



Environmental heterogeneity alters mate choice behavior for multimodal signals

Andrew D. Cronin^{1,2} · Michael J. Ryan^{3,4} · Rachel A. Page⁴ · Kimberly L. Hunter¹ · Ryan C. Taylor^{1,4} 

Received: 10 September 2018 / Revised: 26 February 2019 / Accepted: 4 March 2019 / Published online: 9 March 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Animals frequently experience changes in their environment, including diel and seasonal shifts in abiotic and biotic factors. In addition to physiological and morphological changes, animals alter their behavior in response to environmental variation. This study examined the impacts of heterogeneous environments on mating behaviors. We examined both male and female túngara frog phonotactic responses to multimodal (audiovisual) and unimodal (acoustic) stimuli. We altered aspects of the physical environment by changing substrate (terrestrial and aquatic) and ambient light levels. Females demonstrated a similar preference for the audiovisual stimulus regardless of substrate but decreased latency to choose in an aquatic environment. When ambient light levels were increased (relative to darker control), females reversed their preference, avoiding the multimodal stimulus, but the latency to choose was unchanged. Males demonstrated no preference for the multimodal signal on either substrate, but like females, male latency was reduced in an aquatic environment. Different environments carry their own associated costs, including varying levels of predation risk. Increased light levels and an aquatic environment likely carry higher predation risk and therefore should lead to changes in female and male responses. Interestingly, these two environments do not cause uniform changes in female responses. The addition of an aquatic environment led to a reduction in latency, whereas an increase in ambient light levels induced a change in female mate preference. These findings demonstrate the importance of the environment on mating responses to multimodal signals.

Significance statement

Responses to conspecific mating signals (e.g., mate preferences) have often been assumed to be static. In this study, we tested responses of both male and female túngara frogs to a multimodal (visual + vocal) advertisement signal under varying environmental conditions. Elevated light levels changed female responses to mating signals. When both sexes were required to swim, rather than walk to a signal, their choices did not change. They did respond faster, however. These results indicate that measuring the strength of sexual selection should be considered as a function of both innate preferences and the environmental conditions experienced by the animals.

Keywords Multimodal signaling · Environmental heterogeneity · Sexual selection · Túngara frog

Communicated by C. R. Gabor

✉ Ryan C. Taylor
rctaylor@salisbury.edu

¹ Department of Biology, Salisbury University, Salisbury, MD 21801, USA

² Department of Ecological Science, Vrije Universiteit, Amsterdam, The Netherlands

³ Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA

⁴ Smithsonian Tropical Research Institute, Balboa, Republic of Panama

Introduction

Most animals experience environmental changes during the course of their lifetime. These changes can include seasonal and diel shifts in temperature, precipitation, humidity, and light levels. These abiotic changes may also correspond with changes in the biotic environment, altering population densities, food availability, and predation pressure. In response to these changes, organisms can alter aspects of their morphology (Relyea 2002), physiology (Martin et al. 2008; Campbell-Staton et al. 2017), or

behavior (Sunday et al. 2014) that maximize survival and reproduction. Behavioral modifications in response to environmental variation may include altered activity patterns, foraging behavior, habitat use, and reproductive strategies (Buchanan 1992; Sunday et al. 2014).

Females of many species are selective about the males they choose to mate with, thereby expressing their mate preference as a choice. This in turn exerts strong selection on the phenotype of animal communication signals and male courtship behaviors (Rosenthal 2017). Studies of mate choice have often focused on properties of male advertisement signals and how females respond to variation in these properties (Rosenthal 2017; but see Boughman 2002). We still know little, however, about how short-term environmental changes/heterogeneity (e.g., daily to monthly) influence reproductive behavior such as mate choice. Some recent empirical and theoretical studies, however, have suggested that environmental variability may be an important factor influencing mate choice (Chaine and Lyon 2008; Bro-Jørgensen 2010). For example, mate choice may be altered based on seasonality (collared flycatchers: Qvarnström et al. 2000), search costs (sticklebacks: Milinski and Bakker 1992; túngara frogs: Akre and Ryan 2010a), hormone levels (túngara frogs: Lynch et al. 2006), or social context (túngara frogs: Lea and Ryan 2015). Studies examining the environmental effects on mating behaviors have often focused on signals displayed via a single sensory modality (but see Aspbury et al. 2010; Gordon and Uetz 2011; Wilgers and Hebets 2011; Stafstrom and Hebets 2013; Halfwerk and Slabbekoorn 2015). Due to the ubiquity of multimodal communication (Higham and Hebets 2013), however, the salience of many multimodal signals is likely to be strongly impacted by the environment. A commonly cited hypothesis for the evolution of multimodal signals is that each component provides redundant information (degeneracy in Hebets et al. 2016) that may be used to increase signal detection in a variable environment (Partan and Marler 1999). Because multimodal signals impart information in sensory modalities that are governed by different physical properties, however, the effect of environmental heterogeneity on multimodal communication has the potential to be greater than for unimodal communication. This is most likely to occur in systems where multiple signal components are required or otherwise strongly influence mate attraction.

In this study, we tested the effects of ambient light levels and substrate type on unimodal and multimodal mate preferences in female túngara frogs, *Physalaemus* (= *Engystomops*) *pustulosus*. This is a common species in Middle America, and as is common in frogs, males gather at ponds at night and produce sexual advertisement signals in the form of vocalizations. Females arrive at the ponds and evaluate potential mates, based on properties of their advertisement signals. This species has a multicomponent call, consisting of a frequency-modulated whine and 0–7 harmonically rich

chucks appended to the end of the whine. Females prefer to mate with males producing lower frequency calls and whines with chucks appended (Ryan 1985; Ryan and Rand 1990; Ryan et al. 1990). The production of the male's call also requires the inflation of a large, conspicuous vocal sac. The vocal sac inflation is physically linked to call production adding a visual component to the vocal signal. All else being equal, when females are presented with an acoustic call in isolation vs. an acoustic call combined with an inflating vocal sac, females express a preference for the audiovisual (multimodal) signal (Taylor et al. 2008, 2011).

