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Consistency in responses to conspecific advertisement calls with various signal-to-noise ratios in both sexes of the Anhui tree frog

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Abstract

Environmental noise has a significant negative impact on acoustic communication in most situations, as it influences the production, transmission, and reception of acoustic signals. However, how animals respond to conspecific sounds when there is interference from environmental noise, and whether males and females display convergent behavioral responses in the face of noise masking remain poorly understood. In this study, we investigated the effects of conspecific male advertisement calls with different signal-to-noise ratios on male–male competition and female choice in the Anhui tree frog *Rhacophorus zhoukaiyae* using playback and phonotaxis experiments, respectively. The results showed that (1) female Anhui tree frogs preferentially selected the conspecific calls with higher SNR compared to calls with lower SNR; (2) males preferentially responded vocally to the conspecific calls with higher SNR compared to calls with lower SNR; and (3) males' competitive strategies were flexible in the face of noise interference. These results suggest that preferences of both sexes converge in outcome, and that male competitive strategies may depend on predictable female preferences. This study will provide an important basis for further research on decision-making in animals.

Key words: advertisement call, female choice, male–male competition, noise, signal-to-noise ratio (SNR)

Communication is fundamental for the establishment of most social relationships between animals. However, the use of signals within most sensory modalities is constrained by background noise, including nearby conspecific and heterospecific signals, as well as sounds from the abiotic environment (Brumm and Slabbekoorn 2005). For example, the interference of noise on acoustic communication in vocal animals is mainly auditory masking caused by the temporal overlap of acoustic signals and spectral overlap of their frequency ranges (Brumm and Naguib 2009; Codarin et al. 2009). Noise masking may lead to an increase in the threshold of signal detection by receivers (Narins and Wagner 1989; Wollerman and Wiley 2002; Clark et al. 2009), which in turn can decrease the reproductive success of signalers. For instance, female Mediterranean field crickets *Gryllus bimaculatus* show a significantly reduced preference for high-quality calls under the influence of traffic noise and white noise, when compared with the ambient no-noise condition (Adam et al. 2021). Such mate selection decisions may further result in less-adapted offspring. Noise also indirectly affects communication efficiency by affecting attention and decision-making (Ratcliff and Smith 2010; Bankó et al. 2011). Specifically, noise will increase attention allocated to the auditory nucleus to process noisy information (Cunningham et al. 2002). Because

individual attention is limited, attention attracted by noise will decrease the attention allocated to processing other information, ultimately leading to decreased efficiency or accuracy of processing useful information. On the other hand, noise may reduce attention in other sensory channels, that is, preventing the brain from processing information from other senses and causing cross-sensory interference (Halfwerk and van Oers 2020). In addition, noise can cause hearing damage (McCauley et al. 2003), and long-term exposure to environmental noise can damage animals' auditory system, leading to noise-induced hearing loss (Nelson et al. 2005). Accordingly, noise can have a detrimental effect on animal survival and reproduction by affecting individual behaviors (Wiley 2015) and population distribution (Naguib 2013). However, the discrepancy between males' and females' behavioral responses to noise is poorly understood.

Acoustic communication plays a crucial role in the reproduction of anurans (Rose 2018), since male–male competition and female choice rely almost exclusively on it. Male advertisement calls convey various information, such as individual size, breeding status, location, and resources available to them (Wells and Schwartz 2007). In general, frogs usually gather in choruses to attract conspecific mates. Therefore, the acoustic environment of a chorus is very complex due to the

high levels of background noise, intense competition between males, and temporal overlap among advertisement calls of rivals. Although there is no effect on male–male competition and female choice during exposure to traffic noise for some species (Zaffaroni-Caorsi et al. 2022), intense noise negatively impacts male–male competition and female choice for many other species (Schroeder et al. 2012; Injaian et al. 2018). For instance, female neotropical frogs *Dendropsophus ebraccatus* are less effective at recognizing male call signals when disturbed by heterospecific chorus calls (Wollerman 1999), and frogs in urban environments are often disturbed by anthropogenic noise (Kaiser et al. 2011), with traffic noise being the most common (Vargas-Salinas and Amézquita 2013; Vargas-Salinas et al. 2014; Kruger and Du Preez 2016). Under the influence of traffic noise, female Cope's gray tree frogs *Hyla chrysoscelis* are less able to locate males, and must spend more time assessing male advertisement calls, ultimately making them significantly less efficient at selecting mates (Bee and Swanson 2007). On the other hand, males can modulate their call characteristics in response to changes in the number of conspecific competitors or call rate per unit of time, the presence of heterospecific male calls, female proximity, as well as noise (Wells and Schwartz 2007). In particular, when females' ability to recognize acoustic signals is reduced in the face of high levels of noise interference, males will often adjust their spatial position or characteristics of their calls to prevent them from being obscured and hence maximize their reproductive success (Feng et al. 2006; Ey and Fischer 2009; Shen and Xu 2016; Brown et al. 2021; Zhao et al. 2021). For example, male neotropical tree frogs *Dendrosophus microcephalus* can quickly adjust the spacing between adjacent notes when calls overlap, reducing the probability of their notes being obscured (Schwartz 1987). In addition, male dark-sided frogs *Rana nigrovittata* increase the frequency of their own calls in the presence of anthropogenic noise disturbance, thus increasing their probability of being detected through a series of repetitive and low-intensity short tones (Sun and Narins 2005). Although some previous studies have found that males and females of the serrate-legged small tree frog *Kurixalus odontotarsus* have different behavioral responses to noise disturbance (Zhang et al. 2021), it is unclear whether males and females have convergent behavioral responses to noise masking in other anuran species.

