

Variation in nocturnal light levels does not alter mate choice behavior in female eastern gray treefrogs (*Hyla versicolor*)

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Abstract

Nocturnal light levels vary throughout the course of the lunar cycle, being darkest during the new moon and brightest during the full moon. Many nocturnal animals change their behavior in response to this natural variation in moonlight intensity. Frequently, these behavioral changes can be attributed to the way in which moonlight affects the ability of predators to spot potential prey. Mate sampling females may expose themselves to predators, making mate choice a behavior likely influenced by moonlight. Because mate choice is an important cause of sexual selection, understanding the causes of variation in mate choice decisions can yield a better understanding of the strength and direction of sexual selection under natural conditions. We predicted that female eastern gray treefrogs (*Hyla versicolor*) would prefer longer calls (i.e., more attractive males) and/or be choosier, under darker conditions, because cover of darkness may aid in predator evasion. However, light treatment did not affect how females responded to variation in call duration, nor did it affect female choosiness or aspects of their approach behavior. This suggests that in gray treefrogs, variation in light levels associated with the changing phases of the moon does not alter the sexual selection regime on male call traits.

Significance statement

Looking for the perfect mate can be very dangerous, especially when environmental conditions make it more likely to be spotted by potential predators. Changes in mate choice behavior associated with predator exposure is quite common in nature, yet have rarely been examined in connection with the drastic variation in nocturnal illumination associated with the changing phases of the moon. We investigated whether females of a nocturnal treefrog change their behavior depending on whether they look for mates under simulated new moon or full moon conditions. We found that females preferred longer calls under both conditions, and that they also did not move more stealthily during bright compared to dark conditions. Our results suggest that males with long calls always have a mating advantage, and that sexual selection by female choice is uniformly strong across the lunar cycle.

Keywords Preference · Preference function · Choosiness · Sexual selection · Lunar cycle · Moon

Introduction

Mate choice is an important cause of sexual selection (Darwin 1871; West-Eberhard 1983; Andersson 1994), and understanding the causes of variation in mate choice decisions can yield a better understanding of the strength and direction of sexual selection (Jennions and Petrie 1997; Coleman et al. 2004). Variation in mate choice decisions can arise from a range of sources spanning the gamut from internal factors, such as age, size, or body condition (Hunt 2005; Uetz and Norton 2007), to context-dependent factors such as social experience with conspecifics or with predators (e.g., Johnson and Basolo 2003; Fowler-Finn and Rodríguez 2012).

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Context-dependent variation in mate choice decisions is quite common, particularly changes in female mate choice behavior associated with predator exposure. Interestingly, such effects can occur both when directly confronted with predators (e.g., Forsgren 1992; Godin and Briggs 1996; Gong and Gibson 1996; Johnson and Basolo 2003) as well as when increased predator exposure is merely inferred, such as when being placed in a situation that increases perceived predation risk (i.e., Backwell and Passmore 1990; Hedrick and Dill 1993; Rand et al. 1997; Rundus et al. 2010; Bonachea and Ryan 2011a, b). For example, female crickets are less likely to choose a previously preferred call when it is associated with little or no cover (Hedrick and Dill 1993), and female wolf spiders (*Schizocosa floridana*) appear to forego male assessment and mate more quickly under daylight compared to darkness (Rundus et al. 2010).

The changing phases of the moon can drastically alter the visual environment, and bright moonlight is widely believed to increase predation risk for nocturnal animals by increasing the ability of predators to detect prey (Prugh and Golden 2014). A number of studies have examined moonlight-mediated changes in foraging behavior, often finding that predators increase activity, while prey species decrease activity, during brighter phases of the lunar cycle. For example, cottonmouth snakes increase foraging activity during brighter nights (Lillywhite and Brischox 2012), while many rodents decrease foraging intensity during brighter nights, particularly when foraging in open habitats (Longland and Price 1991; Kotler et al. 2010).

Mate searching involves a number of behaviors that should render the individual more conspicuous to predators, such as traveling long distances and remaining in proximity to conspicuously displaying mates. Consequently, the comparative dearth of studies examining the effect of lunar illumination on mate choice behavior is surprising. To our knowledge, how lunar light affects mate choice behavior has been investigated in only two species (both anuran amphibians) (Backwell and Passmore 1990; Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011a, b, c). The way in which the behavior of female frogs differed between dark and bright nights is consistent with the interpretation that selection has acted to adjust their behavior to less perilous dark conditions. For example, in the dark, female túngara frogs (*Physalaemus pustulosus*) were more likely to engage in phonotaxis, they took more time moving towards the sound source, and they were overall more selective (Rand et al. 1997; Bonachea and Ryan 2011a, b). And female reed frogs (*Hyperolius marmoratus*) preferred to approach a broadcast call via elevated perches during bright moonlit nights, but perches had no effect on phonotaxis in darkness (Backwell and Passmore 1990).

Although the above studies provide an interesting glimpse into the potential importance of moonlight-mediated variation in mate choice behavior, they tend to focus on behaviors associated with female's willingness to approach an attractive

call in the face of danger. Mate choice decisions, however, are the product of a complex interaction between mate preference, i.e., the order in which females rank prospective mates, and choosiness, i.e., the effort females are willing to invest to reach their preferred mate (Jennions and Petrie 1997). Both preference and choosiness may vary with nocturnal light levels, and they could do so either independently or in a correlated fashion. For example, a female's mate preference may remain constant across environmental conditions, but she may be less choosy under increased perceived predation risk. In addition, other aspects of the mate searching process, such as how directly a female approaches a chosen mate, or how careful and stealthy her locomotion movements are, may be influenced by the environmental context in which mate choice takes place.

We were interested in whether mate choice behavior of female eastern gray treefrogs (*Hyla versicolor*) is affected by variation in moonlight intensity. *Hyla versicolor* is a common North American treefrog species, and has been the focus of intense research on neurophysiological (Diekamp and Gerhardt 1995), behavioral (Runkle et al. 1994; Schwartz et al. 2001), and evolutionary (Sullivan and Hinshaw 1992; Gerhardt et al. 2000; Gerhardt 2005; Reichert and Höbel 2015) aspects of mate choice and sexual selection. Studies examining female mate choice behavior in frogs are traditionally conducted in darkness (under infrared light that frogs cannot see). If moonlight-dependent variation in mate choice behavior is indeed widespread in frogs (or nocturnal animals in general), this would suggest that sexual selection regimes inferred from those experiments are only acting during a fraction of the lunar cycle (i.e., new moon), and much about the strength or direction of sexual selection acting in nature is in fact unknown even for well-studied species such as eastern gray treefrogs.