Because mating occurs during the night, these nocturnal frogs experience ambient light levels that vary over several orders of magnitude, dependent on cloud/forest cover and the lunar cycle (Cummings et al. 2008). These light levels can greatly impact the mating choices made by female túngara frogs, with females choosing a closer but “less attractive” male call under higher ambient light levels (Rand et al. 1997). This change in female mating behavior is likely due to an increase in perceived predation risk, which has been previously documented in other taxa (Endler 1987). Although female túngara frogs have a preference for multimodal signals, the synchronized inflating vocal sac also attracts a major predator, the frog-eating bat, *Trachops cirrhosus* (Halfwerk et al. 2014). In many species, increasing predation risk alters both male and female mating behaviors and can cause females to change their preferences for male sexual signals (Endler 1987; Kim et al. 2007; Pilakouta et al. 2017). This is likely due to females incurring a higher probability of predation by mating with highly conspicuous males that are more at risk of predation (Pocklington and Dill 1995; Segami Marzal et al. 2017). Based on the increased perceived predation risk associated with higher ambient light levels, and the increased predatory risk associated with visually conspicuous calling males, we predicted that females would avoid the multimodal signal in higher light levels. Females have also been shown to make faster choices in higher ambient light levels (Bonachea and Ryan 2011b), and we therefore predicted that females would choose more quickly in brighter environments.

Calling sites, like ambient light levels, vary markedly in their physical characteristics; males call from highly ephemeral puddles measuring less than 0.1 m² to the edges of large ponds that may exceed 100 m². Based on the range of calling sites utilized by males, both sexes must traverse terrestrial and aquatic environments when deciding where to call or with whom to mate. These terrestrial and aquatic environments likely have varying costs associated with them. For example, the frog-eating bat should be more effective at localizing and capturing calling males when they are in open water (Halfwerk et al. 2014; Gomes et al. 2016). Snakes are important predators of túngara frogs, foraging both in and out of the water along pond edges. Other predators such as crabs and large fishing spiders forage in the water and have been

observed eating frogs at calling sites (MJR and RCT pers. obs.). Male túngara frogs require water for effective calling (Halfwerk et al. 2017), but they almost always call from the shallow edges of the pond where they may be less conspicuous to bat and crab predators. Thus, overall predation risks are likely higher when frogs venture into open areas of water. We hypothesized that the higher predation risk associated with open water would cause females to select the less conspicuous, unimodal signal. Additionally, as was the case with higher ambient light levels, females may reduce their latency to choice as a means of decreasing their predation risk.

Although less is known about male túngara frog responses to calls, they do perform phonotaxis (Bernal et al. 2009). Like many frog species, male túngara frogs aggregate at water bodies and form choruses. Males are not territorial and often tolerate other males calling within close proximity. They do occasionally engage in physical contests however, if calling sites are limited or another male approaches too closely. Male phonotaxis behavior likely enables males to find suitable calling sites. No previous studies have tested phonotactic responses of male túngara frogs to multimodal signals. If male phonotactic responses function primarily as a means to find calling sites, then it is plausible that the precise spatial information imparted by the visual cue will not be as important for males compared to females. However, it is also possible that integration of the visual and acoustic signal components in females could be conserved in males, in which case males should respond positively to a multimodal signal (e.g., for a more general example see Forstmeier et al. 2011). We tested males in a terrestrial environment to get a basic understanding of their phonotactic behavior and also tested male responses in an aquatic environment. If males do not attend to the visual component of another calling male, then we predicted that the substrate would not impact their choice, and males would randomly choose between the two stimuli. Regardless of choice, males may still choose faster, thereby reducing predation risk.

In this study we tested the following: (1) female response to multimodal stimuli in varying ambient light levels, (2) female response to multimodal stimuli in a terrestrial and aquatic environment, and (3) male response to multimodal stimuli in both a terrestrial and aquatic environment.

Methods

Subjects

All experiments were performed at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Panama. We collected amplexed pairs as well as individual calling male túngara frogs between 1930 and 2230 h (June–Aug 2011 and June–Aug 2016, 2017). Once collected, we brought the frogs to

STRI facilities, and placed them in a light-safe cooler for at least 1 h before testing. This process ensured that frogs' eyes were dark-adapted, after collection with headlamps, and could therefore see the visual stimulus in the low light conditions of the testing arena. All frogs were tested between 2100 and 0330 h and at temperature of 27 °C. After testing, all frogs were toe-clipped to ensure that frogs were not retested with subsequent recaptures.

Experimental setup

We conducted all experiments in a $2.7 \times 1.8 \times 2$ -m sound attenuation chamber (Acoustic System, ETS-Lindgren, Austin, TX, USA). We placed a plastic funnel (ca. 10 cm diameter) in the center of the chamber; the funnel contained a cutout covered with plastic food wrap, rendering the funnel transparent to both acoustic and visual stimuli. Trials were recorded using an infrared video camera placed in the ceiling of the chamber. The data were not collected blindly as the experimenter (ADC) could observe the frogs' behavior during the trials. Scoring accuracy was verified independently post experiment, however, by KLH and RCT. All trials were conducted with a nightlight suspended over the arena (GE model no. 55507; Fairfield, CT, USA). Light intensity varied depending on treatment (Table 1) but was within the natural range of conditions experienced at breeding sites (Cummings et al. 2008; Taylor et al. 2008). In all experiments, both speakers played the same synthetic complex call (whine + chuck) that was digitally created as a centroid of 15 acoustic parameters from recordings of 50 males collected around our study sites (Ryan and Rand 2003) and has been shown to be attractive. Acoustic stimuli were presented antiphonally on a loop and had a duty cycle of 600 ms. The speakers were calibrated such that the peak amplitude of the whine in the center of the arena was at 76 dB SPL (fast, C weighting, re 20 μ Pa) at the beginning of each night and throughout testing. In treatments with elevated ambient light levels, we calibrated the speakers at 82 dB SPL. Frogs have been shown to be responsive to multimodal signals at both sound pressure levels in previous experiments (Taylor et al. 2008, 2011; Stange et al. 2016).