During the breeding season, Anhui tree frogs *Rhacophorus zhoukaiyae* live at the bottom of the ridge of farmlands, as well as in fields. In order to defend territories and attract females, males build burrows and produce advertisement calls mostly from inside these burrows, and rarely from outside. Males usually emit advertisement calls consisting of 10 or more notes (Fang et al. 2019). Moreover, they usually produce more competitive and nonoverlapping calls in response to acoustic stimuli, indicating that male Anhui tree frogs can dynamically regulate their competitive strategies by their social environment (Song et al. 2020). The mixtured pygmy frog *Microhyla mixtura* is sympatric with the Anhui tree frog; the former having a higher number of individuals compared to the sparse Anhui tree frogs. Therefore, advertisement calls of male Anhui tree frogs are inevitably overlapped by intense calls of male mixtured pygmy frogs, due to the overlapping acoustic properties between these species' calls (see Figure S1F). The successful amplexed pair of Anhui tree frogs and females' preference for small-sized males indicates that both sexes might effectively mitigate the effects of noise on their

auditory perception by means of spatial distancing and selective attention. Accordingly, the Anhui tree frog represents an ideal animal model for exploring the effect of noise disturbance on behavior and auditory perception.

In this study, we broadcast conspecific advertisement calls with different SNRs (8, 13, 18, and 23 dB) to male Anhui tree frogs in playback experiments, and also played back different stimulus pairs with various SNRs to females in phonotaxis experiments. We recorded and analyzed subjects' responses in order to investigate the effects of conspecific male advertisement calls with different SNRs on male–male competition, as well as female choice in this species. We predicted that in the face of noise disturbance, (1) male Anhui tree frogs will change their calling behaviors dynamically according to various levels of SNR within the conspecific acoustic signals; and (2) females would prefer the conspecific sound signals with higher SNR.

Materials and Methods

Study site and animals

The present study was carried out in Jinzhai county, Dabie Mountain area, Anhui province, China (31.28° N, 115.72° E and elevation of 870 m above sea level). Playback and phonotaxis experiments were conducted on male and female Anhui tree frogs, respectively, from March to April 2021. The former was conducted near the males' burrows, while the latter was conducted in an open wooden sound-attenuating tank away from the farmland that the frogs inhabited. Behavioral data were collected from 16 calling males (snout–vent length: 36.23 ± 1.64 mm; body mass: 4.92 ± 0.79 g) and 50 gravid females (snout–vent length: 46.45 ± 3.65 mm; body mass: 10.34 ± 3.01 g) that inhabited a local area of 5,000 m². The experiments were conducted under ambient light conditions between 20:00 and 03:00 (the next day) in order to avoid the effects of visual stimulation, with an average temperature of 10.3 ± 2.6 °C and relative humidity of 75.7 ± 17.6%. To avoid recapturing the subjects on subsequent nights, after experiments the frogs were housed in a plastic grid enclosure (500 × 150 cm²; 100 cm deep; composed of soil and vegetation) that was set up in a temporarily unused vegetable field near the farmland. Live crickets (bought from a pet food shop) were provided as food for the frogs in the enclosure every 3 days. At the end of all experiments, the enclosure was removed.

Stimulus

The Anhui tree frog is sparsely distributed under natural conditions. In contrast, its sympatric species, the Mixtured pygmy frog, has a high population density, resulting in dense biological noise. Within the Anhui tree frogs' habitats, there are complex environmental noise sources such as water flow, wind, rain, and traffic noise. All of these biotic and abiotic noises with various spectral components create background noise with a wide frequency range (Figure S1). Therefore, we chose white noise with a wide frequency range to simulate the background noise in our experiments.

