Advertisement Calls of Two Horned Frogs, *Megophrys kuatunensis* and *M. huangshanensis*, from China (Anura, Megophryidae)

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Abstract: Animals communicating by sound face interference from biotic and abiotic sources. Contrasting strategies have been reported in different taxa in the presence of prolonged noise. Some torrent-living frogs of the genera *Odorrana* and *Huia* emit ultrasounds to avoid masking by environmental noise. That strategy, however, might not be the only mode of acoustic communication for inhabiting along fast-flowing streams. To examine this possibility, we analyzed call structures of two horned toads, *Megophrys kuatunensis* and *M. huangshanensis*, which inhabit along streams in eastern China. We investigated variation in call properties within and between the two species and found that the two species show similar call structures but significantly differ in note duration and inter-note interval. Both of the two species concentrate energy on a single, wide harmonic band, and this might be an acoustic strategy against environmental noise.

Key words: Acoustic communication; Advertisement calls; Anurans; Horned frogs; Stream breeding

INTRODUCTION

It is commonly believed that acoustic cues are important for attracting conspecifics and for mediating spacing between individuals and groups of diverse animals such as primates, birds, tailless amphibians, and insects (Altmann, 1959; Marler, 1972; Whitney and Krebs, 1975; Campbell and Shipp, 1979; Doolan and MacNally, 1981; Brenowitz et al., 1984; Robertson, 1984). The vocalization of frogs is an important biological characteristic among extant amphibians, and the mating or advertisement call of male frogs is the medium through which frogs conduct their reproductive activities. However, communicating by sound always faces interference from biotic and abiotic sources since the acoustic signals must first be transmitted through the environment in order to play a role in communication.

Confronting this noise interference, animals have evolved different strategies including
changes in vocalization amplitude (Cynx et al., 1998; Pytte et al., 2003; Brumm, 2004; Kaiser et al., 2011), emission rate (Lengagne et al., 1999; Sun and Narins, 2005), duration (Kaiser et al., 2011; Martinez-Rivera and Gerhardt, 2008), inter-call interval (Martinez-Rivera and Gerhardt, 2008), and frequency structure (Katti and Warren, 2004; Parris et al., 2009), and adjustments in the timing of emissions to intervals when the interference decreases or is absent (Greenfield, 1988).

Stream-dwelling frogs face interference from the wide-band background noise of local, fast-flowing torrents. To avoid masking by that noise, some *Odorrana* and *Huia* frogs communicate with bird-like melodic calls with pronounced frequency modulations that often contain spectral energies in the ultrasonic range (Feng et al., 2006; Arch et al., 2009; Boonman and Kurniati, 2011; Shen et al., 2011). The frog *O. tormota* was demonstrated to produce and detect ultrasounds, and this is the first case of non-mammalian ultrasonic communication. *Huia cavitypanum*, which is phylogenetically remote from *O. tormota* (Pyron and Wiens, 2011), can even use pure ultrasound to communicate between conspecific males (Arch et al., 2009). The convergence of call characteristics of these species implies that similar habitats might be an important factor in shaping the call structures of fast-flowing stream-dwelling frogs.

Basic mating call structures of some horned frog species of the genus *Megophrys*, which also occur around streams, have been reported (e.g., Wang et al., 2014), but to our knowledge no reports have associated their mating call structures with stream noises. In order to understand such associations, we studied *Megophrys kuatunensis* and *M. huangshanensis*, which live along streams and whose males call there in breeding seasons under noisy conditions (Fei et al., 2012).

Female frogs in many species choose mates, at least in part, by recognizing the species-specific component of male calls (Gerhardt, 1991a,b). For accurate recognition, calls of each species must differ from those of the other sympatric species (Wollerman and Wiley, 2002). Various studies have shown that the advertisement call represents a premating isolating mechanism in anurans (Duellman and Trueb, 1986; Hoskin et al., 2005), which makes it a valuable tool in taxonomy (Matsui, 2006; Matsui et al., 2009; Hertz et al., 2012).