In each trial, we also placed a robotic frog with an inflating vocal sac directly in front of one of the speakers. This vocal sac inflated and deflated synchronously with the acoustic

Table 1 Light levels and substrate combination in each treatment

Treatment	Substrate	Sex	Light level (W/cm^2)
Dry	Terrestrial	Male	5.8×10^{-10}
Dry	Terrestrial	Female	5.8×10^{-10}
Wet	Aquatic	Male	5.8×10^{-10}
Wet	Aquatic	Female	5.8×10^{-10}
Light	Terrestrial	Female	1.17×10^{-7}

stimuli via a pneumatic pump controlled by the same computer producing the acoustic stimuli (Klein et al. 2012). Adding the robofrog has been shown to increase female preferences for male advertisement calls in this species, as well as others (Taylor et al. 2007, 2011). We alternated the presentation of the robofrog between the two speakers to avoid any potential side bias.

Dry phonotaxis setup Our terrestrial substrate (= dry) treatments occurred on a beige linoleum surface inside the sound attenuation chamber; frogs were able to walk to the speaker of their choice. These dry treatments effectively served as a control for the wet treatments as this configuration is standard for frog phonotaxis experiments. For each trial, we separated an amplexed pair and placed either the female or male under the funnel, depending on the experiment. The funnel was located 80 cm from two speakers that were separated from each other by 70°. Individuals were confined to the area beneath the funnel but were not otherwise restrained. Once placed under the funnel, we played stimuli and allowed individuals at least 2 min to acclimate. After this acclimation period, we remotely raised the funnel, allowing the frogs to move freely around the chamber. Dry trials were conducted at light levels of ca. 5.8×10^{-10} W/cm², which mimics lighting conditions that occur along forest edges on moonless nights. For ease, we later refer to the treatments with lower light levels as “dark.”

We scored each frog as making a choice once they entered a “choice zone” around the two speakers and remained there for 3 s, or if they touched the speaker/robofrog. For females the choice zone was a 10-cm circle around the speaker (Ryan and Rand 2003). Because males may use phonotaxis to search out calling positions, and often fail to approach a speaker closely, the choice zone for males was designated as a 20-cm circle around both speakers. Males were still required to stay within the choice zone for 3 s for a trial to be scored as a choice. Any frog that did not move out of the middle circle within 2 min, did not make a choice within 10 min, or attempted to climb the wall was deemed a “foul out.” Any frog that fouled out twice was removed from the experiment. Presumably, these individuals failed to respond due to a lack of motivation. For each trial, we also recorded the time it took to make a choice (latency).

Wet phonotaxis setup For aquatic (= wet) treatments, we placed a plastic wading pool (1.6 m diameter) in the acoustic chamber and filled it with 2 cm of tap water treated with a dechlorinating agent. The area under the funnel consisted of an elevated platform to ensure that frogs were still in water but were not forced to float or swim. This allowed the frogs to detect the presence of water but allowed them to remain still until they left the platform to make a choice. Once frogs left the platform, however, they had to swim to reach a speaker. Two small holes were cut into the pool siding to allow the

acoustic stimuli to be broadcast from speakers without interference. To keep the visual stimulus of the robofrog stable at the surface of the water, we placed two stones on the sides of the vinyl platform holding the robofrog. These stones did not interfere with the vocal sac inflation or the propagation of water ripples. We placed pairs of stones around the rest of the pool edge at regular intervals to ensure that females were not moving towards the robofrog/stones because it was a potential resting site.

We followed the same protocols used in dry treatments for placing individuals into the chamber and for acclimation. However, due to constraints imposed by the presence of the pool, we altered our scoring methods in the wet treatments. Instead of creating a choice zone around the speaker (which would have required the speaker to be in the pool), we measured the angle at which each frog touched the side of the pool (referred to as “exit angle”). If an individual’s exit angle was within 10° on either side of a speaker (± 12 cm on either side of the speaker), the individual was recorded as making a choice. This criterion has been employed in previous túngara frog experiments (Farris and Ryan 2011). Choice criteria were the same for all trials for the wet treatment, regardless of sex. If a frog did not leave the platform before 2 min or did not make a choice within 10 min, it was deemed a foul out. Any frog that fouled out twice was removed from the experiment. For each trial, we recorded the latency to choice as the time required to reach the side of the pool.

Light phonotaxis setup Light trials followed the same set up as our dry treatment, with three differences. First, only females were tested under the light treatment. In our light trials, the separation angle between speakers was 50°. Female responses to multimodal vs. unimodal stimuli are robust and are not affected by slight variations in separation angle (Taylor et al. 2008; Taylor and Ryan 2013). Additionally, in our light trials, we increased the ambient light levels to 1.17×10^{-7} W/cm². This level mimicked conditions of a cloudless full-moon night (Cummings et al. 2008). Our choice criteria were identical to the criteria for the dry treatment, with a choice zone of 10 cm around each speaker. Foul outs were designated in the same manner as in the dry treatment. Latency to choice was also recorded.

Statistical analysis

We used a binomial distribution (SISA binomial calculator; Uitenbroek 1997) when examining phonotactic preferences of each sex in each treatment. For all male experiments, we utilized an a priori equal probability value of 0.5. This equiprobable value was selected because no previous work has been done on male phonotactic responses to multimodal stimuli. For all female experiments, we utilized an a priori probability value of 0.70. This value is the average female preference

(range = 60–81%) for multimodal stimuli from experiments conducted over a 10-year period (Taylor et al. 2008, 2011; Taylor and Ryan 2013; Stange et al. 2016; K. O. Wilhite et al. unpubl. data). We also employed a 2×2 contingency table to test if preference for a stimulus changed depending on the treatment. We report statistics from a Fisher's exact test. All differences in latencies were tested using independent sample *t* tests, after transforming data to fit assumptions of normality. A Levene's test showed that all latencies exhibited homogeneity of variance.

Data availability

All raw data analyzed for choice tests are included in this published article. Raw data for latencies generated during the current study are available from the corresponding author on reasonable request.

