

# Mate choice behavior of female Eastern Gray Treefrogs (*Hyla versicolor*) is robust to anthropogenic light pollution

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Editor: W. Koenig

## Abstract

Human activities are drastically changing the amount of artificial light entering natural habitats. Because light pollution alters the sensory environment, it may interfere with behaviors ranging from prey detection and vigilance to mate choice. Here, we test the hypothesis that anthropogenic light pollution affects the mate choice behavior of female Eastern Gray Treefrogs (*Hyla versicolor*). We tested this hypothesis under two experimental light treatments that simulate the light pollution created by streetlights (expansion of lit areas and increased light intensity), and the light pollution created by headlights of passing vehicles (rapid fluctuations between bright and dark conditions). The hypothesis predicts that females tested under conditions simulating light pollution will show behavioral changes geared toward mitigating detection by predators, such as relaxed preferences, decreased choosiness for the normally preferred call, and differences in approach behavior (either more directional, faster, or stealthier movements, or no approach at all). Contrary to our prediction, we found that light pollution did not affect mate choice behavior in Gray Treefrogs, and should therefore neither interfere with population persistence nor affect the sexual selection regimes on male call traits of this species. However, we caution that this result does not imply that anthropogenic light pollution is of no concern for amphibian conservation, because behavioral responses to variation in nocturnal light levels (both in the natural as well as anthropogenically enhanced range) seem to be highly species-specific in anurans. We encourage additional studies to help gauge the vulnerability of anurans to anthropogenic light pollution.

## KEYWORDS

anurans, choosiness, conservation, phonotaxis, preference function, sexual selection

## 1 | INTRODUCTION

Urbanization is rapidly altering natural environments. One of its consequences is ecological light pollution, the alteration of natural light levels due to the introduction of artificial light at night (Longcore & Rich, 2004). In recent years, increases in human population growth, industrialization, and urban and suburban sprawl have drastically changed the amount of artificial light entering natural habitats (Cinzano, Falchi, & Elvidge, 2001). The extent of ecological light

pollution is global (Elvidge, Baugh, Kihn, Kroehl, & Davis, 1997); nearly 20% of land on earth is considered polluted by light (Cinzano et al., 2001), and this pollution is increasing every year (Hölker et al., 2010).

Natural light has strong biological relevance for daily and annual biological rhythms, and artificial lighting may interfere with daily activity, sleep patterns, and the timing of reproduction (Kempnaers, Borgström, Loës, Schlicht, & Valcu, 2010; Nordt & Klenke, 2013; Russ, Rüger, & Klenke, 2015). Because it alters the sensory environment,

artificial light may also interfere with predator–prey dynamics. Predators may take advantage of improved prey detection—bats, for example, are more successful at capturing their insect prey when artificial light is present at night (Minnaar, Boyles, Minnaar, Sole, & McKechnie, 2015; Rydell & Baagøe, 1996). In response, prey may adjust their antipredator strategies. For example, some male frogs change their calling behavior when exposed to artificial nocturnal light (Baker & Richardson, 2006; Tuttle & Ryan, 1982).

The effect of light pollution on mate choice behavior has received little attention so far. This is surprising because mate choice is often thought to drive the evolution of extravagant sexual ornaments and elaborate mating displays (Andersson, 1994; West-Eberhard, 1983), which can foster divergence and reproductive isolation (Seddon et al., 2013; West-Eberhard, 2014; ). Ultimately, this may lead to differences between populations in polluted and unpolluted sites. Moreover, the act of sampling potential mates can be a dangerous undertaking because it exposes mate-searching females to predators, both via spending additional time in the open during the sampling process, and via lingering in the vicinity of conspicuously displaying males (Grafe, 1997; Hughes, Kelley, & Banks, 2012; Pocklington & Dill, 1995).

Most anurans (frogs and toads) are partly or completely nocturnal, and thus likely affected by artificially high or fluctuating illumination from light pollution (Perry, Buchanan, Fisher, Salmon, & Wise, 2008). Despite its potential impact on population maintenance and divergence, the effect of anthropogenic light pollution on anuran mate choice behavior has to our knowledge never been examined. Here, we conduct such a test with female Eastern Gray Treefrogs (*Hyla versicolor*). We focus on three aspects of female mate choice behavior: (a) mate preference functions, which describe the order in which a female ranks prospective mates, (b) choosiness, which indicates how much effort a female is willing to invest in acquiring her preferred mate; and (c) approach behavior, which focuses on the type and pattern of female mate searching movements (Kilmer et al., 2017; Kuczynski, Getty, & Gering, 2017; Underhill & Höbel, 2017). Our general prediction is that when faced with light pollution, females will show behaviors that mitigate the increased danger of being detected by predators, such as relaxed preferences, decreased choosiness, and more directional, faster, or stealthier movements toward potential mates.

Artificial light is created by a multitude of sources that likely have different biological effects, and we took this into account when assessing differences in mate choice behavior. Streetlights, lighted buildings, and security lights result in expansion of lit areas and increased light intensity. These sources thus generate a static increase in light levels, and likely affect a number of behaviors that are normally mediated by the diurnal/seasonal pattern of natural light, or with detection of prey or predators. By contrast, the headlight of cars and other vehicles lead to rapid fluctuations between bright and dark conditions. Changes of illumination over brief periods of time can interfere with the visual capacities of dark adapted eyes and temporarily blind animals (Buchanan, 2006), and its effects thus likely center on vigilance and predator detection.

We tested female mate choice behavior in two experiments that mimic the different temporal patterns of light pollution. In Experiment 1, we examined the effects of static light pollution, such as would be generated by street lamps. This experiment involved three light-intensity treatments, spanning the gamut from light levels comparable to natural moonlight to standing under a streetlight (0.2–15 lux). Consequently, in the static light treatment experiment, we examined whether *presence* and *intensity* of artificial light affect mate choice behavior, and we predict that higher light levels result in relaxed preferences, decreased choosiness, and stealthier phonotactic approach movements. In Experiment 2, we examined whether *temporal fluctuation* in light pollution affects frog behavior. This experiment involved two light treatments with identical light intensity (15 lux), but different temporal pattern (continuous, as under a street light, or intermittent, as from cars driving by). Here, we predict that the occurrence of temporal fluctuations in light levels would result in relaxed preferences, decreased choosiness, and stealthier phonotactic approach movements.