In the field, background noise without any conspecific and heterospecific frog calls was approximately 50 dB. Background noise that included the calls of the mixtured pygmy frogs and other abiotic noise was approximately 70 dB, while the intensity of the conspecific advertisement call was 80 dB (these values were measured approximately 1

m from the test individuals' burrows; relative to 20 µPa, C-weighting; Bruel and Kjær, 2240; Nærum, Denmark). Since frogs can gain sufficient directional information to distinguish between 2 sound sources that are separated by more than 30°(Rheinlaender et al. 1981; Michelsen et al. 1986; Gerhardt and Bee 2007), we superimposed a conspecific advertisement call and each of the 4 exemplars of white noise with different amplitudes to form 4 acoustic stimuli with different SNRs. Specifically, white noise with 55, 60, 65, and 70 dB sound pressure level (SPL) and with durations equal to the average value of conspecific advertisement calls were generated using a custom-made software in Matlab. Each exemplar of white noise and a randomly selected conspecific advertisement call (80 dB SPL) were superimposed to synthesize acoustic stimuli with different SNRs of 23, 18, 13, and 8 dB (Figure 1), according to the formula for calculating SNR (Zaunseder et al. 2022; see Supplementary Material). The selected conspecific advertisement call contained 16 notes, and each note's temporal and frequency parameters were close to the mean of the population. Since pseudoreplication can affect statistical analyses in ecological and animal behavior studies (Lazic 2010), we accounted for the possible influence of pseudoreplication on our conclusions in the present study. To do this, 4 advertisement calls containing 16 notes were randomly selected from 4 different individuals in our dataset.

Thus, there were 4 sets of stimuli, each including 4 acoustic stimuli with different intensities of noise. The sound pressure of each stimulus was adjusted to 80 ± 0.5 dB SPL using a sound pressure meter (measured 1 m from the speaker). In the playback experiments for males, each stimulus set was randomly selected and played back to 4 subjects. Since only 50 females were captured, each of the first 2 randomly selected stimulus sets was used on 13 females, while each of the last 2 sets was used on 12 females.

Phonotaxis experiments for females

An open wooden sound-attenuating tank (270×95 cm²; 100 cm height) containing humid mud to a depth of approximately 5 cm was located outdoors and used for the phonotaxis tests. To reduce acoustic reverberation, small holes (6 mm in diameter, spaced 2 cm apart) were drilled along the 4 walls of the chamber, and the inner wall was pasted with sound-dampening cotton (5 cm thick). Two speakers (SME-AFS, Saul Mineroff Electronics, Elmont, NY, USA) were placed equidistantly at opposite ends of the tank. Before the experiments, a pure tone of 1,000 Hz was used to calibrate the peak output intensity of each speaker to 80 dB SPL (measured 1 m from the speaker).

To investigate whether conspecific advertisement calls with different SNR affect female preference, the 4 stimuli with
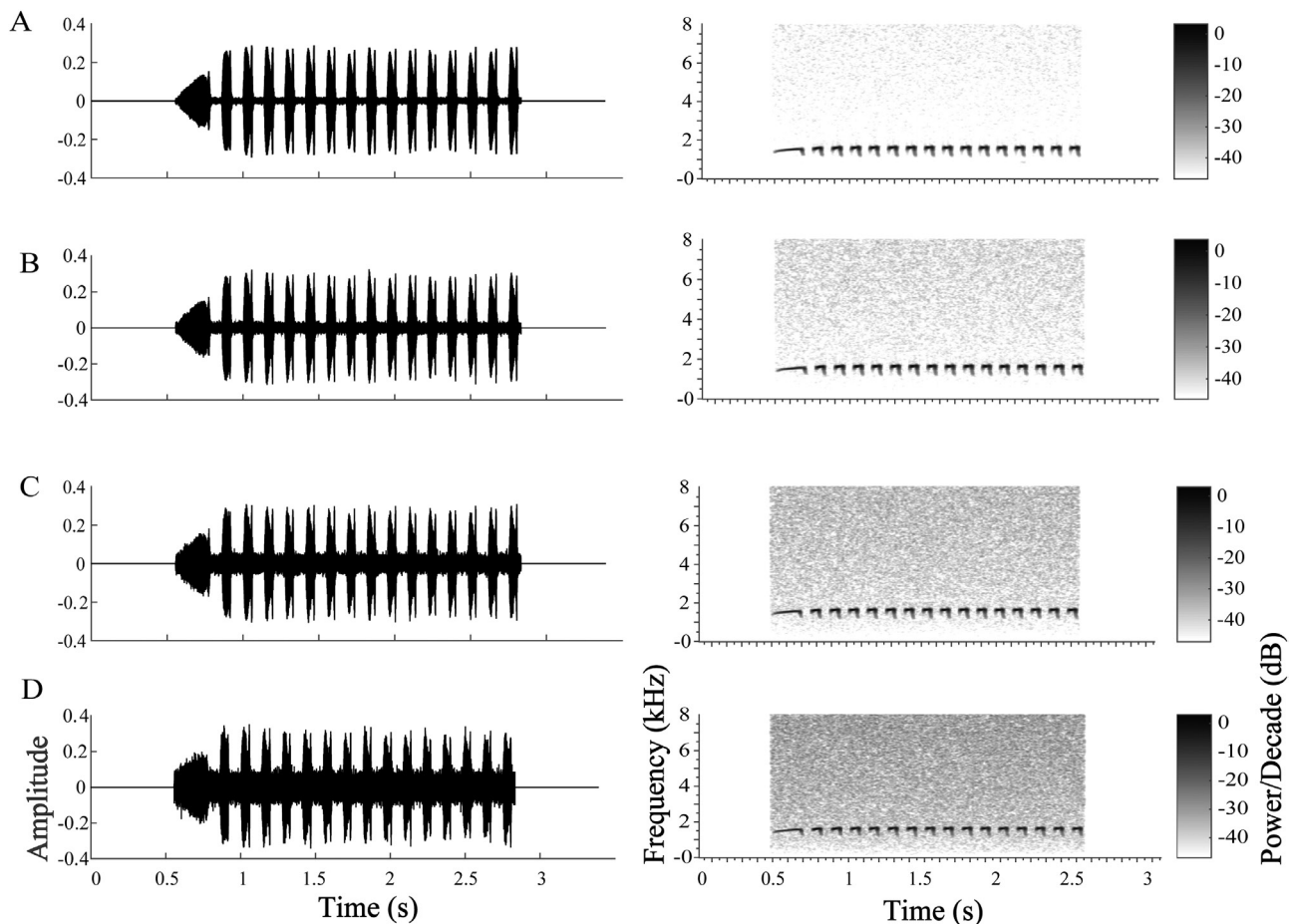

