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Acoustic characteristics of the tiger frog, *Hoplobatrachus rugulosus*, during the breeding season

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Abstract: We recorded and described the calls and acoustic characteristics of the male tiger frog, *Hoplobatrachus rugulosus*, in an artificial pond during mating season. Spectral and temporal call parameters, along with call intensity were analyzed. Three harmonics were distinguishable from the spectrogram. Four patterns of dominant frequency were found in calls produced late at night, i.e, 3 patterns in the first harmonic (located in 500 Hz section, 700 Hz section, and 800 Hz section respectively) and 1 in the second harmonic (located in 1 800 Hz section). Call duration, call duty cycle, call intensity, and pulse rate were highly variable among different patterns of dominant frequency. These call properties could provide valuable evidence for further ecological study of this species.

Key words: Anuran; Advertisement call; *Hoplobatrachus rugulosus*; Mating season

虎纹蛙繁殖季节的求偶鸣叫特征

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摘要：对交配季节虎纹蛙求偶鸣叫进行录制和特征分析。分析结果表明, 虎纹蛙求偶鸣叫主要集中在晚上, 求偶鸣叫声音主要含有 3 个鸣叫谐波, 鸣叫主频率共有 4 种类型, 即在第一谐波上有 3 个主频率段, 分别为 500、700 和 800 Hz 段, 在第二谐波上有 1 个主频率段, 即 1 800 Hz 段。鸣叫时程(call duration)、能环率(call duty cycle)、声强(call intensity)和鸣叫脉冲率(pulse rate)在 4 种主频率中变化很大。这些鸣叫参数特征的分析将对虎纹蛙生理生态学的进一步研究提供有价值的基础数据。

关键词：无尾类; 虎纹蛙; 求偶鸣叫; 交配季节
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Acoustic signaling is the most important form of communication in anuran amphibians (frogs and toads). This is especially true for males during breeding season as the sounds they emit are sexual advertisement signals (Lingau & Bastos, 2007). Research on the functions of such male anuran calls have concentrated on three main areas: 1) How do calls provide females with information for identifying conspecific males; 2) How do females choose their mate among conspecific males; and 3) How is spatial separation between individual males maintained after a male announces the occupation of a territory to other males (Gerhardt, 1994; Halliday & Tejedo, 1995; Krishna & Bosch, 2007; Ryan & Rand, 1993; Tárano, 2001; Wells, 1977). Male anurans may emit a unique species-specific advertisement call to enable females to identify and select conspecific males (Krishna & Bosch, 2007; Lea et al, 2002; McClelland et al, 1996), and females often decide which male to mate with based on

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the quality and structure of the advertisement calls (Gerhardt, 1994; Pröhl, 2003; Yu & Zheng, 2009). Sexual selection among frogs favors males that display at lower frequencies, higher rates, longer durations, greater intensities, and with greater complexity than other males (Bee et al, 1999; Howard & Young, 1998; Pröhl, 2003; Wagner, 1989) and optimize call transmission through the environment and enhance call attractiveness to females (Krishna & Bosch, 2007; McClelland et al, 1996). Due to their species-specificity, anuran advertisement calls have been used as a diagnostic tool for clarifying taxonomy of closely related species (Abrunhosa et al, 2005; Jiang et al, 1995; Jiang et al, 2002; Nunes et al, 2007; Xu et al, 2005; Yu & Zheng, 2009). However, call properties of anurans are influenced by factors such as air temperature, body temperature, body size and weight, and interactions among individuals (Giacoma et al, 1997; Howard & Young, 1998; Navas & Bevier, 2001; Pröhl, 2003; Yu & Zheng, 2009). The acoustic properties of different anuran calls is important in understanding the behavior and evolution of communication and to provide the most comprehensive information on how to separate different call types between species (Krishna & Bosch, 2007).

The tiger frog, *Hoplobatrachus rugulosus*, is usually found in farmland across South and Southeast Asia. It has been recorded in the Chinese provinces of Hubei, Anhui, Zhejiang, Fujian, Guangdong, Hunan, Guangxi, Guizhou, Jiangxi, Jiangsu, Yunnan, Taiwan, Hainan, Sichuan, Shanghai, Shaanxi, and Henan (Fei et al, 2009). Due to the belief that *H. rugulosus* has highly nutritional and medicinal values, a large number of individuals have been captured and killed in the last twenty years, despite its listing as a grade Ⅱ national protection animal in China. The mating season of *H. rugulosus* is from April to August (Fei et al, 2009, 2010). Previous studies of the mating behavior of *H. rugulosus* have identified sexual dimorphism in morphological traits and diet composition (Lin & Ji, 2005; Shao, 2007), body condition index (Wang et al, 2008), feeding habits and behavior (Geng et al, 2002), and genetic diversity (Liu et al, 2005). However, little is known about the call properties of this species. The aim of this study was to describe the spectral and temporal parameters of the advertisement call of *H. rugulosus*.