Results

We first tested if differences in substrate (dry and wet treatments) led to different preferences within each sex. In the dry (dark) treatment, females chose the multimodal signal 65% of the time (13:7, two-tailed binomial test: expected = 0.7 $p = 0.547$). In the wet (dark) treatment, they chose the multimodal stimulus 63.6% of the time (14:8, two-tailed binomial test: expected = 0.7, $p = 0.422$; Fig. 1). There was no significant difference between female preferences in the dry and the wet treatments under dark conditions (two-tailed Fisher's exact test: $p = 1$). When we increased ambient light levels (dry), females significantly preferred the unimodal stimulus. Only two of 20 females respond to the multimodal stimulus, reversing their expected preference (10% response, two-tailed binomial test: expected = 0.7, $p = 0.0001$).

Males failed to demonstrate a preference for either stimulus in the dry (dark) treatment, responding to the multimodal signal in 40.6% of trials (13:19, two-tailed binomial test: expected = 0.5, $p = 0.296$). This lack of preference remained in the wet (dark) treatment with a 42.8% multimodal response rate (9:12, two-tailed binomial test: expected = 0.5, $p = 0.523$). There was no significant difference in male preference between the two treatments (two-sided Fisher's exact test: $p = 1$).

Though we did not find a difference in preference between dry and wet trials (both dark), females exhibited significantly shorter latencies in the wet vs. dry treatment (wet $46.41 \text{ s} \pm 10.387 \text{ SE}$, dry $106.30 \text{ s} \pm 15.282$; two-tailed independent sample *t* test, $df = 34$, $p = 0.003$; Fig. 2). There was no difference in the latency to choice between the dry (dark) and light treatments (light $119 \text{ s} \pm 26.116$, dry $106 \text{ s} \pm 15.282$; two-tailed independent sample *t* test, $df = 29$, $p = 0.667$; Fig. 2).

Males also chose significantly faster in the wet (dark) treatment (wet $38.71 \text{ s} \pm 8.27$, dry $124.28 \text{ s} \pm 12.63$; two-tailed

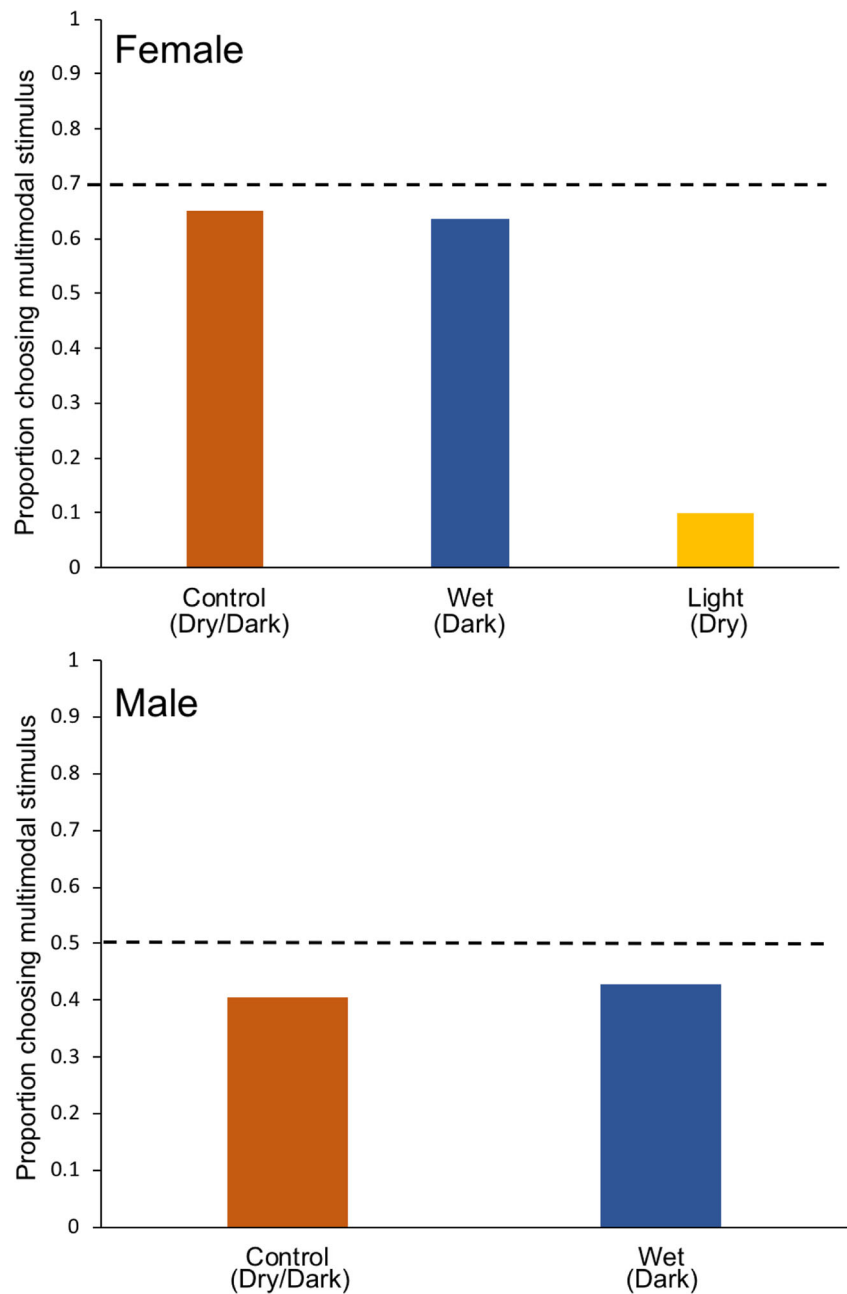
independent sample *t* test, $df = 49$, $p < 0.001$; Fig. 2). Within the dry and wet treatments (both dark), sex did not impact latency to choice (dry male $124.28 \text{ s} \pm 12.63$, female $106.30 \text{ s} \pm 15.28$; two-tailed independent sample *t* test, $df = 42$, $p = 0.379$; wet male $38.71 \text{ s} \pm 8.28$, female $46.41 \text{ s} \pm 10.39$; two-tailed independent sample *t* test $df = 39$, $p = 0.561$).