Figure 1. Waveforms (left) and spectrograms (right) of the 4 acoustic stimuli used in this study: (A) conspecific advertisement call with SNR of 23 dB; (B) conspecific advertisement call with SNR of 18 dB; (C) conspecific advertisement call with SNR of 13 dB; and (D) conspecific advertisement call with SNR of 8 dB.

Table 1. Results of the chi-square test on the number of female choices

| | Stimulus pair | | | | | |
	SNR8–13	SNR8–18	SNR8–23	SNR13–18	SNR13–23	SNR18–23
x^2	9.68	11.52	5.12	8.00	3.92	6.48
P	0.002*	0.001*	0.024*	0.005*	0.048*	0.011*

Note: SNR8, SNR13, SNR18, and SNR23 refer to conspecific advertisement calls with various SNRs of 8, 13, 18, and 23 dB, respectively.
* $P < 0.05$.

SNR of 8, 13, 18, and 23 dB were paired with each other to form 6 stimulus pairs. All 50 females were involved in the phonotaxis tests, during which each animal was covered by a transparent semicircular plastic cup and allowed to habituate for approximately 3 min in darkness. Then, one of the stimulus pairs was randomly selected and played antiphonally with 3.5-s inter-stimulus interval (ISI), approximately equal to the mean of inter-call intervals in nature. After 3 min of habituation, the cup was lifted remotely to allow the animal to move freely in the arena. The behaviors of each subject were monitored via 2 infrared cameras (X4-IPC100WM; Hawkspee, China). A positive response was scored if the female approached a location within 10 cm from the speaker. If the animal failed to choose within 20 min after the cup was lifted, then a "no response" was scored. Each of the 6 stimulus pairs was presented to each subject in this way, resulting in 6 scores and 6 latencies acquired for each animal. For each subject, the assignments between the stimuli and speakers varied randomly to control for possible spatial biases. After the experiments, the subjects' body mass and snout-to-vent length were measured.

Playback experiments for males

Sixteen males were included in these experiments. Before the experiment, each subject was located by its advertisement calls. The acoustic stimuli were simultaneously played by 2 speakers placed approximately 1 m apart along the bottom of the ridge of the farmland, oriented toward the subject located inside his burrow. The burrow and the 2 speakers formed the 3 vertices of an isosceles triangle; therefore, spatial cues of a given stimulus would be eliminated to some extent. Playbacks started approximately 10 min after the male resumed normal calling behavior following the speaker placement. The experiments were conducted under 5 acoustic conditions: First, spontaneous calls of the subject were recorded for 10 min (the control condition, CC), then each of the 4 types of stimuli with different SNRs were played for 10 min with 3.5-s ISI. The interval between each acoustic condition was 3 min. Both the subjects' vocal responses and the playback stimuli were simultaneously recorded with a Sennheiser ME66 microphone (Sennheiser, Wedemark, Germany), which was connected to a laptop (Thinkpad X201; Lenovo, China) with a sampling rate of 44.1 kHz and a 16-bit resolution. The microphone was mounted on a long bamboo rod and placed approximately 0.5 m from the subject. After the experiments, the subjects' body mass and snout–vent length were measured.