Here we test the hypothesis that changes in nocturnal light levels associated with the changing phases of the moon affect female gray treefrog mate choice behavior. Based on previous studies of light effects on frog mate choice behavior (i.e., Backwell and Passmore 1990; Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011a, b, c), we predicted that females would behave differently under simulated dim and bright moonlight. In particular, we predicted that under bright moonlight females would show behaviors that mitigate the increased danger of being detected by predators, such as relaxed preferences, decreased choosiness, and more directional, faster, or stealthier phonotactic approach movements.

Methods

Study site and species

The study was carried out from May to June 2016, during the natural breeding season of *H. versicolor*. We captured females

in a pond adjacent to the University of Wisconsin Milwaukee Field Station (Saukville, WI). Pairs were then brought to the laboratory on the night of capture and placed on melting ice to prevent oviposition prior to testing.

Light treatments

We assessed variation in female mate choice behavior (preference functions, choosiness, approach behavior) under two light levels almost spanning the natural range of nocturnal light (0.2 and 2.0 lx). Light levels vary throughout the course of the lunar cycle from 0.05 lx (new moon) to 2.10 lx (full moon) (Campbell et al. 2008). We set light conditions in the testing chamber using a 25-W incandescent bulb and a dimmer (Leviton TBL03), and measured light levels using an Extech EasyView EA31 Digital Light Meter.

Nota Bene: The vast majority of studies examining the effect of nocturnal light on frog mate choice behavior have been conducted with only one species (túngara frogs), and although light source or light intensity may not be crucial for túngara frogs (see below), we acknowledge that it could be important for other species. Nevertheless, when choosing the light source for our experiments, we used the túngara literature as our guide. Based on those studies, it appears that neither variation in the type of light source used nor variation in light intensity may be crucial for designing experiments investigating light effects in frogs. A range of different light sources, each with different spectral characteristics, have been used to experimentally simulate nocturnal light conditions, including nightlights, LED lights, and incandescent bulbs (Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011a, b, c). Use of different light sources yielded similar results (Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011a, b, c). Light intensity effects show a similar pattern, although comparison of light intensities used in the different studies is not straightforward, because they are reported in different units that are not easily converted (conversion factors vary depending on the wavelength, and thus, the type of light source involved). Our best attempt at conversion yields light intensities ranging from roughly 0.004 lx (irradiance of 5.8×10^{-10} W/cm²; Baugh and Ryan 2010) to roughly 3 lx (0.04–0.05 μ E; Rand et al. 1997). Again, studies obtained similar results although they used light intensities differing by orders of magnitude (0.004–3 lx). Moreover, although the visual acuity of most anurans remains poorly understood, the range of light intensities used in all studies should have been above the visual sensitivity of nocturnal frogs. Cummings et al. (2008) measured visual sensitivity of túngara frogs using the optomotor response and found that the frogs exhibited visual sensitivities corresponding to light intensities below moonless conditions. Thus, while the minimum visual sensitivity of gray treefrogs are unknown, if they are similar to that of túngara frogs, then all light treatments

should have provided females with more light than their sensory system requires for effective orientation and vigilance.

Stimulus generation

Male *H. versicolor* have pulsed advertisement calls consisting of a series of short pulses. At 20 °C, the average temperature of chorus formation of *H. versicolor*, as well as our testing temperature, 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 (1100 and 2200 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 et al. 2000; Reichert and Höbel 2015), and accordingly, we focused on call duration preferences in this experiment.

We generated acoustic stimuli in R (Version 3.1.0) software (R Development Core Team 2015), using the seewave package (Sueur et al. 2008). For all stimuli, we set call frequency and call period to the average values of our study population (i.e., first frequency peak of 1071 Hz, second (dominant) frequency peak of 2142 Hz (2nd peak 10 dB louder); call period of 7750 ms (see Reichert and 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).

General testing procedure

Females were tested in a circular playback arena (2 m diameter), set up inside a semi-anechoic 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 towards 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; C-weighting, fast RMS). Unless otherwise specified, amplitude was set at 85 dB SPL. Because phonotaxis trials require exact knowledge of the presented call alternatives, it was not possible to record data blind.

Prior to testing, females were warmed to a testing temperature of 20 °C. For testing, females were placed in an acoustically transparent release cage in the center of the arena. An acoustic stimulus was broadcast to the female while confined in the release cage; the experimental choice time began when the female was released from the cage by pulling on a rope attached to the lid. A choice was defined as entering a choice zone, marked by tape on the arena floor, within 10 cm of a

speaker. Trials where a female failed to reach the choice zone or did not make a distinct choice within 5 min, but instead crossed the choice zone while wandering around the arena, were considered non-responses. Females that did not respond were rested for 5 min and rerun on that stimulus. Females that had three consecutive non-responses were not tested further, and their data set was discarded. Only 6 females out of the initial 61 tested stopped responding, so our sample size was 55 responsive females (preference function trials: $n = 20$; choosiness 12 vs. 24 trials: $n = 20$; choosiness 6 vs. 18 trials: $n = 15$). Non-responses were not included in the analysis. Female movements were observed and recorded from outside the chamber using a video camera (EQ150, EverFocus USA, Duarte, CA, USA) mounted above the arena. Following testing, pairs were returned to the pond.

It took 1–2 h for a given female to complete her set of trials, and individual females had 5 to 10 min rest between consecutive trials. To ensure that the females' eyes were adapted to the treatment light level, we placed them into the testing chamber (in small individual transparent boxes) at least 5 min prior to starting the experiment. Between tests, we kept females inside the testing chamber, but placed them in individual transparent boxes inside a larger lidless, thick-walled cooler. The cooler was placed in the quietest location inside the test chamber (i.e., outside the actual testing arena and at 90° angle from the speaker(s)). Although this did substantially attenuate the call broadcast to the female currently being tested, it did not completely prevent females in the cooler from hearing the playbacks. Rapid shifts in light intensity (which are unavoidable when transporting frogs to and from the test chamber to a holding area outside the chamber) affect frog behavior (Buchanan, 1998, 1993). Keeping frogs in the test chamber was the only logistically feasible way to maintain females' eyes continuously adapted to experimental light levels for the duration of the experiment.

Testing variation in call duration preferences

We assayed female call duration preferences using a single-speaker design, where call stimuli varying in duration are presented sequentially, and the latency of a female's approach towards the speaker is noted as a measure of preference. To obtain data on approach latency, we used a stop watch to time from the moment the release cage was lifted and the female was free to move around the arena until she reached the choice zone at the speaker.