Discussion

Most animals live in habitats with fluctuating environmental conditions, and adaptations to these fluxes have long been documented (West-Eberhard 1989; Grant and Grant 2002; Relyea 2002; Martin et al. 2008; Campbell-Staton et al. 2017). Preferences for properties of sexual advertisement signals have traditionally been considered to be relatively fixed; only more recently has attention been given to the idea that fluctuating environmental conditions may influence mating preferences (Bro-Jørgensen 2010; Botero and Rubenstein 2012; Stafstrom and Hebets 2013; Halfwerk and Slabbekoorn 2015). Here, we tested the impact of varied environmental conditions on the phonotactic response to multimodal stimuli in both male and female túngara frogs. Females were presented three different environmental conditions: a dry (dark) control, an aquatic substrate (wet and dark), and elevated ambient light levels (dry substrate). Substrate did not alter female choice, but the wet environment led to faster choices in females. When ambient light levels were elevated, female latency to choice remained unchanged, but females showed a reversal of choice and responded strongly to the unimodal stimulus. Under control conditions, males showed no preference for the multimodal stimulus. This lack of preference remained when males were placed in the wet environment, and like females, males responded faster in this setting. Thus, substrate did not impact overall preference for either sex but did decrease latency to choice in both sexes.

Ambient light levels play an important role in the mating behavior of female túngara frogs (Rand et al. 1997; Bernal et al. 2007; Baugh and Ryan 2010). Under relatively high light conditions, female túngara frogs prefer a less attractive vocalization when these calls are closer (Rand et al. 1997) or require less effort to approach (Baugh and Ryan 2010). Our data demonstrate that full moon conditions induce a similar preference reversal in female responses to a multimodal signal. Previous work (Rand et al. 1997; Baugh and Ryan 2010) compared stimuli that were made to represent calling males at different distances and the reversal in female preference is associated with decreasing search effort and cost. In the current study, females were exposed to two calls that were presented with the same amplitude and distance. Therefore, the avoidance of the multimodal signal was not due to differences in search effort. Instead, the audiovisual

Fig. 1 Responses to unimodal and multimodal stimuli in dry, wet, and light treatments, top panel female and bottom panel male túngara frogs. Values represent the proportion of individuals in each treatment choosing the multimodal (visual + acoustic) stimulus. Females are compared to a 0.7 expected value and males are compared to a 0.5 expected value. Female dry/dark ($n = 20$), female wet/dark ($n = 20$), female light/dry ($n = 22$), male dry/dark ($n = 32$), male wet/dark ($n = 21$)

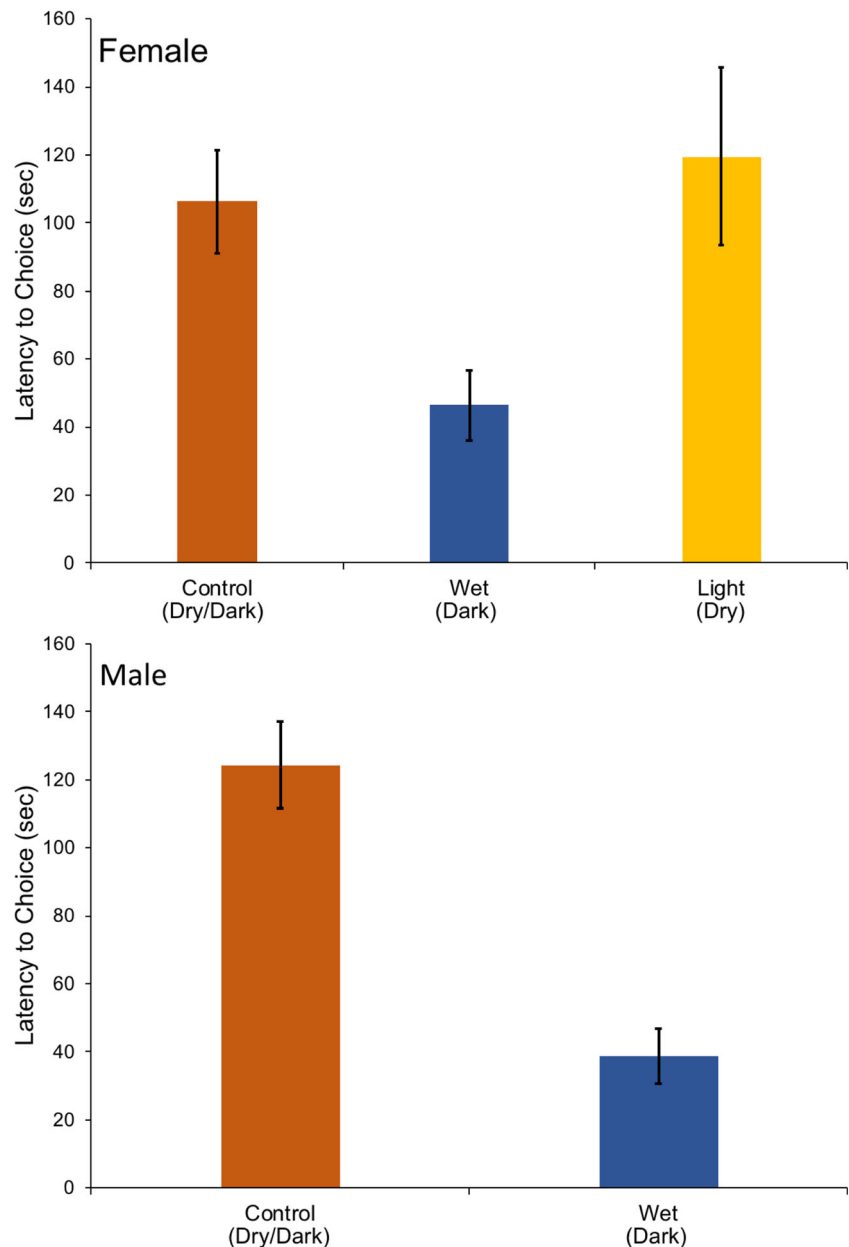


stimulus itself caused females to reverse their preference when in higher light levels.