Data processing and statistical analyses

For each acoustic condition, we used Adobe Audition 3.0 software (San Jose, CA, USA) to measure 4 parameters of male responsive vocalizations: number of advertisement calls;

number of notes for each call; duration of each call; and onset latency of the first responsive call relative to the first stimulus presentation. These values were further averaged for each parameter, each acoustic condition, and each frog. For females, the number of selections and latencies (the delay between when the cup covering the female was lifted and when the female made a choice) for each acoustic stimulus pair were determined.

The normality and homogeneity of variance for all data were estimated using Shapiro–Wilk W and Levene's tests, respectively. For each of the 4 parameters of male vocalizations, the values were statistically analyzed using 2-way repeated-measures ANOVA with the variables of "stimulus set" (the 4 sets of stimuli) and "stimulus type" (the 4 types of stimuli with different SNRs within each stimulus set). There was no significant main effect of "stimulus set," congruent with the idea that the 4 stimulus sets were not significantly different in evoking responses from the males. Therefore, the values were statistically analyzed using 1-way repeated-measures ANOVA with the variable of "acoustic condition" (control condition and playbacks of the 4 types of stimuli with different SNRs). For significant ANOVAs, the data were further analyzed for multiple comparisons using the least significant difference (LSD) test. For the number of female choices, a chi-square test was used to assess whether there were significant differences in the number of female choices among different stimulus sets or among advertisement calls with different SNRs. There was no significant difference for the former, suggesting the 4 stimulus sets were not significantly different in evoking responses from the females. For each stimulus pair, the latencies of female choice for each acoustic stimulus were statistically analyzed using independent samples t-tests. All statistical analyses were conducted using SPSS software (release 23, IL, USA), with $P < 0.05$ as statistically significant.

Results

Phonotaxis experiments for females

In all trials, the signal with the higher SNR was preferred by the females (Table 1). For example, the females significantly preferred the sound with SNR13 when the stimulus pair SNR8 versus SNR13 was played back antiphonally (14 vs. 36; Chi-square test: $\chi^2 = 9.68$, $P = 0.002$; Figure 2A and Table 1). Similarly, females showed a significant preference for the sound with SNR18 in the stimulus pair SNR8 versus SNR18 (13 vs. 37; Chi-square test: $\chi^2 = 11.52$, $P = 0.001$; Figure 2A and Table 1). When the stimulus pair SNR8 vs. SNR23 was played back antiphonally, the females significantly preferred the latter (17 vs. 33; Chi-square test: $\chi^2 = 5.12$, $P = 0.024$; Figure 2A and Table 1), and when

the stimulus pair SNR13 versus SNR18 was played back antiphonally, they also significantly preferred the latter (15 vs. 35; Chi-square test: $\chi^2 = 8.00$, $P = 0.005$; Figure 2A and Table 1). There was a significant preference for the sound with SNR23 in the stimulus pair SNR13 versus SNR23 (18 vs. 32; Chi-square test: $\chi^2 = 3.92$, $P = 0.048$; Figure 2A and Table 1) as well as SNR18 versus SNR23 (16 vs. 34; Chi-square test: $\chi^2 = 6.48$, $P = 0.011$; Figure 2A and Table 1). There were no significant differences in latencies for female choices between the 2 acoustic stimuli within a pair ($P > 0.05$; Figure 2B).

Playback experiments for males

The mean value and standard error of each acoustic parameter of the advertisement calls vocalized under different acoustic conditions are listed in Table 2. For the number of male responsive calls, the main effect was significant for the variable of "acoustic condition" ($F_{4, 60} = 3.677$; $\varepsilon = 0.539$, $P = 0.034$, partial $\eta^2 = 0.197$). Multiple comparisons showed that the number of calls under the conditions of SNR13, 18, and 23 dB was significantly greater than that under the control condition (CC, no sound was played back), and the number of calls under SNR23 dB was significantly greater than that under SNR8 dB ($P < 0.05$, Figure 3A and Table 3). For the number of notes of each call, the main effect was also significant for the variable of "acoustic condition" ($F_{4, 60} = 4.573$; $P = 0.003$, partial $\eta^2 = 0.234$). The number of notes of each call during CC was significantly less than those under the other acoustic conditions ($P < 0.05$, Figure 3B and Table 3), but there were no significant differences among SNR8, 13, 18, and 23 dB. Similarly, the main effects of the variable "acoustic condition" were significant for call duration ($F_{4, 60} = 10.781$; $P = 0.005$, partial $\eta^2 = 0.418$) and the onset latency of the first responsive call ($F_{3, 45} = 7.421$; $P = 0.000$, partial $\eta^2 = 0.331$). Call durations for SNR8, 13, 18, and 23 dB were significantly longer than that for CC ($P < 0.05$, Figure 3C and Table 3), but the differences among SNR8, 13, 18, and 23 dB were not statistically significant. The onset latencies of the first responsive call were significantly longer under SNR8 and 13 dB, compared with SNR18 and 23 dB ($P < 0.001$, Figure 3D and Table 3).

