4 ^a	4.2 ± 1.1	42.5	5/93
FM	26.6 ± 8.4	5.3 ± 1.5–2.3 ± 0.5	44	25/93
Narrow-band	39.5	0.58	3.6	1/93
Staccato	3.5 ± 0.5 ^a	1.3–1.7	5.1	1/93

The descriptive statistics (mean ± S.D.) for diverse types of calls are presented. $n = 16$ males

F_0 fundamental frequency, F_{max} maximum frequency in harmonics of the call (this represents the highest frequency that is visible in the Selena spectrogram, with a range of relative amplitude of 0 to –80 dB, and a sampling rate of 96 or 192 kHz)

^a Durations refer to those of the individual notes within these calls

~35 kHz only. Multi-note calls are characterized by a series of short notes (note duration = 35.4 ± 12.4 ms) in which F_0 shifts progressively from ~3 to ~6 kHz; they show pronounced harmonic stacks up to ~42 kHz. The above calls all have substantial energy in the ultrasonic range. In contrast, the one narrow-band call we recorded (~40 ms duration) had a very low F_0 (~0.6 kHz); the call energy is limited to the audible range, with no ultrasonic components. This narrow-band call is further characterized by the presence of subharmonics and harmonics throughout the call. The single staccato call comprised a series of double pulses (each pulse with a duration of ~3.5 ms) with a repetition rate of ~22.7 Hz—the doublets are repeated over a period of about 1.5 s and have a low F_0 (1.3–1.7 kHz). Like the narrow-band call, the staccato call contains no energy in the ultrasonic range.

Discussion

Sound communication in noisy habitats is challenging. Among vertebrates until recently, only three groups of mammals: whales (Sales and Pye 1974) and dolphins (Au 1993), bats (Fenton 1984; Bohn et al. 2009; Melendez and Feng 2010), and some rodents (Ehret 1992) were known to use ultrasound to communicate. Communication with ultrasounds has the advantage of minimal masking by biotic, as well as abiotic, background sounds which have energy concentrated at low frequencies. Recent studies demonstrate that (a) two species of sunken-tympanum frogs (*O. tormota* and *H. cavitympanum*) are capable of generating and detecting calls with energy extending into the ultrasonic range, and (b) two bird species (*Abroscopus albogularis* and *Lapornis clemenciae*) also produce a variety of calls with strong ultrasonic components (Narins et al. 2004; Pytte et al. 2004; Feng et al. 2006; Arch et al. 2008). The present study provides evidence that males of the large

odorous frog (*O. graminea*) also emit vocal signals containing ultrasonic harmonics. As to its ultrasonic sensitivity, electrophysiological data on the auditory evoked potentials from the frog's midbrain were consistent with an ability to detect ultrasound up to 22 kHz (Feng et al. 2006). Thus, the arboreal, nocturnal frog species *O. graminea* can be added to the select list of anuran amphibians that produce and detect ultrasound, following *O. tormota* and *H. cavitympanum*. However, for *O. graminea*, it is noted that most of the ultrasonic energy present in their calls (e.g., Fig. 1a) is inaudible for them. These findings also support the hypothesis that the upward extension into the ultrasonic range of both the harmonics of the advertisement calls and the hearing sensitivity may be the result of selective pressure to avoid masking by the intense, predominantly low-frequency ambient noise from local streams and therefore benefit short-distance communication.

One question is raised: How do *O. graminea* detect ultrasound? Males of *O. tormota* have highly unusual recessed tympana (i.e. ear canals), which likely facilitate high-frequency hearing. Reduced mass of the middle-ear ossicles and extremely thin tympanic membranes facilitate transmission of high-frequency sounds to the inner ear (Feng et al. 2006). Unlike the males of *O. tormota*, males of *O. graminea* do not have recessed ears, but similar to the males of *O. tormota* they possess very thin (12–15 μm) and transparent tympana. At this time, exactly how *O. graminea* detects ultrasound remains to be determined. It is possible that the middle ear morphology of *O. graminea* represents an intermediate stage of evolution for the overall transformation of thick tympana that are sensitive to low-frequency sounds into recessed and thin tympana that are sensitive to ultrasound. Alternatively, the observed morphology in *O. graminea* may represent a final stage of evolution for detection of high-frequency sound (but not deep into the ultrasonic range)—the high frequency sensitivity is adequate for reducing the masking effects of the ambient noise.

Whereas most vocal signals of male *O. graminea* contain substantial ultrasonic energy, two of their call-types (narrow-band and staccato calls) do not. In addition to having no ultrasonic energy, these calls are weak, apparently designed for short-range communication. In males of *O. tormota*, staccato calls are the predominant call types emitted during male–female interaction, i.e., upon hearing a female's call, a male typically produces staccato calls prior to initiating phonotaxis toward the female (Shen et al. 2008). Also, staccato as well as narrow-band calls are often heard when males are engaged in short-range male–male interactions, e.g., in neighbor–stranger discrimination (Feng et al. 2009a). We speculate that their functions in *O. graminea* are not different than those in *O. tormota*—further study, however, is necessary to validate this tenet.

