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Mate choice decisions of female serrate-legged small treefrogs are affected by ambient light under natural, but not enhanced artificial nocturnal light conditions

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ABSTRACT

Artificial light at night (ALAN) is a widespread anthropogenic stimulus that can significantly alter nocturnal animals' behavior, from migration to foraging to vocal communication. In the present study, we tested the hypothesis that the mate choice decisions of female serrate-legged small treefrogs (*Kurixalus odontotarsus*) were influenced by ambient light intensity. Standard two-speaker phonotaxis tests were conducted in a sound attenuating chamber. We set four light treatments (I-IV, from low to high) based on a range of light intensities from the maximum natural light at night (i.e., full moon) to that of the actual calling sites, which had artificial light. Contrary to our prediction, female frogs showed a preference for calls on the bright side in treatment I when they were exposed to identical stimuli. However, females preferred longer calls on the dim side to shorter calls on the bright side in this treatment. In addition, there were no significant effects of choice side, light treatment or their interaction on leave time or choice time. Our results suggest that females are more attracted to mates in bright light under natural nocturnal light conditions, but the preference for longer calls is not altered in serrate-legged small treefrogs.

1. Introduction

Urbanization is rapidly consuming natural land and dramatically altering natural environments. Among the various pollutants that accompany urban construction, light pollution is one of the most immediate and evident (Longcore and Rich, 2004; Falchi et al., 2016), since artificial light at night (ALAN) is commonly much brighter than natural light at night (e.g., moonlight or starlight). This anthropogenic stimulus can significantly alter nocturnal animals' behavior, from migration (La Sorte et al., 2017) to foraging (Amichai and Kronfeld-Schor, 2019) to vocal communication (Van Doren et al., 2017). In addition, ALAN may facilitate the predation of some nocturnal species (Spoelstra et al., 2017) and, in turn, promote anti-predator strategies (Russart and Nelson, 2018). Consequently, these behavioral alterations can affect an individual's survival and reproductive success and may ultimately influence population dynamics (van Geffen et al., 2015).

It is worth noting that ALAN is so widespread that it exists not only in urban areas but also on roads connecting different cities or in remote buildings. Thus, wild animals might be more likely to be influenced by these kinds of nocturnal light. For example, male forest-breeding songbirds that live near streetlights start singing at dawn significantly earlier than males elsewhere in the forest (Kempenaers et al., 2010), and this effect occurs in regions that differ in natural variation in daylength (Da Silva and Kempenaers, 2017). Furthermore, a long-term study on blue tits (*Cyanistes caeruleus*) suggested that streetlights induce females to start egg laying on average 1.5 days earlier and facilitate males to obtain twofold extra-pair mates (Kempenaers et al., 2010).

Amphibians primarily inhabit places such as forests, mountain streams and farmlands that are far from urban areas (Wells, 2007). However, human activities such as the development of tourism and rural construction may increase their exposure to streetlights. Most anurans (frogs and toads) are nocturnal, and their reproductive activities mainly rely on vocal communication (Gerhardt and Huber, 2002). A study on green frogs (*Rana clamitans*) suggested that brighter light

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conditions induce fewer advertisement calls and more movements in males, reflecting an anti-predator response (Baker and Richardson, 2006). In contrast, Tuttle and Ryan (1982) reported that male *Smilisca sila* reacted to bat models by decreasing calling and behaving more cautiously at lower light levels, because the frogs could detect the predator less effectively under dimmer conditions. It seems that behavioral responses to variations in nocturnal light levels are species-specific in anurans, and the alterations in vocal behavior may have potential effects on population recruitment rates (Baker and Richardson, 2006). However, whether and how ALAN affects female mate choice decisions in anurans has received less attention thus far.