Discussion

The present results showed that female Anhui tree frogs usually choose conspecific calls with higher SNR compared with those with lower SNR (Figure 2A). These results are consistent with previous studies on female Túngara frogs *Physalaemus pustulosus* that show preferences for attractive conspecific calls with medium- and low-density chorus noise or white noise, while failing to express preferences for calls with high-density chorus noise or white noise (Coss et al. 2021; Taylor et al. 2021). Overlapping conspecific and/or heterospecific calls cannot be avoided in dense anuran choruses, which may obscure the fine acoustic features of the calls and further affect phonotactic behavior and signal selectivity of females (Marshall et al. 2006). In other words, noise masking may increase the threshold of signal detection and decrease the signal receiver's ability to recognize signals (Narins and Wagner 1989; Wollerman and Wiley 2002; Clark et al. 2009), ultimately resulting in a decrease in reproductive success of vocal animals. These findings, including ours, suggest that conspecific calls with higher SNR are more attractive to females than those with lower SNR. Therefore, females favor conspecific calls with higher SNR, which may assist them in choosing males that produce louder calls.

We found that there was no significant difference in latencies between choosing various conspecific calls with different SNRs in females (Figure 2B). Since conspecific calls
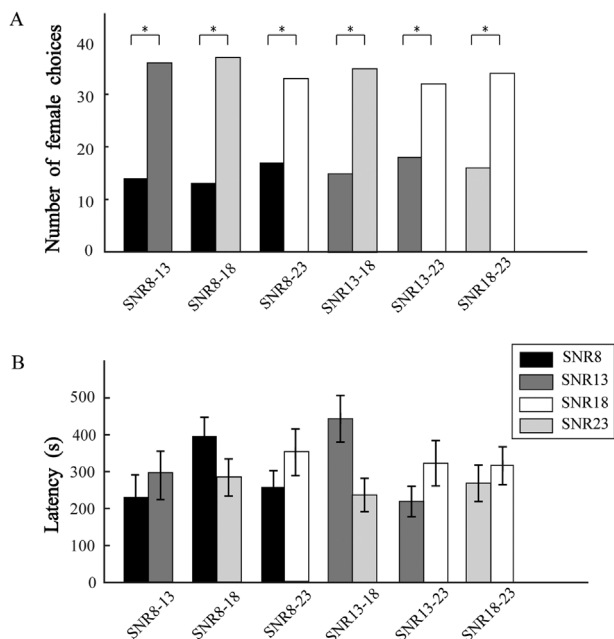

Figure 2. The number of female choices (A) and corresponding latencies (B) for each acoustic stimulus under different stimulus pairs. SNR8, SNR13, SNR18, and SNR23 refer to conspecific advertisement calls with various SNRs of 8, 13, 18, and 23 dB, respectively. *$P < 0.05$.

Table 2. The acoustic parameters of male-evoked vocal responses

	Stimulus				
	CC	**SNR8**	**SNR13**	**SNR18**	**SNR23**
Total calls	30.9 ± 10.9	34.8 ± 12.1	42.8 ± 20.8	40.3 ± 16.3	42.2 ± 14.5
Number of notes	18.1 ± 3.2	20.8 ± 4.0	21.5 ± 4.4	21.6 ± 4.9	21.6 ± 3.6
Call duration	2.5 ± 0.6	3.1 ± 0.9	3.1 ± 0.8	3.1 ± 0.7	3.2 ± 0.8
Responsive latency		1.6 ± 0.9	1.4 ± 0.9	1.4 ± 0.6	1.2 ± 0.6