We examine variation in call duration preferences using preference functions, which are curves that describe female responses as a function of variation in call traits (i.e., Ritchie 1992; Meyer and Kirkpatrick 2005; Rodríguez et al. 2006; Reichert and Höbel 2015). In this function-valued approach, the entire preference function is considered as the trait of interest, which allows us to extract further preference function

traits to describe female preferences (Fowler-Finn and Rodríguez 2012). We visualize preference functions by fitting non-parametric cubic splines to the response data, using the program PFunc (Kilmer et al. 2017). After generating individual preference functions (two functions per female, one for each light treatment), the program also extracts several traits from those preference functions: (1) Peak describes the female's most preferred call duration value (i.e., call duration eliciting the fastest response); (2) strength describes the extent to which a female's response is reduced to signals that deviate from the peak preferred signal; (3) tolerance describes the range of call duration values that still elicits a high level of response (within one third of the function peak); and (4) 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 towards the broadcast call (i.e., a short response latency). 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 latency (in seconds) to $1/\text{latency}$ before generating preference functions.

A total of 20 females contributed to the data set; each female responded to the full complement of nine call duration stimuli (6–30 pulses; presented in random order) under each of two light treatments (randomly assigned to start with the dim or bright treatment). Thus, each female provided two preference functions (one for each light treatment).

Latency is a commonly used measure of preference in studies of anuran mate choice (see Gerhardt 1992; Bosch et al. 2000; Lynch et al. 2005; Reichert and Höbel 2015), and we consequently focused our detailed description of call duration preferences on this measure. However, since we were interested in behaviors that might affect "risk," we also examined other aspects of female phonotaxis behavior. The rationale for this is that a female taking a short, direct path with frequent stops, and another female taking a long, winding path walking continuously does show very different approach behaviors, yet may end up spending the same amount of time in phonotaxis (i.e., same latency value). To obtain data for approach path length and directionality, we used the program Avidemux (<http://fixounet.free.fr/avidemux/>) to break videos into individual frame JPGs, and then used the Extended Depth of Field plugin for ImageJ (Schneider et al. 2012) to stack the single-frame JPGs into a composite image that showed the approach path taken by the female. We then used ImageJ to measure the approach path length (by tracing the path the female took from the release cage to the choice zone) and the leave angle (the angle, relative to the playback speaker, at which the female left the release cage).

Statistical analysis To test for differences in preference function shape between light treatments, we entered the phonotaxis measures (latency, path length, and leave angle,

respectively) 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 two full preference functions (in dim and bright conditions).

We analyzed each preference function trait (derived from the latency-based preference function) using a mixed model (implementing REML) with the preference function trait (i.e., peak, strength, tolerance, or responsiveness) as the response variable, and the treatment (dim or bright condition) as the independent variable. Because each female contributed two data points per preference function trait (one from the preference function under dim and one under bright light), we also included female identity as a random term in the model. All statistical tests were implemented in JMP 11 (SAS Institute Inc., Cary, NC).

Testing variation in choosiness

We assayed choosiness using a two-speaker design that is based on the common observation that female frogs trade off call attractiveness with distance to source (i.e., 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 and an unattractive call are played antiphonally (from speakers separated by 180°, 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.

Females frequently discriminate more strongly against very unattractive calls, while showing only slight preferences between average and attractive calls (Gerhardt et al. 2000; Reichert and Höbel 2015). Thus, females seem to evaluate absolute as well as relative attractiveness of available alternatives. We took this behavior into account by testing choosiness in trials that pitted (i) a very unattractive call against an average call (6 vs. 18 pulses/call), and (ii) a call that was shorter than average against one that was longer than average (12 vs. 24 pulses/call). We expected the 6 vs. 18 pulses/call trials to present a stronger trade-off for the tested females.

A total of 15 females in the 6 vs. 18 and 20 females in the 12 vs. 24 trials contributed to the data set. Females were randomly assigned to start with the dim or bright light treatment. Each female's choosiness was determined within a given light treatment. Depending on the females responses, this required

two to four trials in which attenuation levels were adjusted until the female no longer approached the attractive call. Then the process was repeated in the other light treatment. Thus, each female provided two choosiness measures (one for each light treatment) to the final data set. Following testing, pairs were returned to the pond.

Statistical analysis 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. For each experiment (6 vs. 18, and 12 vs. 24, respectively) 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.

Phonotaxis behavior

Using the phonotaxis videos and the corresponding stacked pictures of the 30-pulse trials from the preference function experiment, we mapped the cumulative distance moved by the females and the cumulative time it took them to reach the speaker (see Murphy and Gerhardt 2002). We distinguished three types of movement: hops, crawls, and repositions. Hops are quick movement that results in a displacement of > 1 body length; crawls are slow movement that results in a displacement of > 1 body length; and repositions are movements that do not result in displacement, such as moving the head to look in a different direction, or adjusting arm/leg position. For each phonotaxis approach, we noted (1) the number of hops, (2) the number of crawls, and (3) the number of repositions. In addition, we noted (4) the time after lifting the lid of the release box until the females started to move towards the speaker, (5) the average time between displacement movements (hops and crawls), and (6) the average distance covered by displacement movements (hops and crawls).

Statistical analysis We used JMP 11 (SAS Institute Inc., Cary, NC) to calculate a series of ANOVAs testing whether phonotaxis movement behaviors, or their timing, were affected by light treatments.

Effect sizes

We calculated effect sizes for the differences in preference function traits, choosiness, and approach behavior, respectively, between dim and bright moonlight treatments. We first calculated Cohen's d by using mean values and a pooled standard deviation between light treatment types and then calculated the correlation coefficient r from Cohen's d (Cohen 1988). 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 to 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 the above effect sizes into context, we also computed some comparisons that focused on the effect of call traits, not light treatment. These values serve to illustrate the differences that we are able to detect with our experiments (at similar or even lower sample size), if they involve variables that are biologically relevant to the frogs. 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 dim/bright moonlight treatments used in the present study. We also calculated effect sizes for a comparison of choosiness during our 6 vs. 18 compared to the 12 vs. 24 pulse trials, focusing on the effect of the auditory stimuli the frogs were presented with.

Results

Preference functions

Female eastern gray treefrogs (*H. versicolor*) prefer longer-duration calls (Fig. 1a), irrespective of the behavior (latency, path length, or angle) that is scored to assess their preferences (significant linear and quadratic stimulus terms in Table 1). However, light treatment did not affect how females responded to variation in call duration (non-significant treatment and stimulus \times treatment interactions terms in Table 1; Fig. 2a–c). 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 ($F_{1,19} = 0.0071$, $p = 0.94$; Fig. 1b), responsiveness ($F_{1,19} = 0.028$, $p = 0.87$; Fig. 1c), tolerance ($F_{1,19} = 0.61$, $p = 0.45$; Fig. 1d), and strength ($F_{1,19} = 0.53$, $p = 0.48$; Fig. 1e). Effect sizes were small in each case: peak preference ($r = 0.04$), responsiveness ($r = 0.02$), tolerance ($r = 0.14$), and strength ($r = 0.12$). For comparison, effect sizes from Reichert and Höbel's (2015) preference function traits were generally of intermediate size: peak preference ($r = 0.18$), responsiveness ($r = 0.40$), tolerance ($r = 0.40$), and strength ($r = 0.37$).