1 Material and Methods

All work was conducted at the herpetological laboratory of Lishui University, Zhejiang province, August 12th to 17th, 2010. We selected a group of 20 female adults and 20 male adults and placed them in an artificial pond (3 m×2 m×1 m). According to our observations, *H. rugulosus* mainly produced mating calls at three different times during the day: from 11:00 to 14:00, 16:00 to 19:00, and 01:00 to 04:00. Due to the frogs producing sounds intermittently, it was difficult to monitor calls successfully between 11:00 to 14:00 and 16:00 to 19:00. Consequently, we recorded advertisement calls emitted by the 20 males for five nights (August 12th to 15th and August 17th) between 01:00 – 04:00. An external directional microphone was held at an approximate distance of 1−2 m from the males and calls were recorded by a Sony IC recorder (ICD-SX950). A total of 52 sound files were recorded successfully. Due to the difficulty in recording the calls and body temperatures of individual males simultaneously, , all sound files representing the 20 males were combined. All sound files were digitized and analyzed using Praat, version 5.1.4.3, at a sampling frequency resolution of 44 100 Hz and 16 bit resolution. Call parameters were defined and illustrated based on Pröhl (2003) and Yu & Zheng (2009). Wide band spectrograms were used to measure dominant frequency (including the first harmonic, second harmonic, and third harmonic frequency). Ten calls in each call sequence within a sound file were analyzed and the following call properties were measured: call duration (ms), call intensity (dB), duty cycle (ms/s), first harmonic frequency (Hz), second harmonic frequency (Hz), third harmonic frequency (Hz), dominant frequency (DF, Hz), and pulse rate (pulses/ms). As amplitude was lower at the beginning than the remaining of the call sequence, the first 10 calls of every call sequence were omitted from the analysis as per Pröhl (2003).

Statistical analyses were performed with SPSS 11.5 for windows. All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (F-max test). Data were analyzed with one way ANOVA. All values were presented as mean ± 1 standard deviation (*SD*), and the significance level was set at $\alpha = 0.05$.

2 Results

Oscillograms and spectrograms of *H. rugulosus* calls are shown in Fig. 1 and Fig. 2. The calls recorded during this study were highly variable across nights (Tab. 1). Three harmonics were clearly discernible from the spectrograms. The males mainly produced four call frequency patterns during the mating period: dominant frequencies were located in the 500 Hz section (Type Ⅰ, Fig.2 A), 700 Hz section (Type Ⅱ, Fig.2 B), 800 Hz section (Type Ⅲ, Fig.2 C) of the 1st harmonic and the over 1 800 Hz section of the 2nd harmonic (Type Ⅳ, Fig.2 D). For Type Ⅰ, the call frequency ranged from 521.16 to 686.41 Hz in the 1st harmonic, 1 843.18 to 2 483.53 Hz in the 2nd harmonic, and 2772.72 to 4 012.11 Hz in the 3rd harmonic, while Type Ⅱ ranged from 707.07 to 748.38 Hz, 1471.36 to 2049.74 Hz and 2359.59 to 3475.04 Hz. The dominant frequency in Type Ⅲ ranged from 810.35 to 851.66 Hz, 1388.73 to 2843.53 Hz and 2380.25 to 3722.92 Hz, whereas in Type Ⅳ,it ranged from 500.50 to 975.6 Hz, 1553.99 to 2 049.74 Hz, and 2937.97 to 3748.53 Hz (Tab. 1). Among all pairwise comparisons of the four call groups, both frequency of the 1st harmonic and dominant frequency were significantly different (Tab. 1). For frequency of the 2nd harmonic, except for comparisons of between Type Ⅰ and Type Ⅱ, Type Ⅲand Type Ⅳ, all other pairwise

