

## Acoustic characteristics of eight common Chinese anurans during the breeding season

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**Abstract:** Anurans often have species-specific vocalizations. To quantify and compare the characteristics of anuran calls in Gutianshan National Nature Reserve, Zhejiang Province, we recorded the advertisement calls of eight species belonging to four families (Ranidae, Microhylidae, Megophryidae and Bufonidae) from June to September 2012 using Sony ICD-FX8 IC recorders. All recordings were analyzed using the “Praat” software. Five acoustics parameters were measured, including temporal traits (call duration, number of notes or pulse number/call) and spectral traits (fundamental frequency, the first three formants and dominant frequency). The characteristic parameters of *Microhyla ornate* and *Fejervarya limnocharis* calls were different as were the calls of some populations of the same species recorded in different regions. The advertisement calls of the eight species were specific. Our study has provided a useful reference for identifying the calls of some common Chinese anurans.

**Keywords:** Anurans; Advertisement calls; Acoustic characteristics

Sound communication is one of the most important means of animal communication. Research on sound communications in insects, birds and mammals indicate that there are intimate connections between sound, social activities and behaviors (Li et al, 1993; Shen et al, 2007; Zhou et al, 2004). Anurans are the largest amphibian taxon and have a mate recognition system based on advertisement call (Marshall, 2005; Stuart et al, 2006). Vocalizations are therefore a useful diagnostic feature for identifying anuran species.

There is a large body of research on anuran vocalizations, including many studies of advertisement calls (McLeod et al, 2001). In some species, the quality and structure of male calls influence the outcomes of male-male contests and female choice (Bee, 1999; Pröhl, 2003; Yu & Zheng, 2009). Female always prefer male with calls of lower fundamental frequency, more variable calls or that can sustain calling longer than their competitors (Howard & Young, 1998; Morris & Yoon, 1989; Pröhl, 2003; Ryan & Drewes, 1990). Call surveys are a widely used and accepted monitoring technique for predicting anuran calling activity (Kirlin et al, 2006; Steelman & Dorcas, 2010).

A three-parameter model for classifying anurans on the basis of their advertisement calls correctly identified

species with an accuracy of >70% (Gingras & Fitch, 2013). Because of their highly species-specific character, some studies have used calls to evaluate relationships between different species and to identify cryptic species (Abrunhosa et al, 2005; Heyer & Barrio-Amorós, 2009; Nunes et al, 2007; Roy & Elepfandt, 1993; Wychwerley et al, 2002; Yu & Zheng, 2009).

To date, research on anuran vocalizations in China has been relatively limited. So far, aspects of the calls of more than 30 species have been analyzed, including pulse rate, dominant frequency, fundamental frequency and formant frequency (Chen et al, 2011; Cui et al, 2011; 2012; Huang et al, 1982; Jiang et al, 1995; Jiang et al, 2002; Matsui & Wu, 1994; Shen et al, 2008; Xu et al, 2005; Yu & Zheng, 2009; Wang et al, 2012; Wei et al, 2011; 2012; 2013). In recent years, research has focused more on the calls of single

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species in one location, rather than addressing differences between the calls of different species, or regional differences in the calls of the same species (Chen et al, 2012; Cui et al, 2011, 2012; Wei et al, 2011). Although the sound analysis software adopted in different studies was various, the results were still relatively consistent. However, the way to define parameters, such as pulse, note, etc., would make it difficult to compare the results from individual research groups.

We here present the results of an analysis of the vocalizations of the advertisement calls of eight common Chinese anurans based on the standard set of parameters used by Cui et al (2012). The species of anurans are extremely rich in China, but the basic datum of the vocalizations of the anurans advertisement calls are limited. This study aims to expand the datum of the vocalizations of anurans communication calls, and to provide a useful reference for identifying the calls of some common Chinese anurans.