Note: CC, control condition, that is, no sound was played back; SNR8, SNR13, SNR18, and SNR23 refer to conspecific advertisement calls with various SNRs of 8, 13, 18, and 23 dB, respectively.
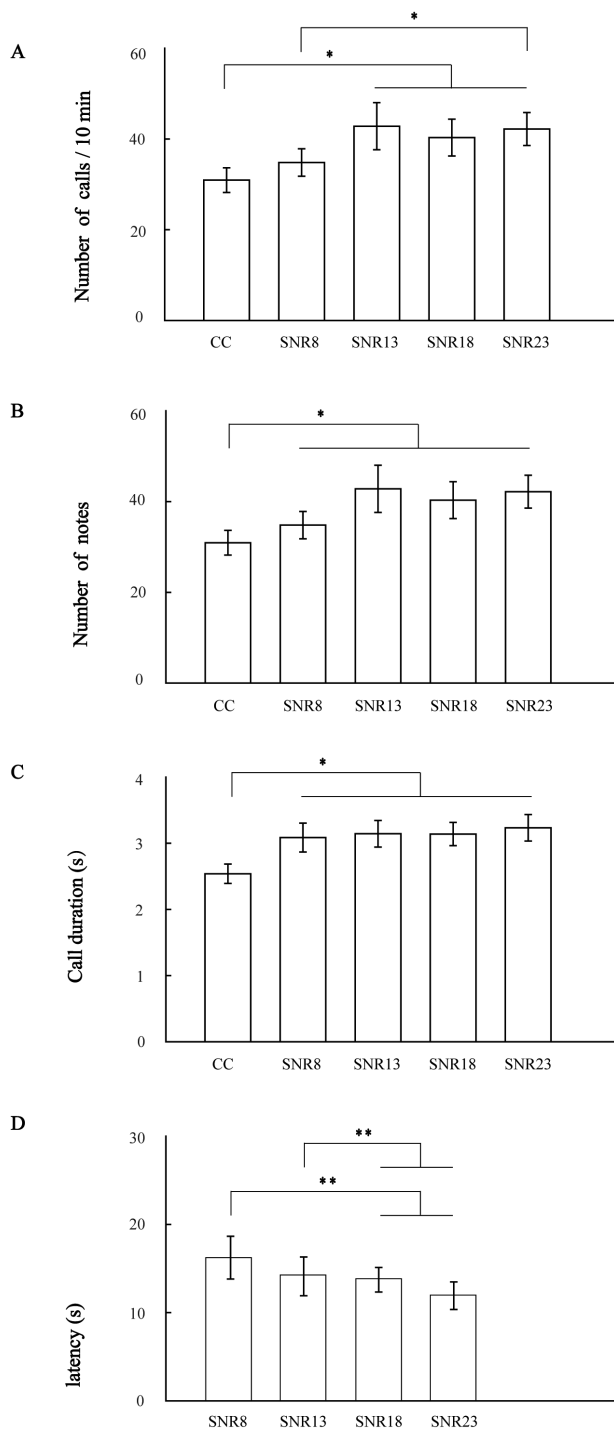
Figure 3. The acoustic parameters of the males' responsive vocalizations, that is, the number of calls (A), the number of notes for calls (B), call duration (C), and the onset latency of the first responsive call related to the onset of first playback (D). CC, control condition, that is, no sound was played back; SNR8, SNR13, SNR18, and SNR23 refer to conspecific advertisement calls with various SNRs of 8, 13, 18, and 23 dB, respectively; $^*P < 0.05$ and $^{**}P < 0.001$.

with higher SNR are more attractive to females, it could be expected that females would decide to select these higher SNR conspecific sounds more quickly than those with lower SNR. For example, when female Cope's gray tree frogs *Hyla chrysoscelis* are exposed to low, medium, and high levels of road traffic noise, they expressed longer latencies in high levels

of road traffic noise and took longer to make decisions (Bee and Swanson 2007). This contradiction between the present results and those of previous studies might result from species differences, or different levels and different types (conspecific calls, heterospecific calls, natural noise, and white noise) of noise. For example, the white noise used in the present study was not identical to any type of noise that the subjects experience in their natural conditions. Thus, the difference between white noise and natural noise might contribute to this contradiction.

The present results showed that presentations of conspecific calls with different SNR evoked males to emit more vocalizations (Figure 3A), increase the number of call notes (Figure 3B), and prolong call duration (Figure 3C) compared with their spontaneous vocalizations under the natural condition. Similar responses have been found in other anuran species. For example, broadcasted conspecific advertisement calls to the green frog *Rana clamitans* (Bee and Perrill 1996) and the Emei music frog *Nidirana daunchina* (Fang et al. 2014) can elicit more calling from males. Calling behavior in male frogs is highly plastic (Brenowitz and Rose 1999; Reichert 2010), and male calling strategies are influenced by social and environmental factors (Halfwerk et al. 2017). This is because male–male competitions in anurans do not usually result in physical contact, but must rely strongly on an assessment of the social environment to optimize their competitive strategy (Wells and Schwartz 2007). These results, including ours, suggest that competitive motivation of the frogs could be promoted by external acoustic stimuli. Interestingly, a similar phenomenon can be found in many vocal animals, such as insects (Schatral and Bailey 1991), birds (Pizzari and Birkhead 2001), and some mammalian species such as humpback whales (Herman 2017). Consequently, males can dynamically optimize their competitive strategies according to changes in social and environmental factors.