On the other hand, some call components show substantial variation within a single population (Sullivan and Wagner, 1988; Briggs, 2010) or even within a single individual (Gerhardt, 1991). As a result, traits can be classified as static (low within-male variation) or dynamic (high within-male variation) call properties, and the variability found in dynamic properties may account for their importance in mate choice (Gerhardt, 1991, 1994b). Static call traits include dominant frequency, which is not expected to change greatly because it is a function of body size (Gerhardt, 1991). Dynamic call properties like call rate and call duration are typically the products of both abiotic and biotic factors (Gerhardt, 1991).

In the present study, both static (dominant frequency) and dynamic (note duration, inter-note interval) call properties of male advertisement calls of two stream-dwelling horned frogs, *M. kuatunensis* and *M. huangshanensis*, were investigated. Based on the spectral structures, we examined how the stream-dwelling *Megophrys* species employ specific acoustic communication modes. Those may be different from the acoustic modes of sympatric *Odorrana* species, which can extend some harmonic energy into the ultrasonic range. We also conducted statistic comparisons within and between species to explore the call traits associated with species recognition and individual variation.

**Materials and Methods**

**Study site**

The study of *M. kuatunensis* was conducted from 16 to 23 March 2011 at the locality of Guadun Village (27°44′41″N, 117°38′05″E, 1360 m asl) in Wuyishan City, Fujian Province,
China, which is the type locality of this species (Pope, 1929). The air temperature varied between 12.5 and 18°C. All recordings were carried out between 19:45 and 0:30 h, when calling activity was most intense.

The study of *M. huangshanensis* was carried out on 21 July 1992 (one individual) near the type locality (Fei and Ye, 2005), and from 26 July to 1 August 2011 (three individuals) and from 18 to 22 July 2012 (one individual) at Fuxi Village (30°05′3″N, 118°08′46″E, 563 m asl) in Huangshan City, Anhui Province, China, about 4 km southwest to the type locality (Fei and Ye, 2005). The air temperature fluctuated between 17 and 22°C. All recordings were carried out between 18:45 and 0:30 h, during the most intensive calling activities. Recordings were made sequentially for each individual encountered, and the temporary number and the time of recording were also noted for later analyses. We did not measure body sizes of frogs.

**Acoustic recording and call digitization**

Vocalizations of *M. kuatunensis* (48 calls from two males) and *M. huangshanensis* (134 calls from five males) were recorded and digitized using a Portable Linear PCM Recorder (Sony PCM-D50). Exceptionally, recording of one *M. huangshanensis* (36 calls) was made using a cassette tape recorder (Sony TC-D5), and the vocalizations were digitized at 16-bits and 44 kHz like other digitized recordings. Sounds were later transferred onto a PC (Lenovo Windows 7 PC), where recordings were stored at a sampling rate of 44.1 kHz.

Calls were then categorized according to the degree of frequency modulation and harmonic structure (Feng et al., 2009). The call properties were extracted with PRAAT software (Boersma and Weenink, 2009). Call attributes, following the definition of Matsui (1997), included mean note duration, mean inter-note interval between separate notes, mean dominant frequency (mean DF), and minimum and maximum dominant frequencies (MinDF and MaxDF) for each male. A note is defined as a complete sequence of pulses separated from the next note by a notable gap (inter-note interval). Dominant frequency of a call is defined as the harmonic frequency that carries the most energy.

**Analysis and statistics**

Differences among groups were analyzed by a Kruskal-Wallis ANOVA on ranks because the data were not normally distributed. All multiple comparison tests were two-tailed. Direct comparisons between two groups were performed with the nonparametric Mann-Whitney U test. We conducted pair-wise comparisons of call traits among all the individuals using Dennett’s post hoc test because equal variances were not assumed. All statistical analyses were performed using SPSS statistical software (SPSS 17.0 for Windows, Release 17.0.0, SPSS Inc., Chicago, IL).

**Results**

**Call characteristics**

We recorded and analyzed spontaneous vocalizations from five males of *M. huangshanensis* and two of *M. kuatunensis*. Just one basic call type was observed (Figs. 1 and

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**Fig. 1.** Sound spectrograms (A) and waveforms (C) of three notes within a call, a slice amplitude spectrum (B), and waveforms of two calls within a call bout (D) of *Megophrys kuatunensis*; vocalizations recorded at Guadun Village.
2): short tonal multi-calls within a call bout that contain shallow or no frequency modulation (FM). The energy of the call was concentrated in one wide frequency band.