## MATERIALS AND METHODS

### Research objectives

Our primary objective was to record, characterize and compare the advertisement calls of eight anuran species, *Bufo melanostictus* (Bufonidae), *Xenophrys boettgeri* (Megophryidae), *Microhyla ornate* (Microhylidae), *Odorrana schmackeri*, *Limnonectes fujianensis*, *Hylarana adenopleura*, *Fejervarya limnocharis* and *Hylarana guentheri* (Ranidae). *X. boettgeri*, *H. adenopleura*, *L. fujianensis* and *O. schmackeri* were recorded in the Gutianshan National Nature Reserve (N29.14°, E118.05°), Quzhou City, Zhejiang Province. *H. guentheri* and *B. melanostictus* were mainly recorded at Daluo Mountain (N27.93°, E120.71°), Chashan Town, and Wenzhou City. *F. limnocharis* and *M. ornata* were recorded in both locations.

### Recording methods

Anuran advertisement calls were recorded between 19:00 and 05:00 from June to September 2012, which was the most active period of the day and part of the year in which the species under investigation. Calls were recorded for 5–30 min by Sony IC recorders (ICD-FX8) without external directional microphone which were usually held 15–20 cm away from the animal being recorded. After the calls of an individual animal were recorded, the subject was photographed to aid subsequent identification (Cui et al, 2012).

### Analysis of call Parameters

*Cooledit Pro 2.0* was used to reduce background noise at a sampling rate of 44.1 kHz and 16 bit resolution after which *Praat 5.3.32* was used to analyze call traits

(Boersma & Wecnick, 2006). Each individual sound file for each species contained at least 10 calls. The acoustic parameters of calls measured including temporal properties, such as call duration, number of notes and note duration, as well as spectral properties, including dominant frequency (DF), fundamental frequency (F0) and harmonics (F1–F3). The Fast Fourier Transformation (FFT) procedure which the frame size is 512 in Matlab software (R2010b) was used to obtain the main peak, or dominant frequency, of each call. We didn't record the acoustic pressure which is affected by the distance of recording. Because of the large variation in number of notes and call duration between species, we analyzed and compared either the first or last note of the call of different species. Data were analyzed with independent-samples *t*-test (*t*-test). Statistics are presented as means  $\pm$  *SD*, the threshold of statistical significance was set at  $\alpha=0.05$ . Statistical analyses were performed by SPSS 13.0.

## RESULTS

Parameters of the calls of the eight species are in Table 1.

### Call Trait Analysis

#### Bufonidae

##### *Bufo melanostictus* (Figure 1a)

Males of this species have an internal vocal sac and call continually at night. Advertisement calls are composed of single notes which increase in amplitude during the call sequence. Variation in call duration was high (mean=47.22, *SD*=49.73, Table 1), which is probably a consequence of the small sample size (*n*=3).

#### Megophryidae

##### *Xenophrys boettgeri* (Figure 1b)

Based on analysis of 140 calls from six individuals, notes are generally frequent but short and the call duration is always long. The fundamental frequency (F0) was the highest among the eight species recorded (Table 2) and higher than the first (F1) frequency.

#### Microhylidae

##### *Microhyla ornate* (Figure 1c)

Males of this species have an internal vocal sac under a single pharyngeal sac. One hundred and twenty recordings of seven individuals from Gutianshan indicate that each call has a relatively long duration of up to 12 notes (mean=12.34, *SD*=2.20). However, 172 recordings of 11 individuals of the same species at Wenzhou (Table 2) indicate a pulse number up to 15 (*SD*=3.87). In addition, the calls of different individuals were found to have either one of two different dominant frequencies (DF) (Table 1).