Under our control conditions, the attractiveness of the multimodal signal is likely in part due to the conspicuousness of the visual component. However, a visually conspicuous male is also known to attract attention from eavesdropping predators (Halfwerk et al. 2014). Higher light levels may make this conspicuous signal even riskier. Therefore, when risk of predation is increased, females should avoid conspicuous males, likely reducing the probability of being captured. Previous work has demonstrated that when acoustic predatory cues are added, increasing perceived predation risk, females also

choose closer individuals, and even become more permissive to heterospecific calls (Bernal et al. 2007; Bonachea and Ryan 2011a). The shift in response by females to the unimodal stimulus suggests that risk associated with a visually conspicuous calling male is substantial. Our data indicate that in a natural chorus environment, which experiences drastic fluctuations in ambient light levels, female preferences may shift depending on weather patterns, forest canopy cover, and position in the lunar cycle. Female túngara frogs prefer a visually conspicuous caller under new moons or cloudy nights but may avoid those same callers on clear, moonlit nights. Interestingly, Underhill and Höbel (2017) found no

Fig. 2 Túngara frog latency to choice in both dry and wet treatments for females (top panel) and males (bottom panel). Error bars are \pm SE. Female dry/dark ($n = 20$), female wet/dark ($n = 22$), female light/dry ($n = 20$), male dry/dark ($n = 32$), male wet/dark ($n = 21$)



influence of light level on female preference for call duration in the gray treefrog, *Hyla versicolor*. The different responses to light levels between these species could stem from different experimental conditions or may simply reflect lower overall predation risk in the larger, temperate-dwelling gray treefrog.

Our finding that light influences mate choice in the túngara frog also has implications for understanding potential impacts from anthropogenic sources of light (Perry et al. 2008). Urban environments are understood to exert selection pressures on sexual signals, impacting communication across a variety of sensory modalities (Longcore and Rich 2004; Halfwerk et al. 2011; Halfwerk and Slabbekoorn 2015). Based on the avoidance of multimodal signals at high light levels found in this study, it is plausible that in developed areas with elevated light

levels, female choice may favor males that remain hidden while calling. This pressure could in turn cause the visual signal to lose its saliency over evolutionary time in certain populations.

The risk of predation can also alter behaviors aside from choice, such as activity level (Lima and Dill 1990). Individuals may be more cautious in their approach and take longer to move or make a choice, as túngara frogs do when a predatory stimulus is presented alongside an attractive call (Bonachea and Ryan 2011b). Reducing movement is a common anti-predatory response (Lawler 1989; Richardson 2001), and female túngara frogs are more likely to remain motionless in higher ambient light levels (Rand et al. 1997). However, when individuals do make a choice in higher light

levels, they tend to have decreased latencies (Bonachea and Ryan 2011b). Surprisingly, we found no significant difference in female latencies between light and dark (dry substrate) treatments. One possible reason for the discrepancy between our findings and those of Bonachea and Ryan (2011b) could be that the intensity of light used in our study was lower. We did, however, find a difference in latencies between our dry and wet treatments. We hypothesize that, like increased ambient light levels, the faster response rates in both sexes are due to an increased predation risk associated with open water. Frogs can move faster when walking/hopping compared with swimming (Peters et al. 1996), but in our experiments, they responded to stimuli significantly faster in wet treatments. After the funnel was lifted in the wet treatments, frogs typically swam directly towards a speaker. In the dry treatments, frogs often stopped and/or changed directions while making a choice. This indicates that frogs of both sexes were reducing the amount of time they spent assessing alternative choices when they were required to swim. One possible influence of decreased latencies in wet treatments could be that added information provided by ripples reduced assessment time regarding the location of the stimuli. Although both sexes responded faster, only females preferentially responded to the multimodal stimulus. Halfwerk et al. (2014) showed that male túngara frogs use water ripples to assess distance to rival callers (Halfwerk et al. 2014); the degree to which females assess ripples is unknown. In wood frogs (*Lithobates sylvaticus*), males respond to ripples but females avoid them (Höbel and Kolodziej 2013). Wood frogs have a short, explosive breeding season where males actively search out females, which is different from túngara frogs where males do not search. Thus, information provided by ripples likely influences sexual decision making in frogs. The different responses to multimodal signals and ripples across sex (present study) and species (Höbel and Kolodziej 2013), however, indicate a need for additional experiments specifically testing the influence of ripples.

Females chose significantly faster in the wet treatments compared to the dry but maintained their preference for the multimodal call. This finding begs the question, “why do females take so long to approach callers when they do not have to swim?” When no immediate predation risk is present, females may opt to spend more time evaluating males. In a natural chorus, there are often several males calling simultaneously, which may make discrimination difficult. Also, females incorporate previous information about male calling into their mating decisions (Akre and Ryan 2010b), and increased sampling time may be advantageous, as male calling characteristics such as call rate and call complexity are not fixed. In bumblebees, a scent added to floral visual signals improved accuracy of bee visits in decreasing light levels but did not increase the rate of visits (Kaczorowski et al. 2012). Thus, the addition of the scent maintained visit

accuracy across different light levels but at a cost to bees of not increasing pollen collection rates. Floral signals to pollinators evolved under different selection regimes than animal sexual signals. The influence of multiple signal components on response time by receivers under variable environmental conditions, however, suggests a need for additional investigations of speed-accuracy trade-offs (Chittka et al. 2009).

Previous studies examining environmental heterogeneity and its impact on mating behavior typically alter a single environmental variable. While these studies tell us a great deal about animal communication and its dependence on the environment, environmental changes occur often and across multiple dimensions. In the current study, we found that changes in female response are not uniform for different types of environmental change. When presented with increased light levels, females reversed their choice for the multimodal stimulus. This avoidance of more conspicuous signals is a common trend when females experience high predatory risk (Forsgren 1992; Hedrick and Dill 1993; Gong and Gibson 1996; Johnson and Basolo 2003; Pilakouta and Alonzo 2013; Pilakouta et al. 2017). In an aquatic environment, females did not alter their choice, instead they responded faster relative to controls. Changes in latency to choice and time spent mate searching are also commonly affected by predation risk (Karino et al. 2000; deRivera et al. 2003; Baugh and Ryan 2010). Our findings demonstrate the importance of not overgeneralizing the influences of different environmental conditions of female and male responses to conspecific signals.