Choosiness

As amplitude differences between an attractive and an unattractive call increased, fewer females continued to approach the attractive call (Fig. 3a, c; Table 2: significant effect of attenuation). Light levels did not affect choosiness (Table 2: non-significant effect of treatment): neither in the 6 vs. 18 (Fig. 3a, b; Table 2, left columns) or in the 12 vs. 24 pulse trials (Fig. 3c, d; Table 2, right

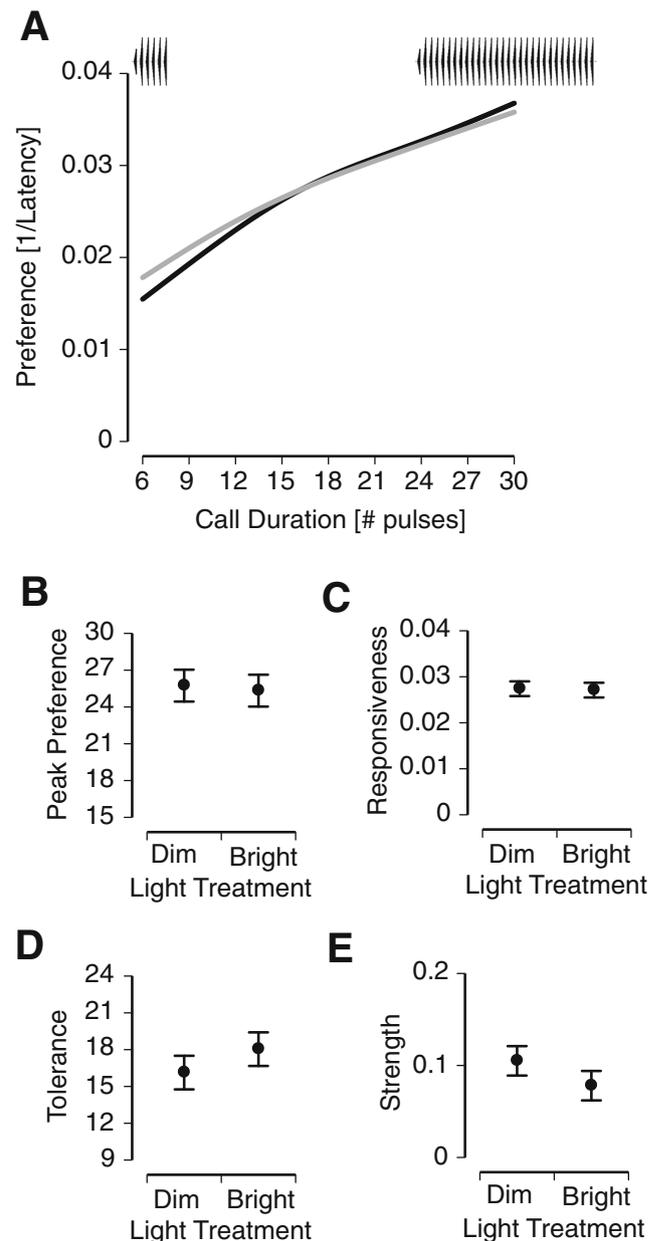


Fig. 1 Call duration preferences of female eastern gray treefrogs (*Hyla versicolor*) under simulated dim and bright moonlight. Shown are preference functions (a) and traits derived from those preference functions (b–e) based on the latency it took females to reach the speaker broadcasting the test calls. Dim light treatment is indicated by a black line, the bright treatment by a gray line. Preference functions were almost identical under the two light conditions (a), and preference function traits did not differ either (b–e). The waveforms in a illustrate the range of call stimuli tested, from a short 6-pulse call to an along 30-pulse call. Shown are means \pm 95% CI (imperceptibly small in a)

columns). Effect sizes were small in each case: 6 vs. 18 ($r = 0.03$); 12 vs. 24 pulse trials ($r = 0.06$).

By contrast, the range of tested call duration values did affect choosiness: choosiness was higher in the 6 vs. 18 pulse duration trials compared to the 12 vs. 24 pulse trials; for example, at an amplitude difference of 9 dB, 90% of females still

Table 1 Effect of variation in nocturnal light levels on preference for call duration in female eastern gray treefrogs (*Hyla versicolor*)

Behavior	Factor	<i>df</i>	<i>F</i>	<i>P</i>
Latency	Light treatment	1335	3.03	0.08
	Call duration	1335	71.77	< 0.0001
	Call duration × call duration	1335	14.15	0.0002
	Treatment × call duration	1335	0.21	0.65
	Treatment × call duration × call duration	1335	2.27	0.13
Path length	Light treatment	1333.1	1.52	0.2189
	Call duration	1333.1	8.12	0.005
	Call duration × call duration	1333	0.84	0.36
	Treatment × call duration	1333.1	0.07	0.79
	Treatment × call duration × call duration	1333.1	1.16	0.28
Leave angle	Light treatment	1333.5	0.35	0.55
	Call duration	1333.4	3.75	0.05
	Call duration × call duration	1333.1	0.23	0.63
	Treatment × call duration	1333.5	0.44	0.51
	Treatment × call duration × call duration	1333.8	0.72	0.40

The assays used to describe preferences are (i) the latency until females reached the sound source); (ii) the length of the path traveled between the release box and the sound source; and (iii) the angle at which the female left the release box (relative to the speaker). Females were faster, walked a shorter path, and were more directional when approaching longer calls, but light treatment did not affect call duration preferences. Significant terms are set in bold

approached the attractive call in the 6 vs. 18 pulse trials, while only 50% still did so in the 12 vs. 24 pulse trials (see Fig. 3a, c), and average choosiness was also different (Fig. 3b, d). Here, effect size was always large: 6 vs. 18 compared to 12 vs. 24 pulse trials in dim light ($r = 0.59$) and 6 vs. 18 compared to 12 vs. 24 pulse trials in bright light ($r = 0.70$).