Table 1 Descriptions of acoustic characteristics of eight anuran species (All data were not adjusted by temperature)

Species	Call duration/s	Note numbers/call	Note duration/s	Dominant frequency (DF, Hz)	Fundamental frequency (F0, Hz)	F1 (Hz)	F2 (Hz)	F3 (Hz)
<i>Bufo melanostictus</i> * (n=3)	6.14±4.86	47.22±49.73	0.14 ± 0.06	1758.50±72.24	874.08±45.28	1545.70±83.66	1806.14±68.21	2661.98±282.54
<i>Xenophrys boettgeri</i> (n=6)	6.26±1.97	25.99±8.64	0.08 ± 0.01	3468.94±129.54	1727.96±128.40	1443.28±176.37	2718.41±297.20	3381.73±188.51
<i>Microhyla ornate</i> (n=7)	0.26±0.06	12.34±2.20**		577.44±55.67 2221.84±235.35	1276.46±262.79	1425.32±69.17	2162.59±230.44	2795.22±228.84
<i>Hylarana adenopleura</i> (n=14)	0.64±0.21	2.15±0.79	0.18 ± 0.02	577.44±55.67 2221.84±235.3	309.85±22.34	813.66±64.38	1882.94±132.10	2361.65±141.08
<i>Limnonectes fujianensis</i> (n=3)	0.30±0.04	6.15±0.76**		531.09±50.76	512.59±15.04	667.29±25.12	1318.65±106.72	2186.64±235.71
<i>Odorrana schinackeri</i> (n=10)	0.04±0.02	1.64±0.52	0.03 ± 0.01	3479.14±406.70	908.45±76.18	1623.77±370.67	2457.01±464.83	3185.98±388.66
<i>Fejervarya limn-ocharis</i> (n=6)	0.16±0.08	1.33±0.49	0.07 ± 0.01	1456.28±83.55 2893.48±115.92	1458.94±65.72	1374.81±87.23	2108.96±422.67	2794.47±241.51
<i>Hylarana guentheri</i> * (n=3)	0.15±0.01	1.00±0.00	0.15 ± 0.01	354.34±6.87	324.38±35.49	618.56±120.47	1189.61±147.98	1679.43±191.43

\*: Calls recorded at Wenzhou, the remaining six species were recorded at Gutianshan; \*\*: Pulse number/call; The first note of *Xenophrys boettgeri* and *Hylarana adenopleura* were used to analyze the last five parameters excluding call duration, note number and note duration; The corresponding parameters of *Bufo melanostictus* were determined by the analysis of the last note; Data are presented as mean±SD; Given that in some species there are two apparent dominant frequencies, for the parameters of dominant frequency, two values were present.

## Ranidae

### *Hylarana adenopleura* (Figure 1d)

Males of this species have paired internal pharyngeal vocal sacs and calls that are relatively easy to identify. One hundred and twenty-one calls from 14 individuals indicate that different individuals have either one of two dominant frequencies (DF), one at 577 Hz and the other at about 2 221 Hz. The fundamental frequency (F0) was the lowest among the eight species recorded (Table 1).

### *Limnonectes fujianensis* (Figure 1e)

Males of this species don't have vocal sacs and occasionally call during the day. The call analyzed was chosen from 33 recordings of 3 individuals. Each note lasted 317~351 ms and the dominant frequency (DF), fundamental frequency (F0) and first frequency were close (Table 1). However, it was very difficult to