## 2 | METHODS

### 2.1 | Study site and species

This study was carried out from May–June 2016 and 2017, during the natural breeding season of *H. versicolor*. During the same time, we also conducted a study examining the mate choice behavior of females under natural nocturnal light conditions (Underhill & Höbel, 2017). The data reported there derive from a separate set of females. All females were captured in amplexus in a pond adjacent to the University of Wisconsin Milwaukee Field Station (Saukville, WI). Pairs were held in containers placed on melting ice to prevent oviposition prior to testing. Following testing, pairs were returned to the pond. All applicable national and institutional guidelines for the care and use of animals were followed. We received ethical clearance from the Institutional Animal Care and Use Committee of the University of Wisconsin–Milwaukee (protocol number 15–16 #43).

### 2.2 | Stimulus generation

Male *H. versicolor* have advertisement calls consisting of a series of short pulses. At a testing temperature of 20°C, pulses are approximately 25 ms in duration, and are repeated after a pause of 25 ms (thus, the duration of the call can be expressed in number of pulses or in milliseconds). Two frequency peaks are emphasized in the call (1,100 Hz and 2,200 Hz). Females are selective for multiple aspects of male calls, including pulse rate, call frequency, and call rate. Call duration, however, is the trait for which females have the strongest preferences (Gerhardt, Tanner, Corrigan, & Walton, 2000; Reichert & Höbel, 2015), and accordingly we focused on this call trait.

Acoustic stimuli were generated in R (Version 3.1.0) software (R Development Core Team., 2015), using the seewave package (Sueur, Aubin, & Simonis, 2008). For all stimuli, we set call frequency and call period to the average values of our study population (first frequency

peak of 1,071 Hz, second (dominant) frequency peak of 2,142 Hz (2<sup>nd</sup> peak 10 dB louder); Call period of  $7,750 \pm 3,027$  ms (see Reichert & Höbel, 2015). Call duration ranged from 6 to 30 pulses per call, increasing in increments of three pulses (i.e., 6, 9, 12, 15, 18, 21, 24, 27, and 30).

### 2.3 | General testing procedure

Females were tested in a circular playback arena (2 m diameter), set up inside a semianechoic chamber. The arena wall was constructed from hardware cloth covered in black fabric (visually opaque but acoustically transparent). We placed speakers (JBL Control 1Xtreme) just outside the arena wall and angled them toward the center of the arena. Call stimuli were played from a laptop computer, and amplified by a Behringer Reference Amplifier (Behringer, A500 Model). Call amplitude, measured at the release point of the female, was adjusted using a 407764 Sound Level Meter (EXTECH instruments, RS232/Data logger). Unless otherwise specified, amplitude was set at 85 dB SPL.

Prior to testing, we warmed females to a testing temperature of 20°C. For testing, females were placed in an acoustically transparent release cage in the center of the arena. We broadcast three repetitions of an acoustic stimulus to the female while confined in the release cage before lifting the lid by pulling on a rope attached to the top of the lid. The experimental choice time began once the female was released from the cage. A choice was defined as entering a 10-cm choice zone (marked by tape on the arena floor), after having shown directed phonotaxis movements (trials where females crossed the choice zone while aimlessly wandering around the arena were not counted). Female movements were observed and recorded outside the chamber using an infrared video camera (EQ150, EverFocus USA, Duarte, CA, U.S.A.) mounted above the arena. There were four infrared light sources (mounted on the chamber ceiling) that provided illumination for the camera at the times the treatment incandescent bulb was switched off. Females were rested 3–5 min between consecutive trials. Females that did not respond were rested for 5 min and rerun on that stimulus. Females that had three consecutive nonresponses were not tested further and their data set was discarded (6 out of 72 tested females did not complete the trials and were removed). Sample size for the four experiments was (# females tested / # responsive females included in study): Exp. 1–Preferences (21/20), Exp. 1–Choosiness (13/11), Exp. 2–Preferences (21/20), Exp. 2–Choosiness (17/15).

### 2.4 | Light treatments

We used a 100 W incandescent bulb mounted on the chamber ceiling (2 m over the arena floor) and over the center of the testing arena to simulate light pollution. We measured light levels using an EasyView Digital light meter (EXTECH instruments, Model EA31) and used a dimmer (Leviton TBL03) to adjust light levels to the desired brightness. (Nota bene: There are a large variety of artificial light sources, and the spectral composition of the light

emanating from them differs from each other, as well as from natural light (Buchanan, 2006; Elvidge, Keith, Tuttle, & Baugh, 2010). Nevertheless, we consider the exact choice of light source used in our experiment of lesser importance. At the light intensities at which the experiments were performed, even light sources whose spectral composition do not match the frog visual system would still provided females with more light than their sensory system requires for effective orientation and vigilance (Cummings et al., 2008). Moreover, studies examining frog mate choice behavior under nocturnal light levels (simulating starlight/moonlight) have used a range of light sources (nightlights, LED lights, incandescent bulbs) without differential effects on female behavior (Baugh & Ryan, 2010; Bonachea & Ryan, 2011; Rand, Bridarolli, Dries, & Ryan, 1997).

We assessed variation in female mate choice behavior in two experiments. In Experiment 1 (*Static light pollution*), we tested females under three light levels: one in the natural nocturnal range (new moon/starlight at 0.2 lux) and two increased light levels (5 lux and 15 lux, comparable to standing about 3.5 m from a streetlight, and directly under a streetlight, respectively). While testing, the light was on continuously.

In Experiment 2 (*Dynamic light pollution*), we assessed variation in female mate choice behavior under two light conditions that were identical in the intensity of the light pollution used (15 lux; comparable to the headlights of an approaching vehicle at 7–10 m distance), but differed in their temporal presentation: (a) static light pollution was simulated by a light source set at a constant brightness of 15 lux; (b) dynamic light pollution was simulated by alternating between darkness and 15 lux. We used a dimmer to steadily increase light to 15 lux over 3 s, then quickly switching the light off and maintaining darkness for 7 s (resulting in a 3 s on/ 7 s off pattern that repeated every 10 s). To verify the consistency of our manual dimmer manipulation, we reviewed the phonotaxis videos of the dynamic trials and scored the proportion of frames that appeared bright and dark, respectively. Mean  $\pm$  SD of bright frames was  $27.1 \pm 3.73\%$ , of dark frames it was  $72.9 \pm 3.73\%$ .