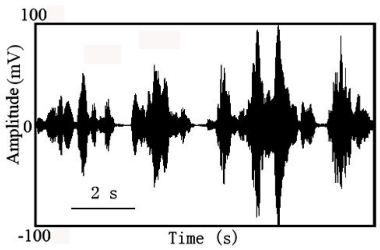

Fig. 1 Oscillogram of *Hoplobatrachus rugulosus* advertisement calls

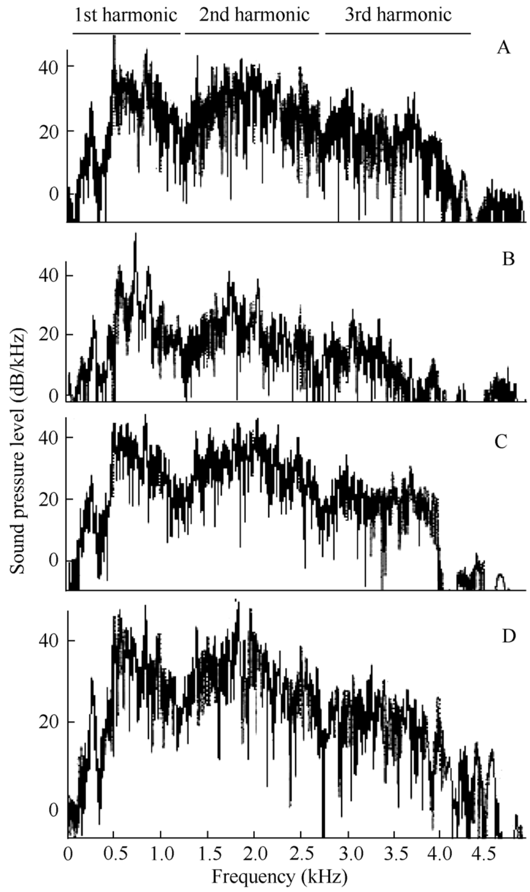

Fig. 2 Spectrogram of *Hoplobatrachus rugulosus* advertisement calls

Dominant frequency (DF) located in 500Hz section (A), 700 Hz section (B), and 800 Hz section (C) respectively in the first harmonic, and one located in the second harmonic (D).

Tab. 1 Characters of the advertisement calls of *Hoplobatrachus rugulosus*

	Dominant Frequency in 500 section (TypeⅠ, Mean ± *SD*, *n* = 336)	Dominant Frequency in 700 section (TypeⅡ, Mean ± *SD*, *n*=68)	Dominant Frequency in 800 section (TypeⅢ, Mean ± *SD*, *n* = 73)	Dominant Frequency in 2nd harmonic (TypeⅣ, Mean ± *SD*, *n* = 52)	Results of One Way ANOVA
Call duration (s)	0.30 ± 0.07 (0.29 − 0.55)	0.28 ± 0.06 (0.18 − 0.39)	0.31 ± 0.07 (0.14 − 0.51)	0.31 ± 0.05 (0.19 − 0.47)	$F_{3,525}$ = 4.26* Ⅰ^{ab}, Ⅱ^{b}, Ⅲ^{a}, Ⅳ^{a}
Duty cycle (ms/s)	0.09 ± 0.03 (0.09 − 0.20)	0.09 ± 0.02 (0.05 − 0.12)	0.10 ± 0.03 (0.04 − 0.19)	0.10 ± 0.02 (0.06 − 0.16)	$F_{3,525}$ = 4.40* Ⅰ^{ab}, Ⅱ^{b}, Ⅲ^{a}, Ⅳ^{a}
Call intensity (dB)	72.78 ± 3.49 (67.83 − 79.92)	76.71 ± 3.22 (69.16 − 81.51)	73.55 ± 4.24 (51.41 − 78.44)	74.30 ± 2.07 (69.49 − 79.01)	$F_{3,525}$ = 25.33** Ⅰ^{c}, Ⅱ^{a}, Ⅲ^{bc}, Ⅳ^{b}
Pulse rate (pulses/ms)	86.10 ± 56.60 (31.00 −279.00)	96.50 ± 30.20 (24.00 − 151.00)	63.40 ± 35.00 (3.00 −146.00)	37.90 ± 30.70 (3.00 − 159.00)	$F_{3,525}$ = 19.72** Ⅰ^{a}, Ⅱ^{a}, Ⅲ^{b}, Ⅳ^{c}
1st harmonic frequency(Hz)	544.64 ± 35.72 (521.16 − 686.41)	724.38 ± 9.17 (707.07 − 748.38)	838.37 ± 10.53 (810.35 − 851.66)	640.33 ± 137.66 (500.50 − 975.60)	$F_{3,525}$ = 764.38** Ⅰ^{d}, Ⅱ^{b}, Ⅲ^{a}, Ⅳ^{c}
2nd harmonic frequency(Hz)	1 787.67 ± 184.12 (1 843.18 − 2 483.53)	1 771.97 ± 97.44 (1 471.36 − 2 049.74)	1 874.00 ± 166.06 (1 388.73 − 2 843.53)	1 854.30 ± 146.26 (1 553.99 − 2 049.74)	$F_{3,525}$ = 7.53** Ⅰ^{b}, Ⅱ^{b}, Ⅲ^{a}, Ⅳ^{a}
3rd harmonic frequency(Hz)	3148.10 ± 318.62 (2 772.72 − 4 012.11)	3016.48 ± 143.40 (2 359.59 − 3 475.04)	3 221.26 ± 285.18 (2 380.25 − 3 722.92)	3 264.40 ± 257.15 (2 937.97 − 3 748.53)	$F_{3,525}$ = 8.86** Ⅰ^{b}, Ⅱ^{c}, Ⅲ^{ab}, Ⅳ^{a}
Dominant frequency(Hz)	544.64 ± 35.72 (521.16 − 686.41)	724.38 ± 9.17 (707.07 − 748.38)	838.37 ± 10.53 (810.35 − 851.66)	1 854.30 ± 146.26 (1 553.99 − 2 049.74)	$F_{3,525}$ = 8 921.77** Ⅰ^{d}, Ⅱ^{c}, Ⅲ^{b}, Ⅳ^{a}