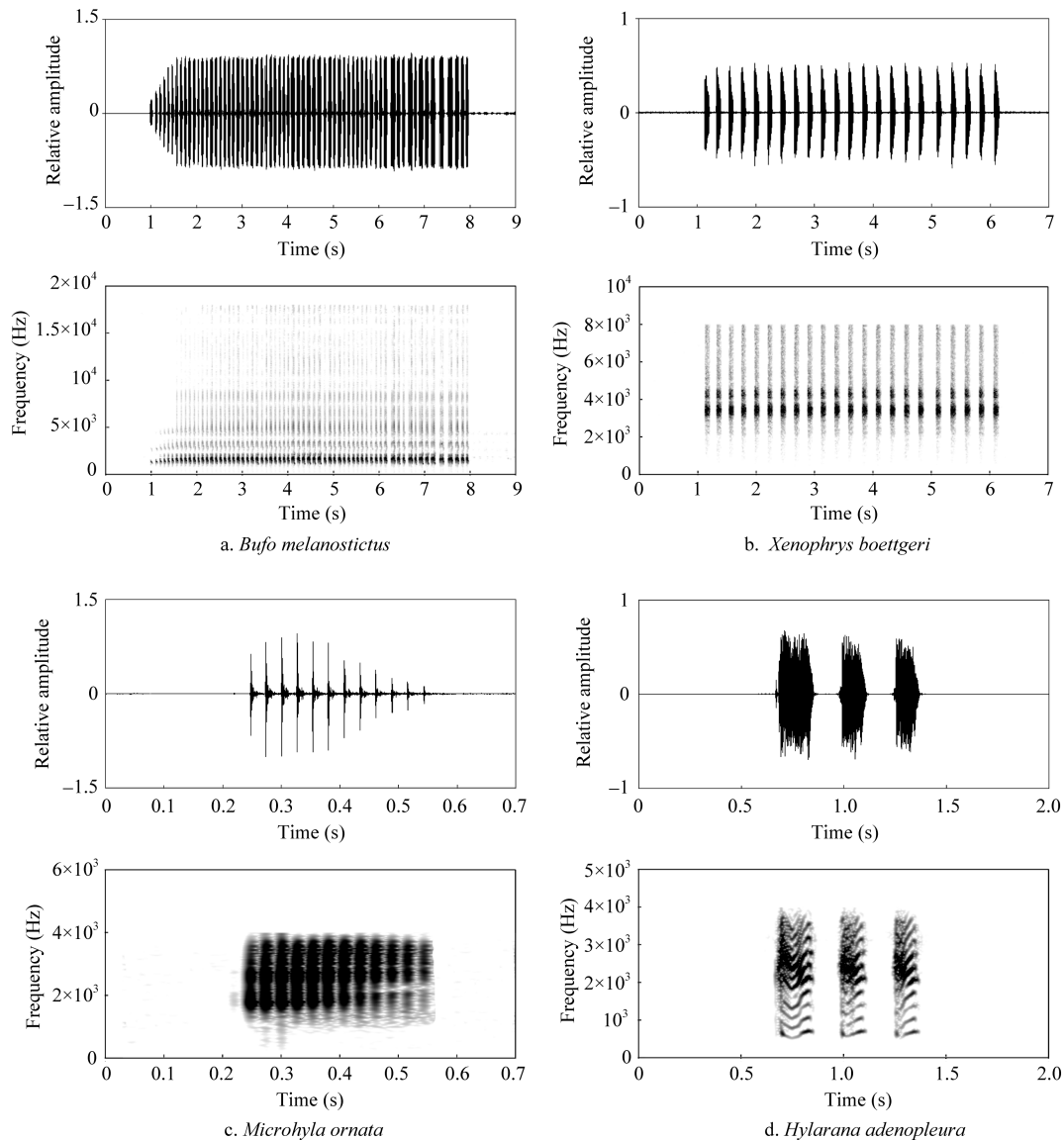
measure the pulse number because the notes were weak.

### *Odorrana schmackeri* (Figure 1f)

During the peak of the breeding season males of this species call *en mass* beginning after nightfall. Based on analysis of 98 recordings from 10 individuals, most male calls had a prelude which was relatively easy to record. This species had the highest dominant frequency (DF) among those recorded (Table 1).

### *Fejervarya limnocharis* (Figure 1g)

The call of this species had fewer notes than the other species and was relatively short. Males and females of this species congregate near water and call day and night during the breeding season. As in *H. adenopleura* and *M. Ornate*, two distinct dominant frequencies (DF) were detected in different individuals (DF), one of which was close to F0 (Table 1).