In the present study, we conducted two experiments to test our hypothesis that the mate choice decisions of a female were influenced by ambient light intensity in serrate-legged small treefrogs (*Kurixalus odontotarsus*). Male frogs usually vocalize in the bushes or on branches to attract mates, and individuals are often observed vocalizing near streetlights or beside buildings that have artificial light (field observations). The calls of *K. odontotarsus* comprise two types of notes: a wideband note A, and a narrowband note B (Zhu et al., 2017a). Commonly, male frogs produce calls with a series of A notes (1–8 notes) to attract females, and our previous study has demonstrated that females prefer calls with more A notes (i.e., a longer call, Zhu et al., 2017b). In experiment 1, we examined whether females are more attracted to mates in dim or bright light. This experiment involved four light treatments and each treatment had a bright side and a dim side. We broadcasted identical stimuli (calls with five A notes, i.e., 5A, Fig. 1) using two speakers, one on the bright side and one on the dim side. We predicted that females would prefer the one on the dimmer side in each treatment because the darkness would be helpful in predator evasion. In experiment 2, we examined whether ambient light intensity of the calling site altered the inherent choice preferences for longer calls of female frogs (Zhu et al., 2017b). Since female preferences for longer calls have been demonstrated in a number of species (Klump and Gerhardt, 1987; Cui et al., 2016), we predicted that females would prefer the speaker that broadcasted longer calls, regardless of the light intensity on that side. Finally, we predicted that females would take more time to leave the release location and less time to make a choice

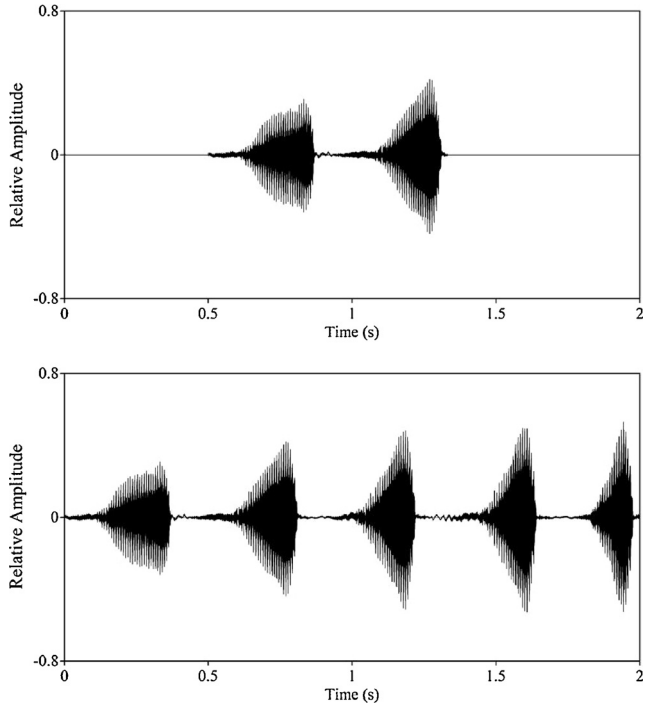

Fig. 1. Oscillogram of call with two A notes (2A, top) and call with five A notes (5A, bottom) of *K. odontotarsus*.

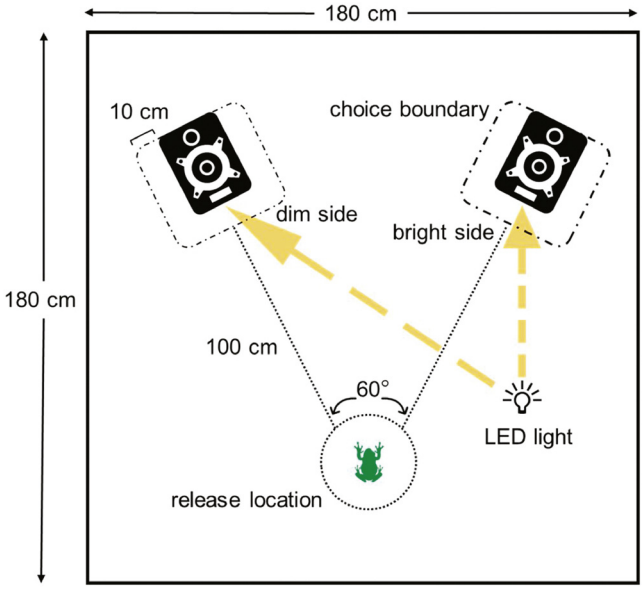

Fig. 2. Apparatus of the phonotaxis tests for *K. odontotarsus* in the sound attenuating chamber.

Table 1
The light intensities of male serrate-legged small treefrogs' calling sites, measuring using Light Meter Pro. (TES-1339, TES Electrical Electronic Corp.).