The call characteristics of *M. kuatunensis*, based on the calls from two individuals recorded at Guadun Village, were: mean (±SE) note duration=0.11±0.01 s (n=48); inter-note interval: 0.71±0.14 s (n=45); MinDF: 3.23±0.02 kHz (n=48); MaxDF: 3.40±0.15 kHz (n=48); and mean DF: 3.27±0.02 kHz (n=48).

The call characteristics of *M. huangshanensis*, based on the calls from five individuals, are: mean note duration=0.10±0.02 s (n=168); inter-note interval: 0.18±0.04 s (n=159); MinDF: 3.24±0.23 kHz (n=168); MaxDF: 3.40±0.223 kHz (n=168); and mean DF: 3.31±0.22 kHz (n=168).

**Analysis and statistics**

There were significant differences between *M. kuatunensis* and *M. huangshanensis* in the note duration and inter-note interval (P<0.01, Mann-Whitney test). However, there was no significant difference between them in MinDF (P=0.16, Mann-Whitney test), MaxDF (P=0.42, Mann-Whitney test), or mean DF (P=0.11, Mann-Whitney test).

Within *M. kuatunensis*, the note duration (P<0.01, Mann-Whitney test), MaxDF (P<0.01, Mann-Whitney test), and mean DF (P<0.01, Mann-Whitney test) showed significant variation between individuals, but the inter-note interval (P>0.05, Mann-Whitney test) and MinDF (P>0.05, Mann-Whitney test) did not differ. In contrast, within *M. huangshanensis*, all the above traits showed significant differences among individuals (P<0.01, Kruskal-Wallis test).

We conducted pair-wise comparisons of call traits among all of the individuals using Dunnett’s post hoc test, without assuming equal variances. Each individual was numbered, numbers 1 and 2 for *M. kuatunensis*, and numbers 3–7 for *M. huangshanensis*. There were no significant intraspecific differences between individuals 1 and 2 in the inter-note interval or MinDF, between individuals 4 and 6 in the inter-note interval, between individuals 3 and 7 in the inter-note interval.
interval, or between individuals 6 and 7 in MinDF (Table 1). In interspecific comparisons, there were no significant differences between individuals 1 and 3, between 1 and 5, or between 2 and 7 in MaxDF; or between individuals 2 and 5, or between 2 and 6 in note duration (Table 1). The remaining pair-wise comparisons all resulted in significant differences (P<0.05, Dunnett’s post hoc test).

**Discussion**

Acoustic signals of male frogs usually contain information regarding species identity, size, fighting ability (Gerhardt and Huber, 2002), quality of parental care (as in some birds; Welling et al., 1997; Buchanan and Catchpole, 2000), and/or species’ adaptation to their acoustic environment. In this study, we report results from statistical analyses of advertisement calls of *M. kuatunensis* and *M. huangshanensis*, which showed possible traits related to acoustic adaption, species identity, and individual condition.

*Adaptation to the acoustic environment*

Communication is shaped in part by the external environment. Signal detectability not only depends on the receiver’s sensory system (Narins and Capranica, 1976; Arch et al., 2012) but also on the signal design of the sender and the conditions of the environment (Endler, 1992; Grafe et al., 2012). During sound transmission through the environment, signals are subject to masking from abiotic and biotic noise (i.e., signals of other noisy animals, including vocal signals of conspecifics). For riparian anurans, a major source of abiotic noise is that of rapidly flowing streams or waterfalls.

Narins (1982) concluded six strategies by which a calling frog could improve its ability to communicate in a noisy environment: 1) spectral separation strategy, namely employing unusual frequencies; 2) temporal separation strategy, calling when the environment is relatively quiet; 3) calling at a greater intensity than its neighbors; 4) producing a periodic, stereotyped call to reduce its variability and hence facilitate localization; 5) adjusting its auditory receptors to match the spectral and temporal features of the species-specific vocalizations; and 6) possessing a high degree of frequency resolution for extracting the species’ call from the background noise.