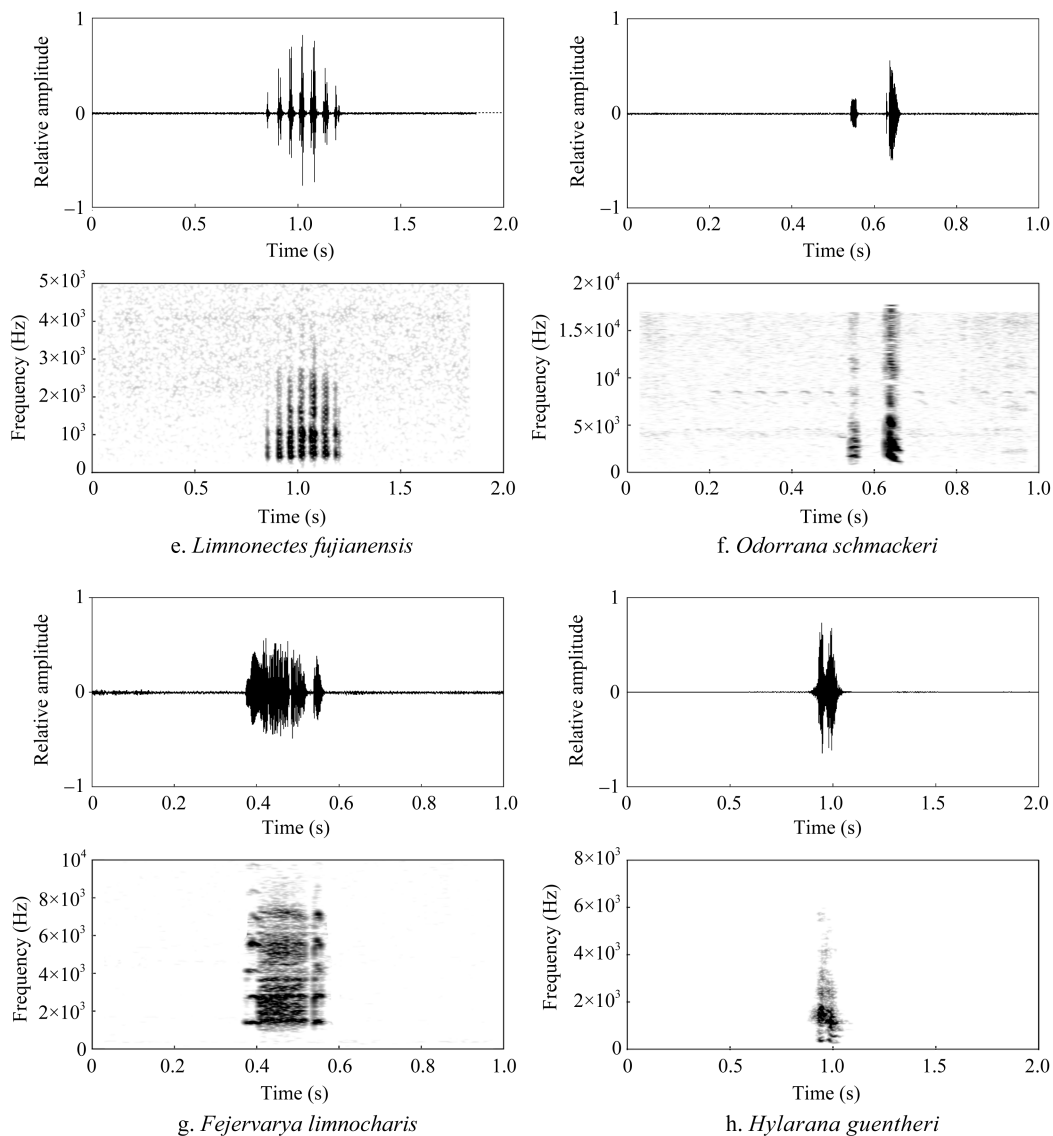


Figure 1 Oscillograms and spectrograms of mating calls of eight anuran species recorded during breeding season

a: *Bufo melanostictus*; b: *Xenophrys boettgeri*; c: *Microhyla ornata*; d: *Hylarana adenopleura*; e: *Limnonectes fujianensis*; f: *Odorrana schmackeri*; g: *Fejervarya limnocharis*; h: *Hylarana guentheri*; Software was Praatt 5.3.32 and frequency range is 0~5 500 Hz

#### *Hylarana guentheri* (Figure 1h)

Males of this species have a pair of internal vocal sacs and call day and night during the breeding season. Its single-note, bark-like call was easy to identify and had the lowest first frequency (F1) among the species recorded (Table 1).