Rapid shifts in light intensity (which are unavoidable when transporting frogs to and from the test chamber to a holding area outside the chamber) may affect frog behavior (Buchanan, 1993, 1998). To ensure that the females' eyes maintained adapted to the treatment light level, we placed them into the testing chamber at least 5 min. prior to starting the experiment, and kept them (in individual transparent boxes inside a large, thick-walled cooler with the top open) inside the testing chamber for the duration of the experiment. The cooler was placed in the quietest location inside the test chamber (outside the actual testing arena and at 90° angle from the speaker(s)). Although this did substantially attenuate the calls broadcast to the female currently being tested, it did not completely prevent females in the cooler from hearing the playbacks. However, keeping frogs in the test chamber was the only logistically feasible way to maintain females' eyes continuously adapted to treatment light conditions.

## 2.5 | Testing variation in female mate choice behavior

### 2.5.1 | Testing variation in call duration preferences

We used a single-speaker design to assay female call duration preferences. Here, call stimuli varying in duration are presented sequentially, and the timing and directionality of a female's approach toward the speaker are noted as a measure of preference. We used a stop watch to measure choice time (time from the moment the release cage was lifted and the females were free to move around the arena until she reached the choice zone at the speaker).

Using the program PFunc (Kilmer et al., 2017), we examined variation in call duration preferences with preference functions. This program generates individual preference function curves, and also extracts several traits from those preference functions: (a) Peak describes the female's most preferred call duration value (duration eliciting the fastest response); (b) Strength describes the extent to which a female's response is reduced to signals that deviate from the peak preferred signal; (c) Tolerance describes the range of call duration values that still elicits a high level of response (within 1/3 of the function peak); and (d) Responsiveness quantifies the mean response across the range of stimuli. In this experiment, a strong preference for a signal is expressed by a fast approach toward the broadcast call (a short choice time). However, because interpretation of results is more intuitive if a female's most preferred value is shown as the highest point in a curve, not the lowest, we converted raw choice time (in seconds) to 1/choice time before generating preference functions.

Each female responded to the full complement of 9 call duration stimuli (6–30 pulses; presented in random order) under each light treatment. Since Experiment 1 had three light treatments (0.2, 5, and 15 lux), each female tested in this experiment provided three preference functions (one for each light treatment) to the final data set. Experiment 2 had two light conditions (static on or dynamic blinking), and accordingly each female provided two preference functions to the final data set.

To test for differences in preference function shape between light treatments, we entered choice time as response variables in a mixed model (standard least squares) implementing REML. As predictor variables, we entered linear and quadratic terms for call duration, a term for light treatment, and a call duration  $\times$  treatment interaction term. We entered both a linear and a quadratic term for call duration to capture the effect of preference function shape: a significant linear term would indicate that females prefer longer calls, while a significant quadratic term indicates that females prefer an intermediate call duration value. We also entered female identity as a random term, to account for each female having provided data for several preference functions (three in Experiment 1 and two in Experiment 2).

We analyzed each preference function trait using a mixed model (implementing REML) with the preference function trait (peak, strength, tolerance, or responsiveness) as the response variable, and

the light treatment (0.2, 5, and 15 lux in Experiment 1, and static and dynamic in Experiment 2, respectively) as the independent variable, and female identity as a random term. All statistical tests were implemented in JMP 11 (SAS Institute Inc., Cary, NC).

### 2.5.2 | Testing variation in Choosiness

We assayed choosiness using a two-speaker design, based on the common observation that female frogs trade off call attractiveness with distance to source (females may approach a less attractive stimulus if it is perceived as being closer, or played at relatively higher amplitude (Gerhardt, 1987). Here, an attractive (18 pulse duration) and an unattractive call (6 pulse duration) are played antiphonally (from speakers set at 180° to each other, each facing the center of the arena). The amplitude of the unattractive call remains constant at 85 dB SPL, while the amplitude of the attractive call is attenuated in successive trials (in 3 dB steps), until the female no longer approaches the attractive call.

Each female's choosiness was determined within a given light treatment; depending on the females' responses, this required 2–4 trials in which attenuation levels were adjusted until the female no longer approached the attractive call. Then, the process was repeated in another light treatment. Since Experiment 1 had three light treatments (0.2, 5, and 15 lux), each female provided three data points to the final data set. Experiment 2 had two light conditions (static on or dynamic blinking), and each female provided two data points to the final data set.

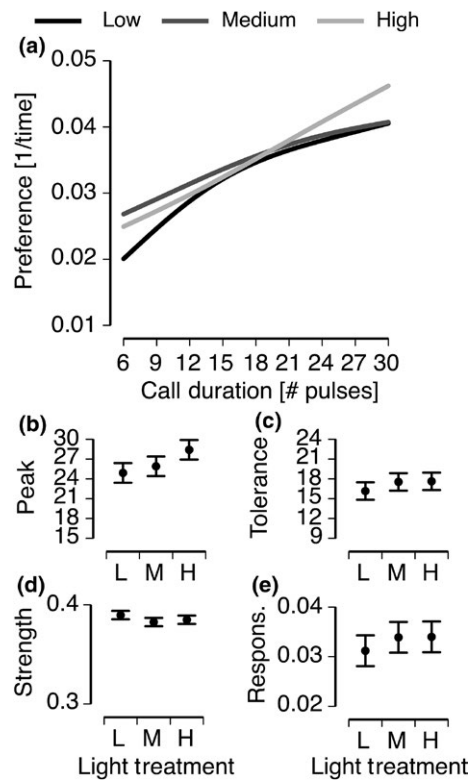
We performed a population-based analysis in which we expressed choosiness data as the percentage of females still approaching the attractive stimulus at each attenuation level. We used a mixed model implemented in JMP 11 (SAS Institute Inc., Cary, NC). We entered the percentage of females approaching the attractive call as the dependent variable, and terms for light treatment, attenuation, and the treatment  $\times$  attenuation interaction as test variables.