Male phonotactic responses to multimodal signals in both dry and wet treatments were random. Males and females demonstrate the same preferences for complex acoustic stimuli when both respond phonotactically (Bernal et al. 2009). It was therefore surprising that males did not show any preference for the multimodal over the unimodal signal. This finding suggests that the importance of information conveyed in the visual component differs between sexes, with males relying less on multimodal signals from other males. Male phonotaxis is thought to be associated with finding suitable calling sites, as opposed to localizing individual males. Differences in the role of phonotaxis between the sexes could explain the differences of preference for the multimodal signal presented. There may be sex-based neurological variances responsible for the apparent differences in behavior. Previous work has found that while male and female túngara frogs similarly process acoustic stimuli in the periphery, central processing has sex-based differences (Hoke et al. 2008). Multisensory integration also occurs in the central nervous system, and potentially in the same brain regions and may be sexually mediated.

Overall, the findings of this study support the hypothesis that mate choice behaviors are not stable across different environmental conditions. These behavioral changes may be as

pronounced as reversing preference for a particular stimulus, or can be more subtle, as in the case of faster mating decisions and responses. Communication via multiple sensory modalities is likely acutely impacted by environmental heterogeneity as each modality will be affected differently by the environmental context. Continued exploration into how the environment (including anthropogenic changes) influences sexual behavior with regard to multimodal communication will provide a better understanding of the role of the environment in shaping communication and complex mate signals.

Acknowledgments Kyle Davis provided help with data collection and logistics. Two anonymous reviewers provided valuable comments, improving the manuscript. The Smithsonian Tropical Research Institute provided logistical support and laboratory space.

Funding information Funding for equipment was provided by NSF grant (no. 0517328) to RCT, MJR, and RAP. Salisbury University provided travel funding for ADC, RCT, and KLH. Travel funds were facilitated by Stephen Gehrich and Clifton Griffin at Salisbury University.

Compliance with ethical standards

Ethical standards Handling and toe clip procedures were conducted in accordance with The American Society of Ichthyologists and Herpetologists' Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. All experiments were conducted in accordance with Smithsonian Tropical Research Institute guidelines and approved by the Institutional Animal Care and Use Committee (protocol no. 2015-0209-2018). All necessary permits were obtained from the Panamanian government including collecting permit numbers SE/A-30-16 and SE/AO-1-17.

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

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Akre KL, Ryan MJ (2010a) Proximity-dependent response to variably complex mating signals in túngara frogs (*Physalaemus pustulosus*). *Ethology* 116:1138–1145
- Akre KL, Ryan MJ (2010b) Complexity increases working memory for mating signals. *Curr Biol* 20:502–505
- Aspbury AS, Espinedo CM, Gabor CR (2010) Lack of species discrimination based on chemical cues by male sailfin mollies, *Poecilia latipinna*. *Evol Ecol* 24:69–82
- Baugh AT, Ryan MJ (2010) Ambient light alters temporal-updating behaviour during mate choice in a Neotropical frog. *Can J Zool* 88:448–453
- Bernal XE, Rand AS, Ryan MJ (2007) Sex differences in response to nonconspecific advertisement calls: receiver permissiveness in male and female túngara frogs. *Anim Behav* 73:955–964
- Bernal XE, Rand AS, Ryan MJ (2009) Task differences confound sex differences in receiver permissiveness in túngara frogs. *Proc R Soc Lond B* 276:1323–1329
- 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
- Botero CA, Rubenstein DR (2012) Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. *PLoS One* 7:e32311
- Boughman JW (2002) How sensory drive can promote speciation. *Trends Ecol Evol* 17:571–577
- Bro-Jørgensen J (2010) Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol Evol* 25:292–300
- Buchanan BW (1992) Bimodal nocturnal activity pattern of *Hyla squirella*. *J Herpetol* 26:521–523
- Campbell-Staton SC, Cheviron ZA, Rochette N, Catchen J, Losos JB, Edwards SV (2017) Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science* 357:495–498
- Chaine AS, Lyon BE (2008) Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science* 319:459–462
- Chittka L, Skorupski P, Raine NE (2009) Speed-accuracy trade-offs in animal decision-making. *Trends Ecol Evol* 24:400–407
- 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
- deRivera CE, Backwell PR, Christy JH, Vehrencamp SL (2003) Density affects female and male mate searching in the fiddler crab, *Uca beebei*. *Behav Ecol Sociobiol* 53:72–83
- Endler JA (1987) Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim Behav* 35:1376–1385
- Farris HE, Ryan MJ (2011) Relative comparisons enable auditory grouping in frogs. *Nat Commun* 2:410
- Forstmeier W, Martin K, Bolund E, Schielzeth H, Kempenaers B (2011) Female extrapair mating behavior can evolve via indirect selection on males. *P Natl Acad Sci USA* 108:10608–10613
- Forsgren E (1992) Predation risk affects mate choice in a gobiid fish. *Am Nat* 140:1041–1049
- Gong A, Gibson RM (1996) Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. *Anim Behav* 52:1007–1015
- Gomes D, Geipel I, Page RA, Taylor RC, Ryan MJ, Halfwerk W (2016) Bats perceptually weight prey cues across sensory systems when hunting in noise. *Science* 353:1277–1280
- Gordon SD, Uetz GW (2011) Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. *Anim Behav* 81:367–375
- Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711
- Halfwerk W, Bot S, Buix J, van der Velde M, Komdeur J, ten Cate C, Slabbekoorn H (2011) Low-frequency songs lose their potency in noisy urban conditions. *P Natl Acad Sci USA* 108:14549–14554
- Halfwerk W, Jones PL, Taylor RC, Ryan MJ, Page RA (2014) Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. *Science* 343:413–416
- Halfwerk W, Slabbekoorn H (2015) Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol Lett* 11:20141051
- Halfwerk W, Smit JAH, Loning H, Lea AM, Geipel I, Ellers J, Ryan MJ (2017) Environmental conditions limit attractiveness of a complex sexual signal in the túngara frog. *Nat Commun* 8:1891