Light intensity (lx)	Max	Min	Mean ± SE	Sample size
Under streetlights	19.27	6.98	12.80 ± 2.10	12
Besides buildings	55.15	22.81	36.50 ± 5.25	12

under brighter light conditions because the predation risk may be increased by the bright light.

2. Materials and methods

2.1. Study site

Our studies were conducted from July to August 2018 at Diaoluo Mountain National Nature Reserve in Hainan, China (18.44 °N, 109.52 °E, elevation of 933 m). The experiments were carried out between 21:00 and 01:00 h, the average temperature was 20.2 ± 0.1 °C and the average relative humidity was 92.5 ± 0.5 (%).

2.2. Experimental apparatus

Standard two-speaker phonotaxis tests were conducted in a sound attenuating chamber under infrared light (Fig. 2). The included angle between the two speakers (Amplified field speaker, Saul Mineroff Electronics, Inc.) was 60°, and the distance between each speaker and the release location was 100 cm (Fig. 2). An LED light that faced the center of the speaker on the same side was placed either on the left or the right side to simulate a streetlight. Therefore, the ambient light intensity on one side was higher and that on the other side was lower. The LED light was tangent to the leading edge of the release location (Fig. 2); thus, the beam was pointing forward to avoid shining directly into the frog's eyes. Four light treatments (I-IV) were set, and the light intensity of the bright side was consistent with a light intensity ranging from that of the full moon (Campbell et al., 2008; Underhill and Höbel, 2017) to that of the actual calling sites that had artificial light (measured using Light Meter Pro. TES-1339, TES Electrical Electronic Corp. Table 1): 2.1, 13, 36 and 55 lx, respectively. Accordingly, the light intensity of the dim side was 0.7, 4.25, 11.85 or 17.85 lx for the four light

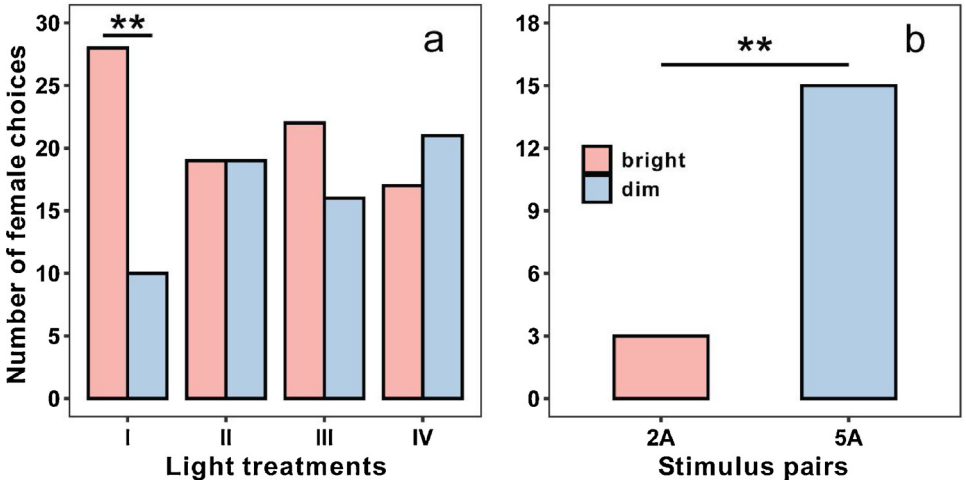

Fig. 3. Results of female *K. odontotarsus* phonotaxis tests. (a) Females only showed a preference for calls on the bright side in treatment I (N = 38). (b) Females preferred longer calls on the dim side in treatment I (N = 18). The asterisk indicates statistically significant difference (< 0.01).

Table 2
LME models for testing the effects of choice side, light treatment and their interaction on leave time and choice time in female *K. odontotarsus*.