### 2.5.3 | Phonotaxis behavior

Reviewing the phonotaxis videos of the 6-pulse (unattractive) and 30-pulse (very attractive) trials from the light pollution preference function data sets (see 2.5.1.), we distinguished two types of movements: hops and crawls. Hops are quick movements that result in a displacement of  $>1$  body length; crawls are slow movements that result in a displacement of  $<1$  body length. For each phonotaxis approach, we noted (a) the number of hops, and (b) the number of crawls, (c) the leave time (the time after lifting the lid of the release box until the females started to move toward the speaker), and (d) the choice time (the time until the female reached the speaker).

We used JMP 11 (SAS Institute Inc., Cary, NC) to calculate a series of mixed models, testing whether phonotaxis movement behaviors, or their timing, were affected by light treatments, call attractiveness, or an interaction of the two.

Finally, we conducted an even more detailed analysis focusing only on the dynamic trials of Experiment 2, since those contained



**FIGURE 1** Call duration preferences of female Eastern Gray Treefrogs (*Hyla versicolor*) under static 0.2 lux (low), 5 lux (medium), and 15 lux (high) light levels. Shown are preference functions (a) and traits derived from those preference functions (b–e) based on the choice time it took females to reach the speaker broadcasting the test calls. Preferences functions were almost identical under the three light conditions (a), and preference function traits did not differ either (b–e). Shown are the least square means  $\pm$  SE; note that SE in panel (a) were so small that they overlap the LSM lines

periods of both bright illumination and darkness. We re-analyzed the phonotaxis videos of the 6- and 30-pulse trials, now noting for the bright and dark portions of the trials whether (a) females moved or not, and (b) how many locomotion movements (hops and crawls) they made in each condition. Because of the 30/70 bright/dark pattern of those trials (see 2.4.), we calculated the observed % of movements in the dark (# movements in dark / total # movements), and used JMP 11 (SAS Institute Inc., Cary, NC) to test whether this differed from the expected % of movements in the dark (using the % of darkness in the bright/dark cycle extracted from each trial video).

### 2.5.4 | Effect sizes

We calculated effect sizes for the differences in preference function traits, choosiness and approach behavior, respectively, under the different anthropogenic light treatments. We calculated the correlation coefficient  $r$  from Cohen's  $d$  (by using mean values and a pooled standard deviation between light treatment types; Cohen, 1988). These values of  $r$  range from 0 to 1 and have similar interpretations as  $r^2$  in a simple linear regression. Correlation values that range from

0–0.3 indicate small effect sizes, 0.3–0.5 indicate intermediate effect sizes, and values greater than 0.5 indicate large effect sizes.

To put these effect sizes into context, we compare them to experiments that focused on the effect of call traits, not light treatment; these values serve to illustrate the differences that we are able to detect (at similar sample size), if experiments involve variables that are biologically relevant to the frogs. For some of these comparisons, we used data from previously published experiments (preference function, choosiness), for others the data set collected for this study allowed for comparisons focusing on the effect of call trait vs. light treatment (approach behavior). In either case, the tested frogs originated from the same study population, and the presented call stimuli were identical across studies. (a) For comparison with our preference function data, we calculated the correlation coefficient  $r$  (from Cohen's  $d$ ) for preference function traits obtained in Reichert and Höbel (2015). Those are based on call duration preference functions obtained using the same call stimuli as used here, but the treatment variable was presence/absence of a co-occurring visual stimulus instead of the anthropogenic light treatments used in the present study. (b) For comparison with our choosiness experiments, we calculated the correlation coefficient  $r$  (from Cohen's  $d$ ) for choosiness obtained in Underhill and Höbel (2017). Those are based on choosiness estimates where the treatment variable was call duration (choosiness when presented with 6 vs. 18 pulses compared to 12 vs. 24 pulses) instead of the anthropogenic light treatments used in the present study. The light level under which those trials were conducted was 0.2 lux, equivalent to our low-light treatments. (c) For comparison of our approach behavior data, we calculated the correlation coefficient  $r$  (from Cohen's  $d$ ) for trials comparing the effect of call duration (6 pulse vs. 30 pulse stimuli) and the effect of light treatment (0.2 vs. 15 lux trials, or static vs. dynamic trials).

## 3 | RESULTS

Across all experiments, response rate (the proportion of females that successfully completed a set of trials) was  $91 \pm 5\%$  (range 85%–95%). This is comparable to the response rate typical for our population (Höbel, pers. obs), and suggests that females were not frightened by the light treatments to the point that they refused to move or tried to flee the arena.

### 3.1 | Static light pollution

In the trials testing the effect of static light pollution on female call duration preferences, the intensity of the light stimuli between treatments differed by almost two orders of magnitude (0.2–15 lux). Nevertheless, while there was almost always a strong effect of call attractiveness on female behavior, light treatments had little if any effect.

The trials testing preference functions showed that female Eastern Gray Treefrogs (*Hyla versicolor*) prefer longer duration calls (Figure 1a, Table 1), irrespective of light treatment (Figure 1a). As

**TABLE 1** Effect of temporal variation in light pollution on preference for call duration in female Eastern Gray Treefrogs (*Hyla versicolor*)

Factor	df	F	p
Light treatment	2, 326	0.9	0.39
Call duration	<b>1, 326</b>	<b>60.2</b>	<b>&lt;.0001</b>
Call duration × Call duration	<b>1, 326</b>	<b>10.5</b>	<b>0.0013</b>
Treatment × Call duration	2, 326	1.6	0.20
Treatment × Call duration × Call duration	2, 326	2.7	0.07

Note. Females preferred longer duration calls, but light treatment did not affect call duration preferences. Significant terms are set in bold. See also Figure 1.