## DISCUSSION

### Species Identification

Many Chinese anuran species are sympatric and have therefore evolved species-specific vocalizations to attract conspecific females. The species-specific nature of anuran advertisement calls means that these calls can provide a useful means of identifying different species.

We found large differences in the calls of different species in features such as call duration, note number, fundamental frequency, dominant frequency and the first three formants. Some of these differences can be attributed to species-specific differences in the morphology and structure of the vocal apparatus (Xiong et al, 2010). For example, *F. limnocharis*, *X. boettgeri* and *M. ornata* all have a single external vocal sac, but differ in call duration and number of notes. As in *X. boettgeri*, the calls of different individuals of these two species appear to have two different dominant frequencies, one of which is close to the fundamental frequency and the other double the fundamental frequency.

The call durations of *H. adenopleura*, *H. guentheri*

and *O. schmackeri* were shorter than those of the other species and had relatively fewer and more distinct notes. *Bufo melanostictus* has a long call duration with many notes, a low fundamental frequency and a high dominant frequency.

Due to the lack of a vocal sac, advertisement calls of male *L. fujianensis* were relatively weak, and the dominant and fundamental frequency of this species were lower than in the other remaining species.

There are obvious differences in the morphology and structure of vocal apparatus between frogs and toads. For example, in the Chinese forest frog *Rana chensinensis* the structure of the *pars lateralis* and middle of the vocal cords resembles the letter “T”, whereas in the toad *Bufo raddei* it looks more like the letter “V” (Zhang et al, 2012). Another influence on the sounds produced by different species is the number of throat muscles (Zhang, 1988). There are four types of throat muscle arrangements in Chinese anurans; two pairs, three pairs, four pairs and five pairs (Zhang, 1988). The *Ranidae* have four pairs of muscles whereas the *Bufonidae* have only three pairs. The number of throat muscles possessed by the *Megophryidae* remains unknown (Zhang, 1988).

It is likely that species-specific differences in call frequency and intensity often reflect such differences in the vocal apparatus between different species. Determining the reasons for these differences will require anatomical investigation of the male vocal apparatus.

### Geographic Variation in Calls within the same Species

We also found evidence of geographic variation in calls of the same species. Xu (2005) found evidence of geographic variation in the calls of *Rhacophorus dugrititei* and *Rhacophorus chenfui*. Wei (2012) found differences in the calls of Chinese and overseas populations of *B. melanostictus*.

Comparing calls of *M. ornata* recorded in Gutianshan

and Wenzhou with those of the same species recorded by Jiang (1995) in Hangzhou and Yishan, and by Wei (2013) in Lishui (Table 2), we found that, although the amplitude and duration of the syllable were similar between locations, the call duration was higher at Yishan (2.4 s, Table 2). Furthermore, call duration and peak frequency differed between locations.

The calls of *M. ornata* recorded in Wenzhou, Gutianshan and Yicheng (Anhui) had two high power peaks, whereas those recorded in Hangzhou and Lishui had only one. There were also significant differences in call duration, note number, fundamental frequency, dominant frequency and first formant between *M. ornata* calls recorded at Gutianshan and Wenzhou (*t*-test,  $P < 0.05$ , Table 2, Figure 2). There was, however, no significant difference in the second and the harmonic (*t*-test,  $P > 0.05$ ). As the same results were reported by Wei et al. (2013), we think it is likely that there is geographic variation in the calls of *M. ornata*. However, the technical differences in sound recording and analyzing may have caused the variations of acoustic characteristic parameters.

We also compared the calls of *F. limnocharis* we recorded at Gutianshan and Wenzhou with those recorded by Jiang et al (1995) in Hangzhou and Shengsi (Figure 3). Although most frequency character of the calls from these four populations were similar, there were still some discrepancies in some call frequency. For example, the dominant frequency (DF) in the Hangzhou population was much lower than that in the other populations. Moreover, significant geographical variation was apparent in call duration, note number, fundamental frequency and the first formant (*t*-test,  $P < 0.05$ ).