By combining strategies 1 and 5, two *Odorrana* species, *O. tormota* and *O. graminea*, use ultrasonic communication in loud stream environments (Feng et al., 2006; Shen et al., 2011), where males can produce calls with prominent energy distribution on high frequency harmonics, in line with their high frequency sensitivity (Feng et al., 2006).

Although we did not analyze environmental noise at the sites of recording, environmental noise of the streams of Huangshan, where *M. huangshanensis* sympatrically occurs with *O. tormota*, have a peak around 0.1 kHz, substantial amplitudes over 0.2–10 kHz, and a progressive decline from 11–22 kHz (Feng et al., 2006). *Megophrys kuatunensis* is also sympatric with the *Odorrana* species, but the two megophryids completely differ in acoustic characteristics from the *Odorrana*. First, the dominant frequencies of the two megophryids are lower than those of the two sympatric *Odorrana* species (Feng et al., 2006; Shen et al., 2011) (strategy 1). Second, the two *Megophrys* species produce a periodic, stereotyped call to reduce its variability and hence facilitate the localization (strategy 4). Finally, the most conspicuous strategy is to concentrate their spectral energy in a single frequency band, which might subserve calling at a greater intensity than the background noise (strategy 3). Thus, it is conceivable that these two megophryid species adopt a combination of these strategies to mask the background noises in a different way from two *Odorrana* species.

**Species identity**

As mentioned above, each species’ calls must differ from those of other species for accurate recognition of conspecifics (Wollerman and Wiley, 2002). Although having similar
acoustic traits, the two *Megophrys* species studied showed significant differences from each other in the note duration and inter-note interval. Statistical comparisons showed that the duration and dominant frequency (including minimum, maximum, and mean) all showed large overlap between the two species. Only the inter-note interval showed clear separation between them. Resemblance in duration and dominant frequency in the two species may be due to phylogenetic constraint. However, our results differ from those reported by Wang et al. (2014) in this regard. For *M. huangshanensis*, they showed note duration of 0.09 s and inter-note interval of 0.17 s, only slightly smaller than our values of 0.10 s and 0.18 s, respectively. In contrast, their values for *M. kuatunensis* (note duration=0.21 s, inter-note interval=1.00 s) are much larger than ours (0.11 s and 0.71 s, respectively). Reasons for the discordance in these temporal characteristics are unknown, but may partly be due to possible differences in temperatures at the time of recording, which they did not provide. Wang et al. (2014) also showed highest and lowest frequencies and the means of such values approximate our results.

**Individual variation**

Frogs’ acoustic signals usually exhibit variation among intraspecific individuals (Gerhardt, 1991; Feng et al., 2009; Briggs, 2010; Chakraborty et al., 2010). On one hand, this intraspecific variation may confer information about an individual’s condition. For example, males of some frog species can assess the size and fighting ability of rivals based on size-related variation in spectral properties of the calls (review in Gerhardt and Bee, 2006). Other studies of sexual selection and female choice have shown that female frogs commonly prefer the low-frequency calls of larger males or differentiate traits that potentially depend on a male’s condition, such as call duration and calling rate (Gerhardt and Huber, 2002; Bee et al., 2010; Richardson et al., 2010). On the other hand, call diversity may be caused by the senders’ acoustic regulation when they encounter variable conditions. For example, some male frogs will change their pulse rate (Schwartz and Wells, 1984; Owen and Gordon, 2005), duration, and interval (Martinez-Rivera and Gerhardt, 2008) when they encounter a conspecific male, and dominant frequency when they encounter background noise (Parris et al., 2009).

Since no aggregation or chorus was observed for either species in this study, the recorded calls are judged to be courtship calls. Intraspecific variations might have been affected by differences in surrounding temperatures that varied somewhat at the time of recording, and variations in body size, which we did not accurately measure. In addition to these possibilities, varied individual condition or calling adjustments to their microhabitat cannot be ruled out. These potential factors need further studies in the future.

**Acknowledgments**

We are grateful to Yuhan Wu of the Chengdu Institute of Biology, Chinese Academy of Sciences for his kind help in conducting the fieldwork. This study was funded by the National Natural Science Foundation of China (NSFC 31172055, 31471964), the Education Department of Guizhou (Nos. 20770066, 23770084), JSPS AA Science Platform Program, and the Kyoto University Foundation.

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Accepted: 2 January 2015