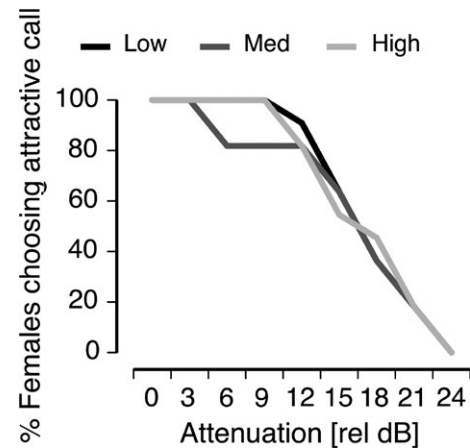
may be expected from the similar shapes of the preference functions, there was also no significant difference in the preference function traits of peak preference ( $F_{2,38} = 1.58$ ,  $p = 0.22$ ; Figure 1b); strength ( $F_{2,38} = 2.29$ ,  $p = 0.12$ ; Figure 1c), tolerance ( $F_{2,38} = 0.46$ ,  $p = 0.63$ ; Figure 1d); and responsiveness ( $F_{2,38} = 0.69$ ,  $p = 0.51$ ; Figure 1e). Effect sizes were small in each case: peak preference ( $r = 0.26$ ), strength ( $r = 0.25$ ), tolerance ( $r = 0.12$ ), responsiveness ( $r = 0.11$ ). For comparison, effect sizes from Reichert and Höbel (2015) were generally of intermediate size: peak preference ( $r = 0.18$ ), strength ( $r = 0.37$ ), tolerance ( $r = 0.40$ ), and responsiveness ( $r = 0.40$ ).

The trials testing choosiness showed that as amplitude differences between an attractive and an unattractive call increased, the proportion of females continuing to approach the attractive call decreased (Figure 2a; Table 2: significant effect of Attenuation). However, light levels did not affect choosiness (Table 2: nonsignificant effect of Treatment), and the effect size was small ( $r = 0.03$ ). For comparison, effect sizes were large ( $r = 0.59$ ) in trials that had compared choosiness based on differences in call duration (Underhill & Höbel, 2017).

The attractiveness of the call stimuli frequently affected approach behaviors (females left the release box faster when presented with a more attractive call, they approached the attractive call faster, and they used fewer hops to reach the speaker). By contrast, treatment light levels only affected choice time (Table 3). Overall, choice time increased significantly with more intense light pollution. However, this statistical effect was likely driven by the very long choice time in response to unattractive calls at low light levels (Figure 3). Effect sizes were small for comparisons testing the effect of light treatment (Leave time ( $r = 0.21$ ), choice time ( $r = 0.13$ ), # hops ( $r = 0.27$ ), # crawls ( $r = 0.07$ ), but more often intermediate for comparisons testing the effect of call duration (Leave time ( $r = 0.32$ ), choice time ( $r = 0.39$ ), # hops ( $r = 0.19$ ), and # crawls ( $r = 0.20$ )).

### 3.2 | Dynamic light pollution

In the trials testing the effect of dynamic versus static light pollution on female call duration preferences, the intensity of the light stimuli



**FIGURE 2** Choosiness of female Eastern Gray Treefrogs (*Hyla versicolor*), expressed as the percentage of females still choosing the more attractive stimulus as it is gradually attenuated, did not differ under 0.2 lux (low), 5 lux (medium), and 15 lux (high) light levels

**TABLE 2** Effect of attenuation and light pollution treatments on choosiness: As amplitude difference increases, fewer females choose the attractive but softer call

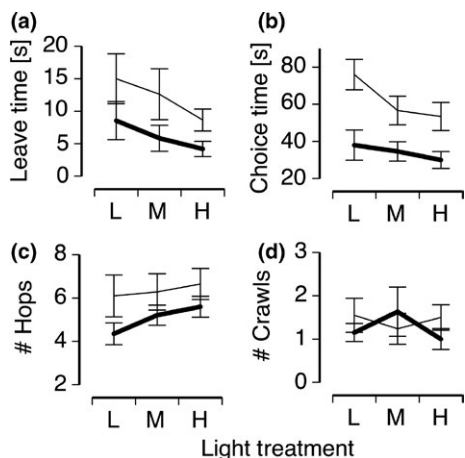
Factor	df	F	p
Light treatment	2, 21	0.3	0.73
Attenuation	<b>1, 21</b>	<b>154.0</b>	<b>&lt;.0001</b>
Treatment × Attenuation	2, 21	0.1	0.93

Note. Light treatment had no effect on choosiness. See also Figure 2.

**TABLE 3** Results of mixed models testing whether phonotaxis movements of female Eastern Gray Treefrogs (*Hyla versicolor*) differed as a function of light treatments and call duration (unattractive 6 pulse call and attractive 30 pulse call)

Behavior	Factor	df	F	p
Leave time	Light treatment	2, 95	2.0	0.14
	Call duration	<b>1, 95</b>	<b>7.4</b>	<b>0.007</b>
	Light treatment × Call duration	2, 95	0.1	0.89
Choice time	Light treatment	2, 95	4.4	<b>0.015</b>
	Call duration	<b>1, 95</b>	<b>39.7</b>	<b>&lt;.0001</b>
	Light treatment × Call duration	2, 95	1.3	0.28
# hops	Light treatment	2, 95	1.2	0.31
	Call duration	<b>1, 95</b>	<b>7.2</b>	<b>0.009</b>
	Light treatment × Call duration	2, 95	0.2	0.79
# crawls	Light treatment	2, 95	0.1	0.88
	Call duration	1, 95	0.3	0.56
	Light treatment × Call duration	2, 95	0.9	0.40

Note. Note that there was generally a significant effect of call duration (females approached the attractive call faster and with fewer movements), while light treatment only affected choice time. See also Figure 3.



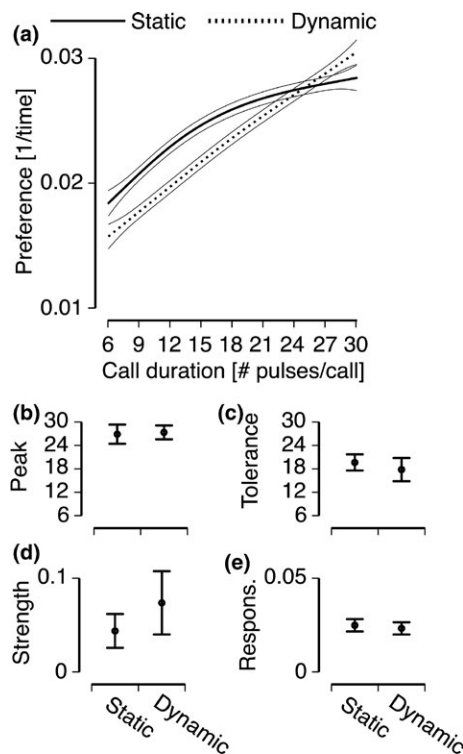
**FIGURE 3** Light treatments (0.2, 5, and 15 lux) had little effect on how female Eastern Gray Treefrogs (*Hyla versicolor*) moved toward the playback speaker. Leave time (a), the number of hops (c), and the number of crawls (d) did not differ between light treatments. Only choice time (b) was faster under higher light pollution. Shown are least square means  $\pm$  SE. Thick lines indicate responses to the attractive 30 pulse call, thin lines responses to the unattractive 6 pulse call

was set to 15 lux in all trials, but in the static trials the light remained constantly switched on (100% of each trial was illuminated), while in the dynamic trials light was switched on about 30% of the time, but the frogs spent 70% of each trial in darkness. Nevertheless, while there was almost always a strong effect of call attractiveness on female behavior, light treatments had few effects.