- Hebets EA, Barron AB, Balakrishnan CN, Hauber ME, Mason PH, Hoke KL (2016) A systems approach to animal communication. *Proc R Soc B* 283:20152889
- Hedrick AV, Dill LM (1993) Mate choice by female crickets is influenced by predation risk. *Anim Behav* 46:193–196
- Higham JP, Hebets EA (2013) An introduction to multimodal communication. *Behav Ecol Sociobiol* 67:1381–1388
- Höbel G, Kolodziej RC (2013) Wood frogs (*Lithobates sylvaticus*) use water surface waves in their reproductive behaviour. *Behaviour* 150: 471–483
- Hoke KL, Ryan MJ, Wilczynski W (2008) Candidate neural locus for sex differences in reproductive decisions. *Biol Lett* 4:518–521
- Johnson JB, Basolo AL (2003) Predator exposure alters female mate choice in the green swordtail. *Behav Ecol* 14:619–625
- Kaczorowski RL, Leonard AS, Dornhaus A, Papaj DR (2012) Floral signal complexity as a possible adaptation to environmental variability: a test using nectar-foraging bumblebees, *Bombus impatiens*. *Anim Behav* 83:905–913
- Karino K, Kuwamura T, Nakashima Y, Sakai Y (2000) Predation risk and the opportunity for female mate choice in a coral reef fish. *J Ethol* 18:109–114
- Kim TW, Christy JH, Choe JC (2007) A preference for a sexual signal keeps females safe. *PLoS One* 2:e422
- Klein BA, Stein J, Taylor RC (2012) Robots in the service of animal behavior. *Commun Integr Biol* 5:466–472
- Lawler SP (1989) Behavioural responses to predators and predation risk in four species of larval anurans. *Anim Behav* 38:1039–1047
- Lea AM, Ryan MJ (2015) Irrationality in mate choice revealed by túngara frogs. *Science*. 349:964–966
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Longcore T, Rich C (2004) Ecological light pollution. *Front Ecol Environ* 2:191–198
- Lynch KS, Crews D, Ryan MJ, Wilczynski W (2006) Hormonal state influences aspects of female mate choice in the túngara frog (*Physalaemus pustulosus*). *Horm Behav* 49:450–457
- Martin LB, Weil ZM, Nelson RJ (2008) Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philos Trans R Soc B* 363:321–339
- Milinski M, Bakker TCM (1992) Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proc R Soc Lond B* 250: 229–233
- Partan SR, Marler P (1999) Communication goes multimodal. *Science* 283:1272–1273
- Perry G, Buchanan BW, Fisher RN, Salmon M, Wise SE (2008) Effects of artificial night lighting on amphibians and reptiles in urban environments. In: Mitchell JC, Jung Brown RE, Bartholomew B (eds) *Herpetological conservation 3: urban herpetology: ecology, conservation and management of amphibians and reptiles in urban and suburban environments*. Society for the Study of Amphibians and Reptiles, Salt Lake City, pp 239–256
- Peters SE, Kamel LT, Bashor DP (1996) Hopping and swimming in the leopard frog, *Rana pipiens*. I. Step cycles and kinematics. *J Morphol* 230:1–16
- Pilakouta N, Alonzo SH (2013) Predator exposure leads to a short-term reversal in female mate preferences in the green swordtail, *Xiphophorus helleri*. *Behav Ecol* 25:306–312
- Pilakouta N, Correa MA, Alonzo SH (2017) Predation risk reduces a female preference for heterospecific males in the green swordtail. *Ethology* 123:95–104
- Pocklington R, Dill LM (1995) Predation on females or males: who pays for bright male traits? *Anim Behav* 49:1122–1124
- Qvarnström A, Pärt T, Sheldon BC (2000) Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature* 405: 344–347
- Rand AS, Bridarolli ME, Dries L, Ryan MJ (1997) Light levels influence female choice in túngara frogs: predation risk assessment? *Copeia* 1997:447–450
- Relyea RA (2002) Local population differences in phenotypic plasticity: predator induced changes in wood frog tadpoles. *Ecol Monogr* 72: 77–93
- Richardson JM (2001) A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behav Ecol* 12:51–58
- Rosenthal GG (2017) *Mate choice: the evolution of sexual decision making from microbes to humans*. Princeton University Press, Princeton
- Ryan MJ (1985) *The túngara frog: a study in sexual selection and communication*. University of Chicago Press, Chicago
- Ryan MJ, Fox JH, Wilczynski W, Rand AS (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* 343:66–67
- Ryan MJ, Rand AS (1990) The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* 44:305–314
- Ryan MJ, Rand AS (2003) Sexual selection in female perceptual space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. *Evolution* 57:2608–2618
- Segami Marzal JC, Rudh A, Rogell B, Ödeen A, Løvlie H, Rosher C, Qvamström A (2017) Cryptic female strawberry poison frogs experience elevated predation risk when associating with an aposomatic partner. *Ecol Evol* 7:744–750
- Stafstrom JA, Hebets EA (2013) Female mate choice for multimodal courtship and the importance of the signaling background for selection on male ornamentation. *Curr Zool* 59:200–209
- Stange N, Page RA, Ryan MJ, Taylor RC (2016) Interactions between complex multisensory signal components result in unexpected mate choice responses. *Anim Behav* 134:239–247
- Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *P Natl Acad Sci USA* 111:5610–5615
- Taylor RC, Buchanan BW, Doherty JL (2007) Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. *Anim Behav* 74:1753–1763
- Taylor RC, Klein B, Stein J, Ryan MJ (2008) Faux frogs: multimodal signalling and the value of robotics in animal behaviour. *Anim Behav* 76:1089–1097
- Taylor RC, Klein BA, Stein J, Ryan MJ (2011) Multimodal signal variation in space and time: how important is matching a signal with its signaler? *J Exp Biol* 214:815–820
- Taylor RC, Ryan MJ (2013) Interactions of multisensory components perceptually rescue túngara frog mating signals. *Science* 341:273–274
- Uitenbroek DG (1997) SISA binomial, <http://home.clara.net/sisa/binomial.htm>
- Underhill VA, Höbel G (2017) Variation in nocturnal light levels does not alter mate choice behavior in female eastern gray treefrogs (*Hyla versicolor*). *Behav Ecol Sociobiol* 71:151
- West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278
- Wilgers DJ, Hebets EA (2011) Complex courtship displays facilitate male reproductive success and plasticity in signaling across variable environments. *Curr Zool* 57:175–186