Phonotaxis behavior

Females hopped/crawled on average (\pm SD) 6.2 ± 3.2 (range 3–17) times before reaching the speaker located 1 m away

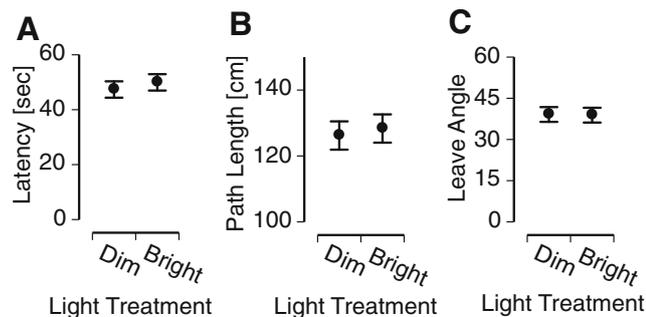


Fig. 2 Light treatments (simulated dim or bright moonlight) did not affect how fast or how directly female eastern gray treefrogs (*Hyla versicolor*) approached the speaker broadcasting the call stimulus. Neither response latency (a; the time it took females to reach the speaker broadcasting the test calls), nor path length (b; the distance females walked until they reached the speaker), nor the angle (relative to the sound source) at which they left the release box to start their approach to the speaker (c) differed between light treatments. Shown are least square means \pm SE

from them. Average (\pm SD) distance moved per movement was 22 ± 9 cm (range 8–40 cm), and the average (\pm SD) time between successive movements was 10 ± 16 s (range 2–23 s). Females also adjusted the head position during phonotaxis (without forward movement); there were an average (\pm SD) of 2.8 ± 1.7 (range 0–7) head movements during each phonotaxis trial. These head movements resulted in the female either facing the speaker (58%) or facing away from the speaker, such that one ear was turned towards the speaker (42%), suggesting that these movements are involved in sound source localization.

Although there was substantial individual variation in approach behavior (see Fig. 4a), none of the sampled approach behaviors were affected by light treatments (Fig. 4b–d; Table 3). Effect sizes were small in each case (see Table 3, right column).

Discussion

Although not widely appreciated, mate choice decisions in frogs are surprisingly flexible. Female frogs generally show strong preferences for particular call traits, often favoring calls of lower frequency (associated with larger male size) or calls that are longer or more frequently repeated (associated with higher energy expenditure) (Gerhardt and Huber 2002; Wells 2010). Yet, both the preference as well as the choosiness aspects of these call trait preferences can be modified. For example, female treefrogs generally become more tolerant

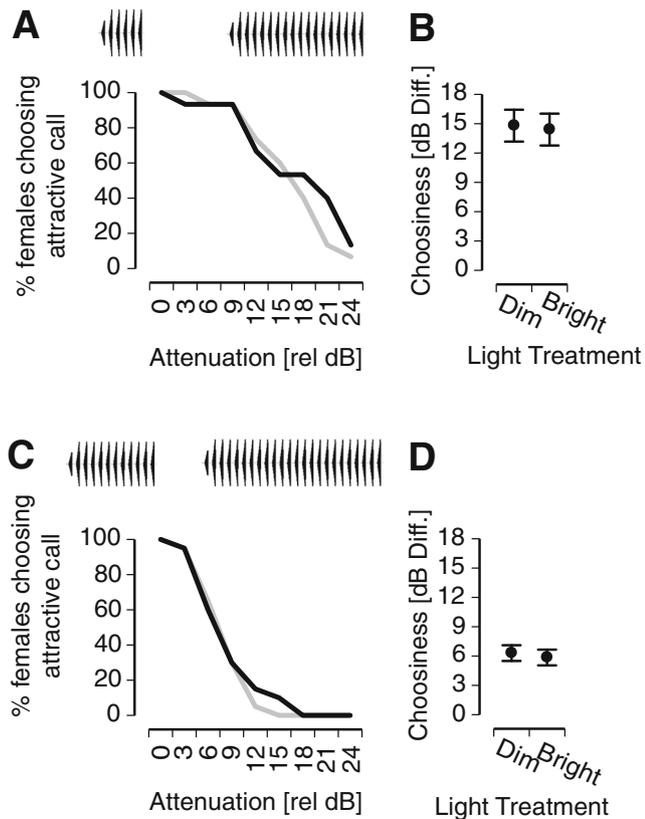


Fig. 3 Choosiness of female eastern gray treefrogs (*Hyla versicolor*), expressed as the percentage of females still approaching the more attractive stimulus as it is gradually attenuated, did not differ under simulated dim and bright moonlight. Waveforms show the call stimuli females were offered to choose from. The call alternatives tested in each trial differed by 12 pulses, but in one (a, b) they represent an unattractive (6p) and an attractive (18p) call, while in the other (c, d) they represent two attractive (12p and 24p) calls. In a, c, dim light treatment is indicated by a black line, the bright treatment by a gray line; b, d show least square means \pm SE

towards accepting less attractive calls if those are paired with a visual signal component (Gomez et al. 2009; Taylor et al. 2011; Reichert and Höbel 2015; Reichert et al. 2016). And female green treefrogs (*Hyla cinerea*) adjust choosiness as a function of variation in the social environment they experience when approaching the breeding chorus to mate: presence

of attractive males increases choosiness, while absence of attractive males reduces choosiness (Neelon and Höbel 2017). Evidently, female frogs are perfectly capable of adjusting their mate choice behavior in contexts in which selection has favored such behavioral plasticity.

Here we tested the hypothesis that female eastern gray treefrogs show context-dependent variation in mate choice behavior, in particular whether their behavior is affected by variation in nocturnal light levels. Contrary to our prediction that females would prefer more attractive males and/or be choosier under dimmer moonlight, we actually found that none of the mate choice behaviors scored during our experiments were affected by the treatment light intensity. Females approached a simulated male call in the same way in dim and bright conditions, showing similar speed, directionality, and even similar number and timing of phonotaxis movements (i.e., hops, crawls, and head scans). Females also had similar call duration preferences, including details of preference function shape such as peak preference and tolerance for deviation from the peak. Finally, females also showed similar effort in obtaining their preferred mate, i.e., choosiness did not vary with light treatment.

The choosiness trials are particularly instructive in terms of the importance female gray treefrogs seem to give to ambient light. We tested choosiness in two experiments in which the overall difference between tested alternatives was the same (call duration differed by 12 pulses), but in one experiment females were offered an unattractive (6 pulse) and an attractive (18 pulse) call, while in the other experiment they had to choose between two attractive calls (12 and 24 pulses). Choosiness was much higher in the experiment that involved the unattractive call, indicating that females put a premium on call traits, but disregard ambient light conditions, at least within the range of light intensities tested here. This is also corroborated by effect size calculations: while effect sizes comparing light treatment effects were small, the effect sizes comparing call quality were of large magnitude.

As is tradition in anuran mate choice experiments, our experiments were conducted in a comparatively small playback arena (i.e., Rand et al. 1997; Reichert and Höbel 2015).