The trials testing preference functions showed that female Eastern Gray Treefrogs (*Hyla versicolor*) prefer longer duration calls (Figure 4a, Table 4), and the temporal pattern of the light pollution treatment did not affect how females responded to variation in call duration (Figure 4a). As may be expected from the similar shapes of the preference functions, there was also no significant difference in the preference function traits of peak preference ( $F_{1,19} = 0.07, p = 0.80$ ; Figure 4b), strength ( $F_{1,19} = 1.6, p = 0.19$ ; Figure 4c), tolerance ( $F_{1,19} = 0.79, p = 0.39$ ; Figure 4d), and responsiveness ( $F_{1,19} = 1.00, p = 0.33$ ; Figure 4e). Effect sizes were small in each case: peak preference ( $r = 0.05$ ), strength ( $r = 0.24$ ), tolerance ( $r = 0.15$ ), and responsiveness ( $r = 0.11$ ). For comparison, effect sizes from Reichert and Höbel (2015) were generally of intermediate size: peak preference ( $r = 0.18$ ), strength ( $r = 0.37$ ), tolerance ( $r = 0.40$ ), and responsiveness ( $r = 0.40$ ).

The trials testing choosiness showed that as amplitude differences between an attractive and an unattractive call increased, the proportion of females continuing to approach the attractive call decreased (Figure 5; Table 5: significant effect of Attenuation). However, the temporal pattern of the light pollution treatment did not affect choosiness (Table 5: nonsignificant effect of Treatment), and the effect size was small ( $r = 0.16$ ). For comparison, effect sizes were large ( $r = 0.59$ ) in trials that had compared choosiness based on differences in call duration (Underhill & Höbel, 2017).

The attractiveness of the test stimuli frequently affected approach behaviors; females approached the attractive call faster and with fewer hops (Table 6). Light treatment, on the other hand, only affected the number of hops during phonotaxis. Females moved less during the dynamic light pollution treatment, although this effect was likely driven by the low variance of female movements toward attractive calls in the dynamic trials (Figure 6). Effect sizes were mostly small for comparisons testing the effect

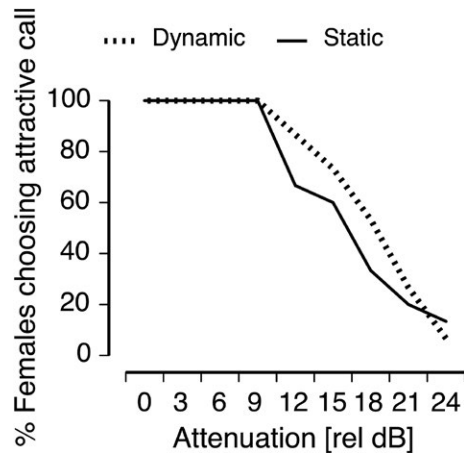


**FIGURE 4** Call duration preferences of female Eastern Gray Treefrogs (*Hyla versicolor*) under static and dynamic light treatments. Preference functions were almost identical under the three light conditions (a), and preference function traits did not differ (b-e). Shown are least square means  $\pm$  SE

**TABLE 4** Effect of temporal variation in light pollution on preference for call duration in female Eastern Gray Treefrogs (*Hyla versicolor*)

Factor	df	F	p
Light treatment	1, 335	1.9	0.16
Call duration	1, 335	<b>37.8</b>	<b>&lt;0.0001</b>
Call duration $\times$ Call duration	1, 335	1.6	0.20
Treatment $\times$ Call duration	1, 335	1.6	0.21
Treatment $\times$ Call duration $\times$ Call duration	1, 335	0.8	0.38

Note. Females preferred longer duration calls, but call duration preferences did not differ whether light pollution was static or dynamic. Significant terms are set in bold. See also Figure 4.



**FIGURE 5** Choosiness of female Eastern Gray Treefrogs (*Hyla versicolor*), expressed as the percentage of females still choosing the more attractive stimulus as it is gradually attenuated, did not differ under static and dynamic light treatments

**TABLE 5** Effect of attenuation and light pollution treatments on choosiness: As attenuation difference increase, fewer females choose the attractive but softer call

Factor	df	F	p
Light Treatment	1, 14	0.9	0.37
Attenuation	1, 14	99.9	<0.0001
Treatment x Attenuation	1, 14	0.1	0.76

Note. Light treatment had no effect on females choosiness. See also Figure 5.

of light treatment—Leave time ( $r = 0.15$ ), choice time ( $r = 0.11$ ), # hops ( $r = 0.32$ ), # crawls ( $r = 0.17$ ), but generally intermediate or large for comparisons testing the effect of call duration—Leave time ( $r = 0.41$ ), choice time ( $r = 0.54$ ), # hops ( $r = 0.25$ ), # crawls ( $r < 0.01$ ).

Focusing on the behaviors that females exhibited during the light and dark portions of the same phonotaxis approach (only the dynamic trials) gave somewhat conflicting results. Qualitatively, there seemed to be a preference for moving in the dark: while all 20 females performed at least one locomotion movement during the dark portion of the trials, only about half of them also moved during the bright periods, and none of the females moved only during bright periods (Figure 7a). Quantitatively, however, movement frequency did not appear to be influenced by light. Once taking into account that there was more darkness during the trials than bright periods (roughly 7:3 dark:bright), the expected and observed proportion of movements performed in darkness did not differ significantly (6P:  $F_{1,39} = 1.26$ ,  $p = 0.27$ ; 30P:  $F_{1,39} = 1.45$ ,  $p = 0.24$ ; Figure 7b).