Anurans emit a variety of sounds during the breeding season. In most anuran species, only males call, only in a few species the females also vocalize. For example, female Emei music frogs, *Babina daunchina*, call to stimulate inter-male competition (Cui et al, 2010).

**Table 2** Acoustic features of *Microhyla ornata* recorded at different sites

	Site	Pulse number/call	Call duration (s)	Fundamental frequency (F0, Hz)	DF (kHz)	F1 (Hz)
<i>Microhyla ornata</i>	Hangzhou (Jiang et al, 1995)	NA	0.31±0.02	NA	1.36±0.02	NA
	Yicheng (huang et al, 1982)	NA	2.40	NA	2.80-2.90	NA
	Lishui (Wei et al, 2013)	NA	0.30±0.03	NA	2.50±0.50	NA
	Gutianshan (this work)	12.34±2.19	0.26±0.05	1276.46±262.79	1.73±5.74 2.80±0.22	1425.32±69.17
	Wenzhou (this work)	15.66±3.87	0.27±0.03	1359.71±12.95	1.31±0.05 2.72±0.12	1369.25±40.35

Data are presented as mean±SD; The different methods of sound recording and analyzing would cause the variations of acoustic characteristic parameters.

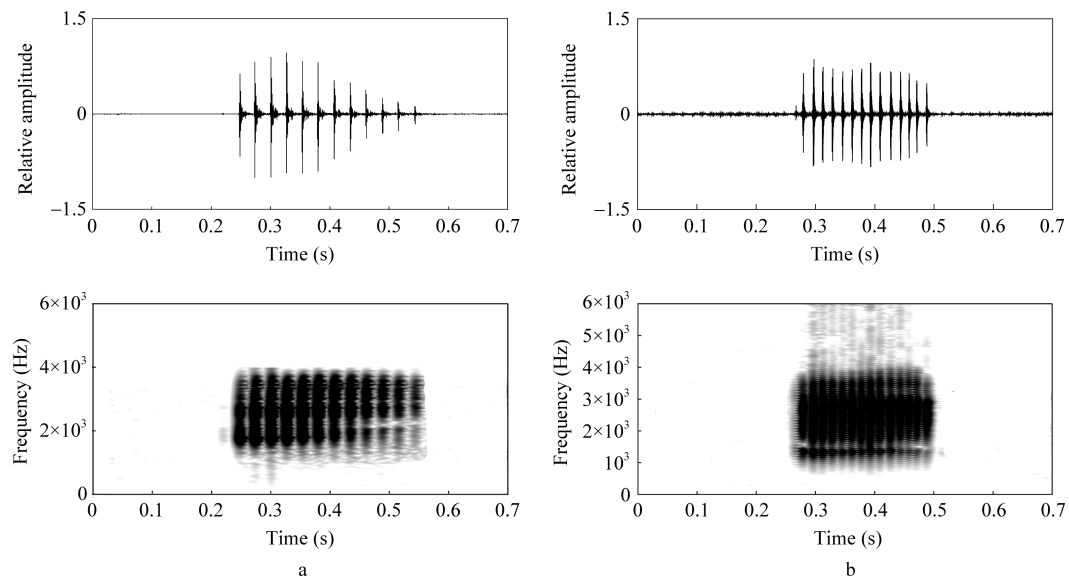


Figure 2 Differences in calls of *Microhyla ornata* recorded at (a) Gutianshan and (b) Wenzhou

a: Recorded at Gutianshan (the inter-note intervals are average and the envelope reduces gradually); b: Recorded at Wenzhou (the inter-note intervals are smaller and the envelope is linear)