Although most differences in the number of notes (Figure 3B) or call durations (Figure 3C) among various advertisement calls with different SNRs were not significantly different, these acoustic parameters showed a tendency to increase in correspondence with the levels of SNR in sounds. Moreover, the latencies for the first responses in males decreased gradually along with the ordinal decline of the levels of noise in sounds (Figure 3D). These results suggest that competitive motivation of male Anhui tree frogs would be promoted by higher SNR in conspecific calls. Similarly, a previous study on the Bornean foot-flagging frog *Staurois parvus* has shown a significant decrease in the frequency of male calls under high-intensity flowing water noise, compared with the condition of low-intensity flowing water noise (Grafe and Tony 2017). Although low-frequency noise generated by motorbikes in motion might increase call rate in some species such as the Taipei frog *Rana taipehensis* (Sun and Narins 2005), reverse effects can be found in other species such as Butler's pigmy frog *Microhyla butleri*, dark-sided frog *Rana nigrovittata*, and banded bullfrog *Kaloula pulchra* compared with quiet conditions (Sun and Narins 2005). Similar negative effects of noise on male vocalizations could also be found in other vocal animals, including birds (McLaughlin and Kunc 2013) and insects (Bent et al. 2021). Accordingly, it seems reasonable to speculate that conspecific calls with higher SNR would promote the competitive motivation of males of some species, though this speculation remains to be further elucidated.

Table 3. Results of ANOVAs for number of calls, number of notes, call duration and latency in responding to stimulus playbacks

	F	*P*	ε	η^2	LSD
Total calls	3.677	0.034[*]	0.539	0.197	SNR13,18, 23 > CC; SNR23 > SNR8
Number of notes	4.573	0.003[*]	NA	0.234	SNR8,13,18, 23 > CC
Call duration	10.781	0.005[*]	NA	0.418	SNR8,13,18, 23 > CC
Latency	7.421	0.000[**]	NA	0.331	SNR8,13 > SNR18,23

Note: The symbols ">" denote that the values on the left side of ">" is significantly greater than that on the right side, and no significant difference exists among the corresponding conditions on the same side of ">". *F*, the *F*-value from ANOVA; ε, the values of epsilon for the Greenhouse–Geisser correction; LSD, the least significant difference test; CC, control condition, that is, no sound was played back; SNR8, SNR13, SNR18, and SNR23 refer to conspecific advertisement calls with various SNRs of 8, 13, 18, and 23 dB, respectively; NA, not applicable.
[*]$P < 0.05$, [**]$P < 0.001$.

The present results show that female Anhui tree frogs preferentially selected the conspecific calls with higher SNR compared to calls with lower SNR (Figure 2A), while males preferentially responded vocally to the conspecific calls with higher SNR (Figure 3). These results suggest that males may assess the quality of their rivals and then dynamically adjust their competitive strategy according to females' preferences. A similar phenomenon has been found in birds (McMullen et al. 2014) and insects (Sathyan and Couldridge 2021), where dominant males preferentially compete with the remaining dominant males to optimize energy consumption (Leonard and Hedrick 2009). In general, sexual selection is mainly controlled by females (female choice or male–female interaction). Bateman's principles suggest that females are choosier in mate choice because they are more energy intensive in egg production and more restricted in their reproductive success (Cox and Leboeuf 1977; Bernal et al. 2007). The presence of high-intensity noise may obscure key information conveyed by the call, further reduce the quality of the call, and negatively affect reproductive success (Potvin and Macdougall-Shackleton 2015; de Jong et al. 2018; Blom et al. 2019). Accordingly, females tend to prefer nonoverlapping conspecific calls with high SNR (Bernal et al. 2007). On the other hand, male vocalization is a very energy-intensive behavior that greatly increases the risk of predation (Walker and Marzluff 2017), so males will often aim to minimize the cost of attracting females' attention while maximizing the probability of mating (Byrne 2008). It would be expected that competing with rivals according to female preferences would be an optimized strategy in a chorus, in which mating success would theoretically be maximized (Cotton et al. 2006; Stoltz et al. 2008).

In conclusion, the present results show that noise, especially high-intensity noise, has a negative impact on the reproductive behaviors of Anhui tree frogs. Male Anhui tree frogs prefer to respond to conspecific calls with high SNR, and are less motivated to compete with conspecific calls with low SNR, while females also prefer conspecific calls with high SNR. These results imply that the competitive strategy of males depends on female preferences. Since the white noise used here is not completely similar to any type of noise that the frogs are subjected to in nature, further studies will be needed to determine whether both sexes of the Anhui tree frog exhibit similar behavioral responses to the conspecific advertisement calls when there is interference from biotic or abiotic noise.

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Ethics approval

All experimental procedures were approved by the Animal Care and Use Committee of Chengdu Institute of Biology.

Conflict of interest

The authors declare that they have no competing of interests.

Author contributions

G.Z.F. and B.W.Z. conceived and designed the study. Z.Y.W., H.H.M., C.C., R.L.S., and K.L. performed the experiments. Z.Y.W analyzed the data and drafted the manuscript. G.Z.F. and B.W.Z. corrected the manuscript. All authors read and approved the final version of the manuscript.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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