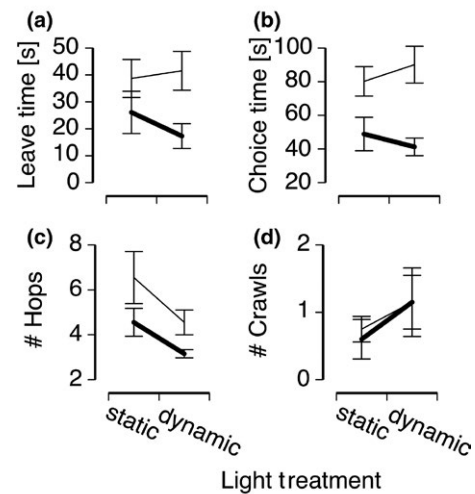
## 4 | DISCUSSION

We tested the hypothesis that mate choice behavior of female Eastern Gray Treefrogs (*Hyla versicolor*) is affected by anthropogenic

**TABLE 6** Results of mixed models testing whether phonotaxis movements of female Eastern Gray Treefrogs (*Hyla versicolor*) differed as a function of light treatments (static vs. dynamic light pollution at 15 lux) and call duration (unattractive 6 pulse call and attractive 30 pulse call)

Behavior	Factor	df	F	p
Leave time	Light treatment	1, 57	0.3	0.61
	Call duration	1, 57	10.1	0.002
	Light treatment × Call duration	1, 57	1.0	0.32
Choice time	Light treatment	1, 57	0.01	0.91
	Call duration	1, 57	21.0	<0.0001
	Light treatment × Call duration	1, 57	1.0	0.32
# hops	Light treatment	1, 57	6.0	0.017
	Call duration	1, 57	6.1	0.017
	Light treatment × Call duration	1, 57	0.2	0.67
# crawls	Light treatment	1, 57	1.9	0.17
	Call duration	1, 57	0.05	0.83
	Light treatment × Call duration	1, 57	0.05	0.83

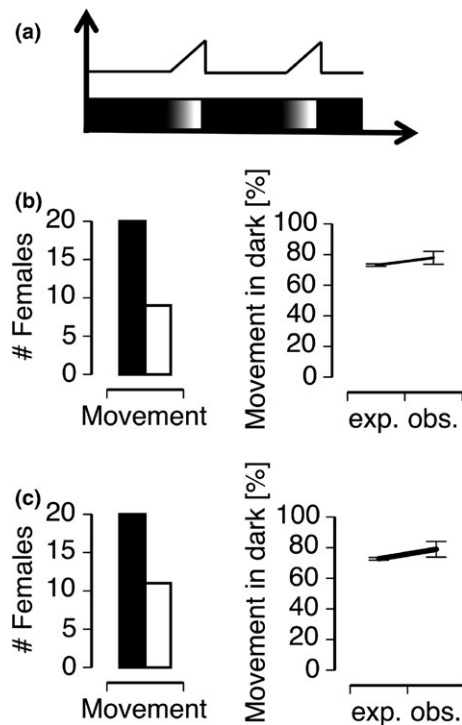
Note. Note that there was generally a significant effect of call duration (females approached the attractive call in faster times and with fewer movements), while light treatment only affected the number of hops (more hops during static light treatment). See also Figure 6.



**FIGURE 6** Light treatment (static vs. dynamic) had little effect on how female Eastern Gray Treefrogs (*Hyla versicolor*) moved toward the playback speaker. Leave time (a), choice time (b), and the number of crawls (d) did not differ between light treatments. Only the number of hops (c) was higher in the static light treatment. Shown are least square means  $\pm$  SE. Thick lines indicate responses to the attractive 30 pulse call, thin lines responses to the unattractive 6 pulse call

light pollution. Contrary to our prediction that females would show relaxed preferences, decreased choosiness, and stealthier phonotactic approach movements under brighter and / or fluctuating light





**FIGURE 7** Temporal pattern of dark and bright periods during the dynamic light pollution trials (a). Movement pattern during the 6 Pulse (b) and 30 Pulse (c) stimuli of the dynamic trials. More females moved during the dark periods than the light ones (left panels): All females moved at least once during the dark portion of the trial (black bar), while only about half the females also moved during the bright portion of the trial (white bar). Observed frequency of movement in the dark (right panels; means  $\pm$  SE), however, did not differ from expected (given that 70% of trials time was in the dark)

conditions, we actually found that almost none of the mate choice behaviors scored during our experiments were affected by light treatment.

#### 4.1 | Experiment 1: Static light pollution

In this experiment, we examined whether *presence* and *intensity* of artificial light affect mate choice behavior in *Hyla versicolor*. In our focal species, persistently increased nocturnal light levels spanning the gamut from light levels comparable to natural moonlight to standing under a streetlight (0.2–15 lux) did not change acoustic call preferences or modify choosiness for longer duration calls. This is in line with the species' response to variation in natural light levels, where female mate choice behavior is similar under conditions equivalent to starlight and to full moon (Underhill & Höbel, 2017).

To our knowledge, only one other study has examined female mate choice behavior in responses to artificially high light levels (roughly 3 lux, Rand et al., 1997). There, female Túngara Frogs changed their behavior under different light conditions in a way that suggested that they felt safer under darker conditions (Rand et al., 1997). Interestingly, female Túngara Frogs are also more cautious when tested under conditions simulating variation in natural light

levels (equivalent to full moon; Baugh & Ryan, 2010; Bonachea & Ryan, 2011), suggesting that there are genuine differences between female Gray Treefrogs and Túngara Frogs, and that the effect of light on mate choice behavior may be highly species-specific.

Some frogs use visual cues/signals during mate choice (Gomez et al., 2009; Taylor, Buchanan, & Doherty, 2007) and during male–male competition (Hödl & Amézquita, 2001). However, there is substantial between-species variation in the use of visual cues, as well as the relative reliance on visual versus acoustic signal components. In *H. versicolor*, visual mate choice is weak (Reichert, Galante, & Höbel, 2014; Reichert & Höbel, 2015), and we therefore expect that females would not attend more to visual cues under increased light levels. Nevertheless, there are species that do attend to visual cues (Gomez et al., 2009; Taylor et al., 2007), and for them increased nocturnal light should facilitate the use of inter- or intrasexual displays. A possible consequence of light pollution on visually signaling species may therefore take the form of altered selection regimes, potentially resulting in divergence between natural and polluted sites. Increased light may facilitate the use of visual over acoustic signal components, thus bringing visual components under stronger selection and/or lowering the strength of selection on acoustic traits. We would predict that a relaxation of acoustic mate preferences is the more likely outcome, because studies examining multimodal communication in frogs frequently report that colocalization of a visual cue makes a call more attractive (Gomez et al., 2009; Reichert & Höbel, 2015; Taylor et al., 2007).