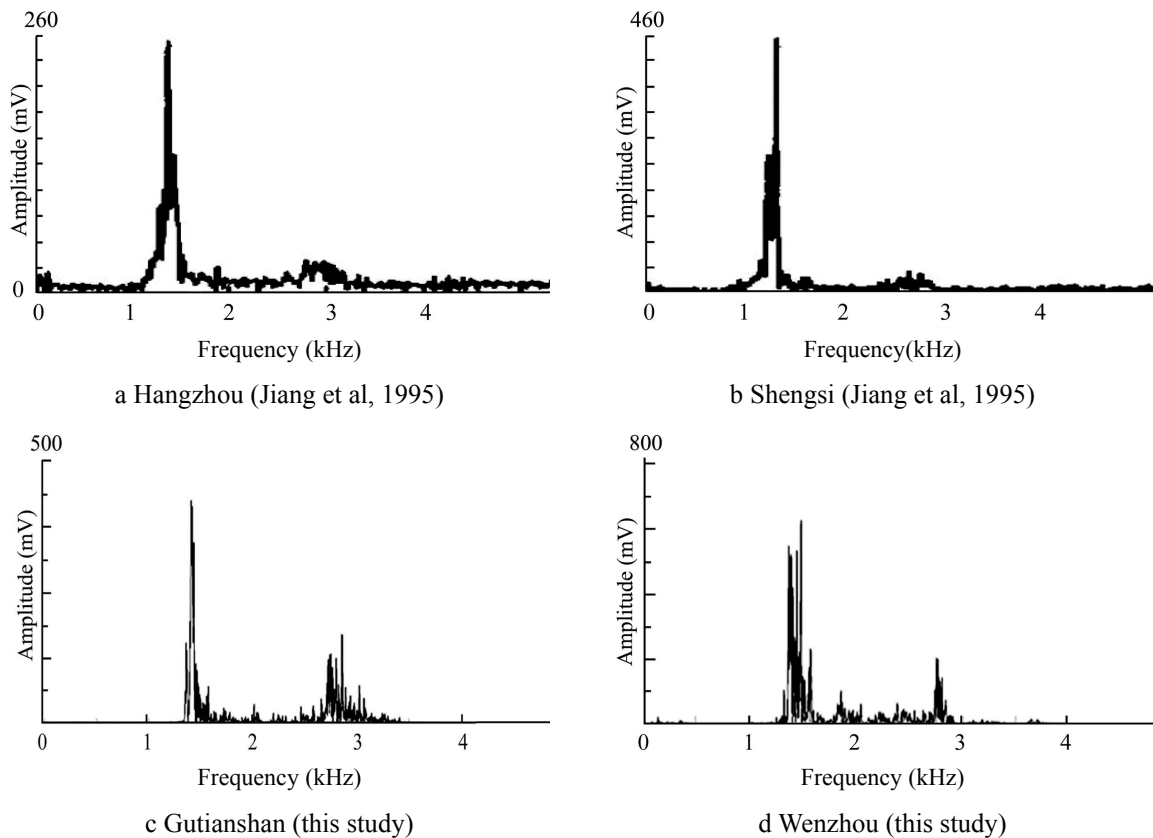


Figure 3 Frequency spectrum analysis of calls of *Fejervarya limnocharis* recorded at four different locations

Anurans' calls can convey quite complex information. For example, male *B. daunchina* can convey not only the possession of, but the quality of a

cave territory to females which preferentially mate with males holding higher quality territories (Cui et al, 2012). On the other hand, environmental conditions such as

temperature can also cause individual variation in vocalization within a species or population. For example, Chen et al (2012) found that call duration and syllable length were negatively correlated with environmental temperature. In addition, individual variation in body mass and body size can also affect call traits (Tárano, 2001; Wang et al, 2012).

Further research is required to clarify the relationship between calls and behaviors. For example, we found that male *L. fujianensis* protected their partner's eggs during the breeding season. Therefore,

the possibility that male's may vocalize differently when they are protecting eggs as opposed to attract mates requires further investigation. The significance of the two types of dominant frequencies in different individual *X. boettgeri*, *M. ornate* and *H. adenopleura* also requires further investigation.

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