	Factor	Estimate	SE	*t*	*P*
Leave time	Side-dim	22.4949	28.1530	0.7990	0.4261
	Light treatment-I	−24.1930	24.3443	−0.9937	0.3227
	Light treatment-II	−24.9393	27.1186	−0.9196	0.3599
	Light treatment-III	27.4045	26.4182	1.0373	0.3020
	Side * treatment-I	−7.7024	41.6561	−0.1849	0.8537
	Side * treatment-II	−9.7918	40.1230	−0.2440	0.8077
	Side * treatment-III	−63.1957	39.7318	−1.5905	0.1148
Choice time	Side-dim	−8.8878	42.7529	−0.2078	0.8357
	Light treatment-I	−60.4193	37.5970	−1.6070	0.1110
	Light treatment-II	−53.0933	41.9081	−1.2668	0.2079
	Light treatment-III	−18.0203	40.1759	−0.4485	0.6547
	Side * treatment-I	49.3170	62.3352	0.7911	0.4306
	Side * treatment-II	52.8826	60.6503	0.8719	0.3852
	Side * treatment-III	−8.5415	60.4245	−0.1413	0.8879

treatments, respectively.

2.3. Procedures in the behavioral tests

In experiment 1, we examined whether females were more attracted to mates in dim or bright light. Each female completed four consecutive phonotaxis tests with different light treatments presented in a random order and was allowed a 3-min rest between tests. Two identical calls (calls with five A notes, i.e., 5A, Fig. 1) were presented antiphonally with 5-s interstimulus intervals, and the amplitude of the stimulus pairs was equalized for an 80 dB SPL (re 20 μPa) (Zhu et al., 2017b), measured at the release location of the female using a sound pressure levels meter (AWA 6291, Hangzhou Aihua Instruments Co., China). We scored a choice as occurring when female frogs approached within 10 cm of a speaker without simply following the wall. The leave time (the time from lifting the lid until the female came out of the release location) and the choice time (the time until the female entered the choice boundary) were recorded in all tests. If a female did not reach any speaker within 10 min, we reran the test after a 5-min rest. Females that had three consecutive nonresponses were not tested further on that day. In total, 40 gravid females (eggs were visible through the skin) were used in the phonotaxis tests, but two of these females had no responses to male calls.

Females showed a preference for calls on the bright side (2.1 lx) in treatment I. Consequently, we examined whether ambient light intensity altered the inherent choice preference of female frogs only in this treatment. Since female *K. odontotarsus* prefer long call to short call (Zhu et al., 2017b), a short call (2A, Fig. 1) was presented by the speaker on the bright side (2.1 lx), and a long call (5A, Fig. 1) was presented by the speaker on the dim side (0.7 lx). A total of 20 gravid females were used in this experiment, and 2 of 20 tested females did not

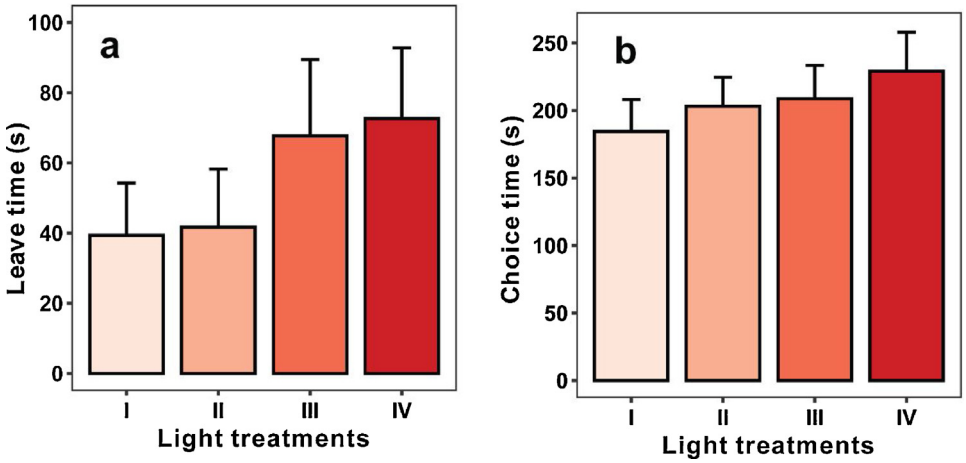

Fig. 4. Bar chart illustrating the mean (± SE) values of leave time (a) and choice time (b) of female *K. odontotarsus* in different light treatments in phonotaxis tests (N = 38).

complete the trials.

All stimuli we used in this study were from Zhu et al. (2017b), which were constructed using natural calls.