Another aspect of static light pollution we did not test in our experiments is phototaxis, the movement in response to light. In a series of studies, Jaeger and Hailman (1973, 1976) tested more than 120 species of frogs and toads and found that the vast majority were photopositive and approached the light source (there were also species that avoided light and those that preferred intermediate light levels). Thus, while some frog species may avoid artificial lights, many others may be attracted to them. This could lead to denser aggregations around light sources, with unexplored or unknown consequences (Buchanan, 2006). For example, denser aggregations of displaying males may attract more predators, thus leading to higher predation pressure on both the males and the females attracted by them. Denser choruses may also lead to increased male–male aggression, and the resulting increase in males producing aggressive instead of advertisement signals may hinder the ability of females to engage in acoustic mate choice. Then again, higher density choruses may facilitate mate choice or decrease the cost of mate assessment, because potential mates are spatially concentrated.

It is also important to remember that female mate preferences are only half of the equation of successful mate choice and population maintenance. Chorus wide effects of light pollution that merit more attention are male calling behavior, including male spacing and male calling perch choice, as all can affect the ability of females to localize and assess potential mates. Detailed observations and experimental tests on changes of male calling behavior are largely missing (but see Baker & Richardson, 2006; Tuttle & Ryan, 1982). However, a number of observations suggest that changes in ambient light

levels may affect how frequently males call, how often they move between calling perches, and how exposed or hidden in vegetation they choose to call (Baker & Richardson, 2006; Onorati & Vignoli, 2017; Tuttle & Ryan, 1982).

## 4.2 | Experiment 2: Dynamic light pollution

In this experiment, we examined whether *temporal fluctuation* in light pollution affects frog behavior. This experiment involved two light treatments with identical light intensity (15 lux), but different temporal pattern (continuous, as under a street light, or intermittent, as from cars driving by). In our focal species, rapid fluctuations in light levels did not change acoustic call preferences or modify choosiness for longer duration calls.

Frogs conduct their reproductive behavior at night, and their eyes are expected to be adjusted to ambient condition and be dark-adapted. Rapid changes in light level require dark or light adaptation, respectively, or frogs will suffer reduced visual capabilities (Fain, Matthews, Cornwall, & Koutalos, 2001; Fite, 1976). When dark-adapted eyes are suddenly exposed to light, the dark-adapted, dilated pupil allows more light to enter the eye, and excessive amount of light can cause photopigment bleaching, which can take hours to return eyes to a dark-adapted state (Cornell & Hailman, 1984; Donner & Reuter, 1962). This suggests that quick changes in light intensity should affect visual acuity of nocturnal frogs. A study examining the effect of rapidly increased ambient light levels on prey capture found that frogs behaved consistent with the interpretation that they were temporarily blinded by the light and required a substantial recovery period: frogs took significantly longer to detect or to attempt to capture prey after rapid increases in illumination (Buchanan, 1998). This was the case when frogs were exposed to 12 lux, which is less bright than our experiments, suggesting that if females during phonotaxis suffer similar consequences than during foraging, we should have observed slower or more hesitant approach movements. This suggests that mate choice, at least in the Gray Treefrogs tested here, is mediated predominantly by acoustic cues. This is in line with previous studies on multimodal communication in this species, that showed that although females are not indifferent to visual cues colocalized with an acoustic signal (Reichert & Höbel, 2015), there is no evidence for visual mate choice (Reichert et al., 2014).

## 5 | CONCLUSION

We have shown that in Gray Treefrogs, light pollution does not affect mate choice behavior, and should therefore neither interfere with population persistence nor affect the sexual selection regimes on male call traits. However, there are several reasons why we consider it premature to assume that anthropogenic light pollution is of no concern for anuran conservation. First, as is tradition in anuran mate choice experiments, (Gerhardt, 1987; Rand et al., 1997; Reichert & Höbel, 2015), our trials were

conducted in a comparatively small playback arena (2 m diameter). Observations thus only encompass a small fraction of the distance a mate-searching female will cover in the night she approaches the breeding pond. Consequently, not documenting behavioral differences in phonotaxis-related behaviors during the very last portion of approach still leave many aspects of female mate choice behavior unexamined. For example, the approach path from the surrounding forest toward the breeding pond may still differ between natural and polluted sites, or females may simply choose not to approach brightly illuminated sites. Second, behavioral responses to variation in nocturnal light levels (both in the natural as well as anthropogenically enhanced range) seem to be highly species-specific, with some species showing behavioral changes (Backwell & Passmore, 1990; Rand et al., 1997), and others not (this study). Interestingly, the species that did change its behavior in response to variation in the natural range (starlight to moonlight; Baugh & Ryan, 2010; Bonachea & Ryan, 2011) also changed behavior (in similar ways) when confronted with artificially high light levels (Rand et al., 1997), while the one that did not change behavior in response to light variation in the natural range (Underhill & Höbel, 2017) was also unaffected by artificially increase light levels. Potentially, knowledge of a species' response to variation in the natural range might help gauge its vulnerability to anthropogenic light pollution.

## ACKNOWLEDGEMENTS

We thank D. Neelon, K. Stratman, R. Gremminger, L. Hayward, A. Heesacker, B. Muransky, C. Robertson, E. Ruder, T. Trinklein, and R. Trout for assistance in the field and in running playback trials. Staff at the University of Wisconsin-Milwaukee Field Station gave logistical support, R. Rodriguez and P. Dunn provided comments on the manuscript, and Nancy and Michael Byers graciously allowed us to collect frogs on their land.

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**How to cite this article:** Underhill VA, Höbel G. Mate choice behavior of female Eastern Gray Treefrogs (*Hyla versicolor*) is robust to anthropogenic light pollution. *Ethology*. 2018;124:537–548. <https://doi.org/10.1111/eth.12759>