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References

- Arch VS, Grafe TU, Narins PM (2008) Ultrasonic signaling by a Bornean frog. *Biol Lett* 4:19–22
- Au WWL (1993) *The sonar of dolphins*. Springer, New York
- Boersma P, Weenick D (2008) PRAAT: doing phonetics by computer (version 5.2). <http://www.praat.org>. Accessed August 2010
- Bohn KM, Schmidt-French B, Schwartz C, Smotherman M, Pollak GD (2009) Versatility and stereotypy of free-tailed bat songs. *PLoS ONE* 4:e6746
- Brenowitz EA, Wilczynski W, Zakon HH (1984) Acoustic communication in spring peepers: environmental and behavioral aspects. *J Comp Physiol A* 155:585–592
- Cai HX, Che J, Pang JF, Zhou EM, Zhang YP (2007) Paraphyly of Chinese *Amolops* (Anura, Ranidae) and phylogenetic position of the rare Chinese frog, *Amolops tormotus*. *Zootaxa* 1531:49–55
- Capranica RR (1965) *The evoked vocal response of bullfrog: a study of communication by sound*. Res Monograph 33. MIT, Cambridge
- Christensen-Dalsgaard J, Ludwig TA, Narins PM (2002) Call diversity in an Old World treefrog: level dependence and latency of acoustic responses. *Bioacoustics* 13:21–35
- Ehret G (1992) Categorical perception of mouse-pup ultrasounds in the temporal domain. *Anim Behav* 43:409–416
- Feng AS, Narins PM, Xu CH (2002) Vocal acrobatics in a Chinese frog, *Amolops tormotus*. *Naturwissenschaften* 89:352–356
- Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX (2006) Ultrasonic communication in frogs. *Nature* 440:333–336
- Feng AS, Arch VS, Yu ZL, Yu XJ, Xu ZM, Shen JX (2009a) Neighbor-stranger discrimination in concave-eared torrent frogs, *Odorrana tormota*. *Ethology* 115:851–856
- Feng AS, Rieder T, Arch VS, Yu ZL, Xu ZM, Yu XJ, Shen JX (2009b) Diversity of the vocal signals of concave-eared torrent frogs (*Odorrana tormota*): evidence for individual signatures. *Ethology* 115:1015–1028
- Fenton MB (1984) Echolocation: implications for ecology and evolution of bats. *Q Rev Biol* 59:33–53
- Glaw F, Vences M (1994) *A fieldguide to the amphibians and reptiles of Madagascar*, 2nd edn. M. Vences & F. Glaw Verlags GbR, Köln
- Melendez KV, Feng AS (2010) Communication calls of little brown bats display individual-specific characteristics. *J Acoust Soc Am* 128:919–923
- Narins PM, Capranica RR (1977) An automated technique for analysis of temporal features in animal vocalizations. *Anim Behav* 25:615–621
- Narins PM, Feng AS, Yong HS, Christensen-Dalsgaard J (1998) Morphological, behavioral and genetic divergence of sympatric morphotypes of the treefrog *Polypedetes leucomystax* in Peninsular Malaysia. *Herpetologica* 54:129–142
- Narins PM, Lewis ER, McClelland BE (2000) Hyperextended call note repertoire of the endemic Madagascar treefrog *Boophis madagascariensis* (Rhacophoridae). *J Zool Lond* 250:283–298
- Narins PM, Feng AS, Lin WY, Schnitzler HU, Denzinger A, Suthers RA, Xu CH (2004) Old World frog and bird vocalizations contain prominent ultrasonic harmonics. *J Acoust Soc Am* 115:910–913
- Pytte CL, Ficken MS, Moiseff A (2004) Ultrasonic singing by the blue-throated hummingbird: a comparison between production and perception. *J Comp Physiol A* 190:665–673
- Ryan MJ (1985) *The túngara frog, a study in sexual selection and communication*. University of Chicago Press, Chicago
- Sales GD, Pye JD (1974) *Ultrasonic communication by animals*. Chapman and Hall, London
- Schwartz JJ, Wells KD (1986) Interspecific acoustic interactions of the neotropical treefrog *Hylaebaccata*. *Behav Ecol Sociobiol* 14:211–224
- Shen JX, Feng AS, Xu ZM, Yu ZL, Arch SV, Yu XJ, Narins PM (2008) Ultrasonic frogs show hyperacute phonotaxis to female courtship calls. *Nature* 453:914–916
- Stuart BL (2008) The phylogenetic problem of *Huia* (Amphibia: Ranidae). *Mol Phylogenet Evol* 46:49–60
- Wu GF (1977) A new species of frogs from Huang-Shan, Anhui, *Rana tormotus* Wu. *Acta Zool Sinica* 23:113–115