2.4. Statistical analysis

A binomial test was used to evaluate the phonotaxis data. Linear mixed effects (LME) models were used to determine whether leave time and choice time were affected by the choice side and light treatment. Choice side and light treatment were treated as fixed effects, and female identity was treated as a random effect. The results were expressed as the mean ± standard error (SE). $P < 0.05$ was considered to indicate statistical significance. LME models were analyzed using the lme4 package (Bates et al., 2013). All statistical analyses were performed with R software (R Core Team, 2016).

3. Results

When exposed to identical stimuli, sixty-four percent of the females approached the speaker on the bright side instead of the speaker on the dim side in treatment I ($P = 0.005$), but not in treatments II, III or IV, in which the light intensities of both sides were higher than 2.1 lx ($P > 0.05$, Fig. 3a). However, more than 80 percent of females preferred 5A on the dim side to 2A on the bright side in treatment I ($P = 0.008$, Fig. 3b). In addition, there were no significant effects of choice side, light treatment or their interaction on leave time (all $P > 0.05$, Table 2, Fig. 4a) or choice time (all $P > 0.05$, Table 2, Fig. 4b).

4. Discussion

Contrary to our prediction, female *K. odontotarsus* showed a preference for calls on the bright side (2.1 lx) in treatment I when facing the same simulated male calls. This result suggests that females were more attracted to mates in bright light under natural light conditions. Although the reproductive activities of most anurans mainly rely on vocal communication (Gerhardt and Huber, 2002), visual cues also play an important role in signal transmission and reception in some species (Summers et al., 1999; Rosenthal et al., 2004; Narins et al., 2005; Taylor et al., 2007; Gomez et al., 2009). For instance, the vocal sac of male túngara frogs (*Physalaemus pustulosus*) may serve as a visual signal to females searching for mates (Rosenthal et al., 2004), and this visual stimulus can enhance call attractiveness (Stange et al., 2017). Thus, we speculated that the reason why female *K. odontotarsus* preferred calls on the bright side was that ambient light facilitated females to detect and locate potential mates.

However, this preference was not found in treatments II, III or IV, indicating that the effects of ambient light on choice preference were constrained by light intensity. Studies on eastern gray treefrogs (*Hyla versicolor*) suggested that females' call preference and choosiness are not influenced by ambient light intensities, either under natural light levels (Underhill and Höbel, 2017) or under artificially high light levels (Underhill and Höbel, 2018). Thus, additional studies are needed to investigate whether nocturnal light affects female mate choice decisions in anurans.

In experiment 2, females preferred longer calls (5A) on the dim side to shorter calls (2A) on the bright side in treatment I. Like some other species of frogs (Kelley, 2004; Bernal et al., 2009; Cui et al., 2016), female *K. odontotarsus* exhibit preferential phonotaxis to longer calls (Zhu et al., 2017b). The present study further demonstrates that vocal signals play a key role in decision-making in the mate choice of anurans, and ambient light conditions are insufficient to alter the inherent choice preferences for longer calls of female *K. odontotarsus*.

In addition, choice side, light treatment and their interaction had no significant effects on leave time and choice time. Generally, exposure to light may increase the risk of predation for nocturnal frogs (Rand et al., 1997). A study on túngara frogs suggested that females are less choosy in dim light conditions than in darkness (Baugh and Ryan, 2010), and field observations demonstrated that females make faster approaches under full moon conditions than under a new moon (Baugh and Ryan, 2010). However, nocturnal light also allows prey to detect predators, thereby reducing predation risk (Rodriguez et al., 2018; van Grunsven et al., 2018). In the present study, females spent a similar amount of time on decision-making in different light treatments, regardless of which side they chose. Consequently, we speculate that nocturnal light may have similar effects on prey detection and predator detection.

Taken together, our results demonstrate that females are more attracted to mates in bright light under natural nocturnal light conditions, but the preference for longer calls is not altered in serrate-legged small treefrogs. In addition, we have shown that neither dim nor bright light conditions affect the decision-making time of female frogs. The effect of light on mate choice behavior may be highly species-specific and sex-specific (Baker and Richardson, 2006; Sun et al., 2017; Underhill and Höbel, 2018). Male frogs that call from conspicuous or bright places might benefit from enhanced detection by females, but this behavior likely comes at the cost of increased predation risk. Future studies are needed to investigate how males choose the sites from where they display to improve signal efficacy, and to assess the costs and benefits in order to advance our understanding of the adaptation to artificial light in anurans.

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