Table 2 Effect of light treatment on choosiness

	6 vs. 18 pulses			12 vs. 24 pulses		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Light treatment	1.14	0.37	0.55	1.14	0.04	0.85
Attenuation	1.14	154.25	< 0.0001	1.14	73.39	< 0.0001
Treatment \times attenuation	1.14	1.69	0.21	1.14	0.01	0.92

As amplitude differences between an attractive and an unattractive alternative increased, fewer females continued to approach the attractive call. Light treatment, however, had no effect on choosiness. Shown are results from trials that pitted a very unattractive call against an average call (6 vs. 18 pulses; left columns), and a call that was shorter than average against one that was longer than average (12 vs. 24 pulses; right columns). Significant terms are set in bold. See also Fig. 3

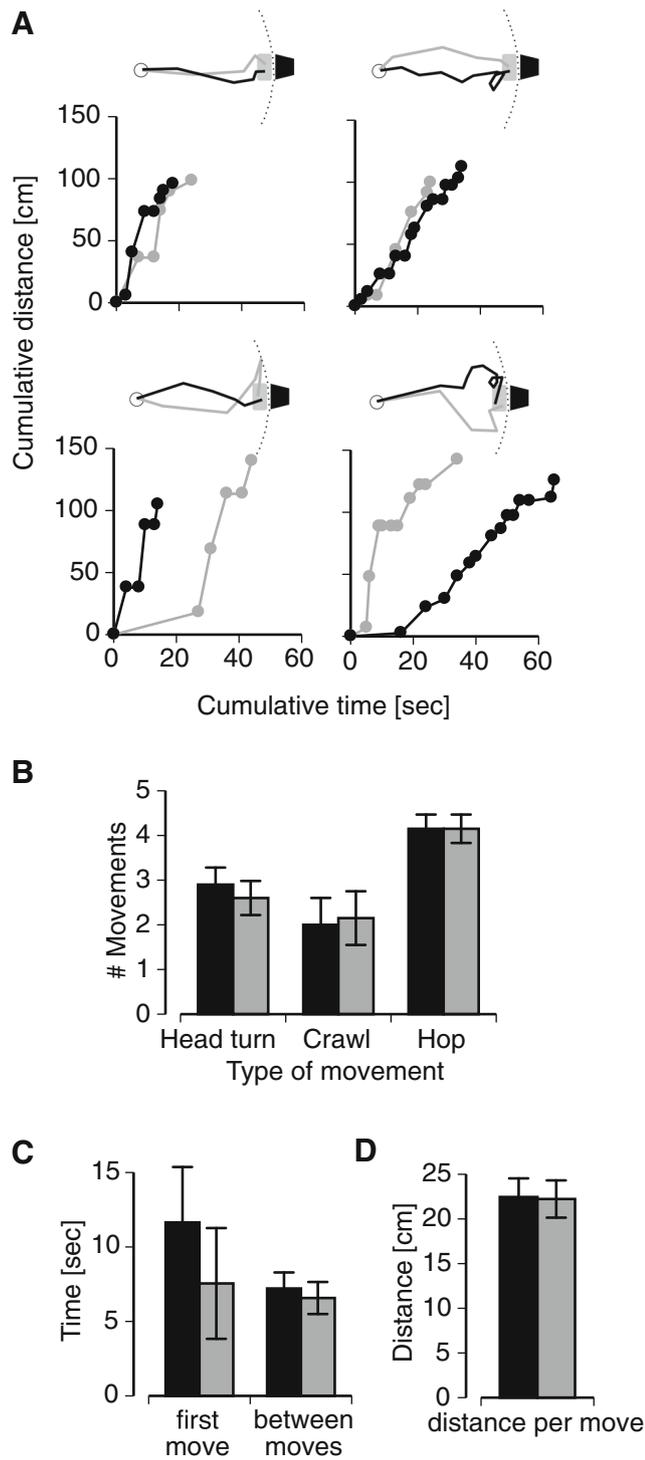


Fig. 4 Movement patterns of female eastern gray treefrogs (*Hyla versicolor*) during phonotaxis under simulated dim and bright moonlight. **a** Paths taken by four females approaching the playback speaker under dim (black line) and bright (gray line) simulated moonlight. The dashed outer lines represent the edge of the playback arena; the inner circle depicts the position of the release box, and the gray square indicates the target zone in front of the speaker. Graphs below path diagrams depict the cumulative distance moved by females plotted against cumulative time since leaving the release box. **b** Average numbers of stationary movements (repositions) or locomotion movements (crawls, hops) did not differ between light treatments. **c** Neither the time until the females left the release box nor the average times between forward locomotion movements differed between light treatments. **d** The average distance covered by a forward locomotion movement did not differ between light treatments. Shown are least square means \pm SE

differ between dark and moonlit nights. In addition, we know that the night of migration towards our pond may be affected by lunar light: although breeding occurs during any point in the lunar cycle (and thus, under the full range of natural nocturnal light levels), it is statistically more likely to occur during intermediately bright nights (incidentally, male calling activity mirrors this pattern; VAU and GH, unpubl. data). Potentially, selection for when to migrate to the breeding pond, and how to get there from a distance, is stronger than how to move during the last meter before reaching the male.

At first glance, the lack of nocturnal light effects documented for gray treefrogs differs markedly from previous studies examining the effect of nocturnal light on anuran mate choice behavior. However, studies are difficult to compare because of differences in experimental design. For example, we tested choosiness in a relatively benign scenario (i.e., asking females to choose between two conspecific calls). The experimental design used by Bonachea and Ryan (2011a, b) to test risk assessment during mate choice in female túngara frogs included light treatments, but additionally placed females in more challenging choice scenarios, such as making females choose between conspecific and heterospecific calls, or adding predator sounds to the calls females were asked to approach. Potentially, the effects of nocturnal light may manifest in gray treefrogs under more challenging conditions as well. On the other hand, there are comparable trial conditions where

Observations thus only encompass the last few meters of phonotactic approach, which is only a 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 leaves many aspects of females' mate choice behavior unexamined. For example, the approach path through the surrounding forest towards the pond may still

Table 3 Results of ANOVAs testing whether phonotaxis movements differed between light treatments (simulated dim and bright moonlight)

Factor	df	F	P	Effect size r
No. of head turns	1.39	0.31	0.58	0.09
No. of crawls	1.39	0.03	0.86	0.03
No. of hops	1.39	0.00	1.00	0.00
No. of movements (hop + crawl)	1.39	0.02	0.89	0.02
Time to first move	1.39	0.61	0.44	0.12
Time between successive moves	1.39	0.18	0.68	0.07
Distance per move	1.39	0.006	0.94	0.01

túngara frogs nevertheless behaved differently from gray treefrogs. For example, Rand et al. (1997) showed that the willingness of female túngara frogs to move and perform phonotaxis in dark and illuminated trials was quite different: almost half the tested túngara frog females did not respond in the illuminated trials, while the vast majority of gray treefrogs tested in our trials approached the playback speaker irrespective of light conditions.