* and ** indicated significant differences at $P < 0.01$ and $P < 0.001$, respectively (One-way ANOVA); Types with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, a > b > c > d).

comparisons were significantly different (Tab. 1). Likewise, except for comparisons of between Type Ⅰ and Type Ⅲ, and Type Ⅲ and Type Ⅳ, all other pairwise comparisons were significantly different in frequency of the 3rd harmonic (Tab. 1).

Pairwise comparisons of call duration, duty cycle, call intensity, and pulse rate were also different among the four call groups (Tab. 1). Call duration was significantly different between comparisons of Type Ⅱ and Type Ⅲ, and Type Ⅱ and Type Ⅳ (Tab. 1). For duty cycle, except for comparisons of between Type Ⅱ and Type Ⅲ, Type Ⅱ and Type Ⅳ, all other pairwise comparisons were not significantly different (Tab. 1). For pulse rate, except for comparisons between Type Ⅰ and Type Ⅱ, all other pairwise comparisons showed significant differences (Tab. 1). For call intensity, except for comparisons of between Type Ⅰ and Type Ⅲ, Type Ⅲ and Type Ⅳ, all other pairwise comparisons showed significant differences (Tab. 1).

3 Discussion

Characteristics of anuran male calls are important for mate attraction, female mate choice, and male mating success (Pröhl, 2003). Not only do calls and reproductive behavior allow us to understand how selection has shaped (and is shaping) a species' ecology, but anuran vocalization is also a tool for monitoring and is therefore useful in conservation. Advertisement calls in anurans are distinctive and are used in species-specific acoustic communication (Wells, 1977). In this present work, *Hoplobatrachus rugulosus* produced multi-harmonic (comprising of over three harmonics) modulated frequency (FM) calls during the breeding season at night, a type of call commonly seen in anurans (Fig.1). The advertisement calls of *H. rugulosus* were highly variable in dominant frequency. Four patterns of dominant frequency calls occurred during courtship: three in the 1st harmonic frequency (500 Hz, 700 Hz and 800 Hz) with the fourth in the 2nd harmonic. This high variation in dominant frequency late at night may be due to an increase in mating calls. As male-male congregate choruses for attracting females were frequently heard, *H. rugulosus* may produce mating calls in a courtship field. As anurans court and mate with females at night (Gerhardt, 1994; Wells, 1977), calls produced during this time may be concentrated on breeding success. This calling behavior requires further study in the field.

For temporal parameters, call duration, call duty cycle, and pulse rate along with call intensity, also showed significant differences among the four types of calls. Individuals produced calls of various temporal features at courtship fields for mate attraction at night. As call duration and call duty cycle are strong predictors of energy expense, this may be an indicator of fitness and therefore play a role in female selectivity (Grafe, 1996; Welch et al, 1998). Other studies have also documented that female frogs prefer to mate with males who emit longer calls with high duty cycles (Navas & Bevier, 2001; Pröhl, 2003). In this study, pulse rate seemed to be higher in the 1st harmonic frequency than the 2nd harmonic frequency: that is, the lower the dominant frequency, the higher the pulse rate. This suggests that environment variation, as well as the physical condition of male frogs, may be influencing calling behavior. On the other hand, environmental disturbance (such as factitious disturbance and natural enemies) emerged beside the pond and therefore the frogs may have produced more call pulses and more patterns of dominant frequency to detect circumambient complexion. In addition, we found call intensity also showed significant difference among the four patterns of dominant frequency calls. We also recorded various clangorous click calls late at night. This calling behavior also requires further study.

In this present work, we recorded and described the characteristics of advertisement calls of *H. rugulosus* in a man-made pond, and obtained ecological information of this species. Further work needs to be carried out to determine the level of call variation in individual males and between males, female preference and mating success.

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