Despite the difficulty of comparing studies, there seems to be genuine differences between female gray treefrogs and the two other species studied before, suggesting that mate choice behavior in relation to lunar light may be highly species-specific. Given what is known about lunar responses in amphibians in general, such between-species variation is not unexpected. In their review paper, Grant et al. (2012) found 79 examples across diverse amphibian taxa where behavior and ecology in relation to the moon had been studied—note that *any* response (ovulation, breeding migrations, phonotaxis behavior, etc.) to *any* lunar cue (moon phase, lunar light) was included in the study. The majority of species were affected by lunar cues, but there was no significant difference between the numbers of species that increased and those that decreased activity during a full moon. There was also no clear trend as to which behavioral responses (i.e., breeding migrations, spawning events, calling, etc.) were particularly frequent during specific moon phases. And there was no taxonomic signature either, as the same family or even genus can contain species that increased or decreased activity under full moon (Grant et al. 2012).

The three frog species whose mate choice behavior in relation to nocturnal light has been studied (see above) are members of different anuran families (Hylidae, Hyperoliidae, Leptodactylidae); they hail from different geographic areas (North America, Central America, Africa) and inhabit tropical and temperate regions. There is thus a multitude of reasons that may explain the observed between-species differences in how mate choice behavior is affected by nocturnal light.

Different predator regimes may select for species differences in moonlight-related behavior. Cover of darkness may provide respite from visually orienting predators, but should not protect from acoustically or chemically orienting ones. In the case of the Central American túngara frog, it seems that visually orienting frog-eating bats are a major threat (Ryan 1985), and there are also frog-eating bat species in Africa that eat reed frogs (Channing 2001). By contrast, this predator guild is absent in North America, and consequently gray treefrogs may not have been under selection to avoid predators during bright nights. Gray treefrogs are preyed upon by a diverse array of nocturnal predators [raccoons, snakes, larger frogs (bullfrogs, green frogs), and even giant water bugs], encompassing a range of prey detection mechanisms (acoustic, chemical, visual). Maybe the lack of a predominant predator did not result in selection for increased vigilance under

brighter conditions, at least not in females. In this regard, it is interesting to note that male gray treefrogs from our pond seem to use visual cues to detect potential predators and adjust their calling behavior accordingly: while the majority of males in the predator-absent trials did call, only a fraction of the ones in the predator-present trials did (Höbel and Barta 2014). Then again, broadcast of bullfrog vocalizations (another known predator on gray treefrogs) failed to influence female phonotaxis as well as male calling behavior in gray treefrogs from another population (Schwartz et al. 2000). These observations suggest that acoustic predator cues are less salient for gray treefrogs than visual cues, and also that there may be a sex difference in response to visual predator cues. However, more research is needed to tackle these hypotheses.

The distribution range of eastern gray treefrogs is enormous, from the Gulf of Mexico in the south to some parts of Canada in the north, and it is possible that the geographic location of our study population affected our results. First, frogs from our more northern population may experience overall reduced predation pressure, particularly from snakes and mammals, and may thus not experience strong selection for increased predator vigilance during brighter nights. Second, although gray treefrogs are categorized as prolonged breeders, in our Wisconsin population breeding takes place only during a portion of the 4–6-week-long breeding season. This is mostly because warm nights amenable to reproduction are separated by cold spells during which frogs are inactive (GH, pers. obs.). Gray treefrogs at our site thus have a shorter period of breeding activity, and may actually be under selection to disregard variation in nocturnal light in order to take advantage of best climatic conditions. In addition, females from our population have a comparatively low life expectancy (1–3 years) and likely only reproduce once in their lifetime (GH et al., unpubl. data), which may put a premium on obtaining the best possible sire for their offspring. Overall, few chances to reproduce in conjunction with potentially low predation pressure may constitute a combination of environmental factors that did not select for differential mate choice behavior under dark and moonlit conditions at our study site. The behavior of our northern frogs may thus not reflect the behavior across the species' range. Comparative studies in sites with higher predation pressure, and/or longer-duration breeding seasons could yield interesting results on the importance of additional environmental factors on the evolution of female mate choice behavior.

In conclusion, we have shown that neither call duration preferences, nor choosiness for longer over shorter calls, nor the minutia of phonotaxis behavior change when female gray treefrogs search for mates under different nocturnal light levels. A key take-home message from our study is thus that in gray treefrogs, variation in light levels associated with the changing phases of the moon does not affect the sexual selection regime on male call traits. In a previous study we have

shown that female gray treefrogs breed under a wide range of nocturnal light levels, but that there is a slight increase in the numbers of females arriving to breed during intermediately bright nights (VAU and GH, unpubl. data). However, this should not lead to lunar cycle-driven variation in the strength or direction of sexual selection, because female gray treefrogs have similarly strong preferences for longer calls irrespective of the moonlight available during the night they come to breed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement 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).

Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

References

- Andersson MB (1994) Sexual selection. Princeton University Press, Princeton
- Backwell PR, Passmore NI (1990) Suitable approach perches affect female phonotaxis in an arboreal frog. *Herpetologica* 46:11–14
- Baugh AT, Ryan MJ (2010) Ambient light alters temporal updating behaviour during mate choice in a Neotropical frog. *Can J Zool* 88:448–453
- Bonachea LA, Ryan MJ (2011a) Predation risk increases permissiveness for heterospecific advertisement calls in túngara frogs, *Physalaemus pustulosus*. *Anim Behav* 82:347–352
- Bonachea LA, Ryan MJ (2011b) Simulated predation risk influences female choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 117:400–407
- Bonachea LA, Ryan MJ (2011c) Localization error and search costs during mate choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 117:56–62
- Bosch J, Rand AS, Ryan MJ (2000) Signal variation and call preferences for whine frequency in the túngara frog, *Physalaemus pustulosus*. *Behav Ecol Sociobiol* 49:62–66
- Buchanan BW (1993) Effects of enhanced lighting on the behaviour of nocturnal frogs. *Anim Behav* 45:893–899
- Buchanan BW (1998) Low-illumination prey detection by squirrel treefrogs. *J Herpetol* 32:270–274
- Campbell SR, Mackessy SP, Clarke JA (2008) Microhabitat use by brown treesnakes (*Boiga irregularis*): effects of moonlight and prey. *J Herpetol* 42:246–250
- Channing A (2001) Amphibians of central and southern Africa. Comstock Publishing Associates, Ithaca, NY
- Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Lawrence Erlbaum Associates Inc., Hillsdale, NJ
- Coleman SW, Patricelli GL, Borgia G (2004) Variable female preferences drive complex male displays. *Nature* 428:742–745
- Cummings ME, Bernal XE, Reynaga R, Rand AS, Ryan MJ (2008) Visual sensitivity to a conspicuous male cue varies by reproductive state in *Physalaemus pustulosus* females. *J Exp Biol* 211:1203–1210
- Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London
- Diekamp B, Gerhardt HC (1995) Selective phonotaxis to advertisement calls in the gray treefrog *Hyla versicolor*: behavioral experiments and neurophysiological correlates. *J Comp Physiol A* 177:173–190
- Forsgren E (1992) Predation risk affects mate choice in a gobiid fish. *Am Nat* 140:1041–1049
- Fowler-Finn KD, Rodríguez RL (2012) Experience-zediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* 66:459–468
- Gerhardt HC (1987) Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Anim Behav* 35:1479–1489
- Gerhardt HC (1992) Conducting playback experiments and interpreting their results. In: McGregor PK (ed) Playback and studies of animal communication. Springer, New York, pp 59–77
- Gerhardt HC (2005) Advertisement-call preferences in diploid-tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): implications for mate choice and the evolution of communication systems. *Evolution* 59:395–408
- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans: common problems and diverse solutions. University of Chicago Press, Chicago
- Gerhardt HC, Tanner SD, Corrigan CM, Walton HC (2000) Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behav Ecol* 11:663–669
- Godin J-GJ, Briggs SE (1996) Female mate choice under predation risk in the guppy. *Anim Behav* 51:117–130
- Gomez D, Richardson C, Lengagne T, Plenet S, Joly P, Léna JP, Théry M (2009) The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proc R Soc Lond B* 276:2351–2358
- Gong A, Gibson RM (1996) Reversal of female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. *Anim Behav* 52:1007–1015
- Grant R, Halliday T, Chadwick E (2012) Amphibians' response to the lunar synodic cycle—a review of current knowledge, recommendations, and implications for conservation. *Behav Ecol* 24:53–62
- Hedrick AV, Dill LM (1993) Mate choice by female crickets is influenced by predation risk. *Anim Behav* 46:193–196
- Höbel G, Barta T (2014) Adaptive plasticity in calling site selection in grey treefrogs (*Hyla versicolor*). *Behaviour* 151:741–754
- Hunt J, Brooks R, Jennions MD (2005) Female mate choice as a condition-dependent life-history trait. *Am Nat* 166:79–92
- Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72:283–327
- Johnson JB, Basolo AL (2003) Predator exposure alters female mate choice in the green swordtail. *Behav Ecol* 14:619–625
- Kilmer JT, Fowler-Finn KD, Gray DA, Höbel G, Rebar D, Reichert MS, Rodríguez RL (2017) Describing mate preference functions and other function-valued traits. *J Evol Biol* 30:1658–1673
- Kotler BP, Brown J, Mukherjee S, Berger-Tal O, Bouskila A (2010) Moonlight avoidance in gerbils reveals a sophisticated interplay

- among time allocation, vigilance and state-dependent foraging. *Proc R Soc Lond B* 277:1469–1474
- Lillywhite HB, Brischoux F (2012) Is it better in the moonlight? Nocturnal activity of insular cottonmouth snakes increases with lunar light levels. *J Zool* 286:194–199
- Longland WS, Price MV (1991) Direct observations of owls and heteromyid rodents: can predation risk explain microhabitat use? *Ecology* 72:2261–2273
- Lynch KS, Rand AS, Ryan MJ, Wilczynski W (2005) Plasticity in female mate choice associated with changing reproductive states. *Anim Behav* 69:689–699
- Meyer K, Kirkpatrick M (2005) Up hill, down dale: quantitative genetics of curvaceous traits. *Philos T Roy Soc B* 360:1443–1455
- Murphy CG, Gerhardt HC (2002) Mate sampling by female barking treefrogs (*Hyla gratiosa*). *Behav Ecol* 13:472–480
- Neelon DP, Höbel G (2017) Social plasticity in choosiness in green tree frogs, *Hyla cinerea*. *Behav Ecol* (published online), <https://doi.org/10.1093/beheco/axx103>
- Prugh LR, Golden CD (2014) Does moonlight increase predation risk? Meta-analysis reveals divergent responses of nocturnal mammals to lunar cycles. *J Anim Ecol* 83:504–514
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna <http://www.R-project.org>
- Rand AS, Bridarolli ME, Dries L, Ryan MJ (1997) Light levels influence female choice in túngara frogs: predation risk assessment? *Copeia* 1997:447–450
- Reichert MS, Höbel G (2015) Modality interactions alter the shape of acoustic mate preference functions in gray treefrogs. *Evolution* 69:2384–2398
- Reichert MS, Symes LB, Höbel G (2016) Lighting up sound preferences: cross-modal influences on the precedence effect in treefrogs. *Anim Behav* 119:151–159
- Ritchie MG (1992) Setbacks in the search for mate-preference genes. *Trends Ecol Evol* 7:328–329
- Rodríguez RL, Ramaswamy K, Cocroft RB (2006) Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proc R Soc Lond B* 273:2585–2593
- Rundus AS, Santer RD, Hebets EA (2010) Multimodal courtship efficacy of *Schizocosa retrorsa* wolf spiders: implications of an additional signal modality. *Behav Ecol* 21:701–707
- Runkle LS, Wells KD, Robb CC, Lance SL (1994) Individual, nightly, and seasonal variation in calling behavior of the gray tree frog, *Hyla versicolor*: implications for energy expenditure. *Behav Ecol* 5:318–325
- Ryan MJ (1985) The túngara frog: a study in sexual selection and communication. University of Chicago Press, Chicago
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675
- Schwartz JJ, Bee MA, Tanner SD (2000) A behavioral and neurobiological study of the responses of gray treefrogs, *Hyla versicolor* to the calls of a predator, *Rana catesbeiana*. *Herpetologica* 2000:27–37
- Schwartz JJ, Buchanan BW, Gerhardt HC (2001) Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behav Ecol Sociobiol* 49:443–455
- Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18:213–226
- Sullivan BK, Hinshaw SH (1992) Female choice and selection on male calling behaviour in the grey treefrog *Hyla versicolor*. *Anim Behav* 44:733–744
- Taylor RC, Klein BA, Ryan MJ (2011) Inter-signal interaction and uncertain information in anuran multimodal signals. *Curr Zool* 57:153–161
- Uetz GW, Norton S (2007) Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behav Ecol Sociobiol* 61:631–641
- Wells KD (2010) The ecology and behavior of amphibians. University of Chicago Press, Chicago
- West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. *Q Rev Biol* 58:155–183