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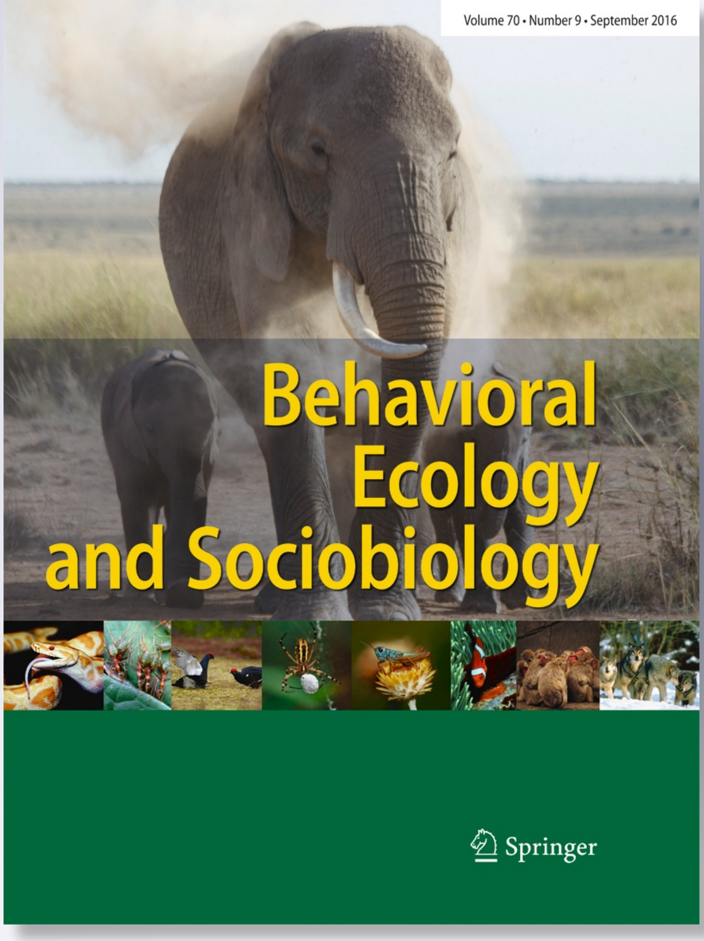

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ORIGINAL ARTICLE

Multimodal signaling improves mating success in the green tree frog (*Hyla cinerea*), but may not help small males

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Abstract
Many anuran amphibians are challenged with the detection of courtship signals in noisy chorus environments. Anurans and other animals partially solve this discrimination challenge by employing auditory mechanisms such as grouping sounds by frequency, time, or spatial location. Animals are also known to employ visual cues as a mechanism of improving auditory signal detection. In this study, we examined the effect of acoustic and visual stimuli on female mate choice preferences in the green tree frog, *Hyla cinerea*. We used a series of two choice playback tests and added a robotic frog, with an inflatable vocal sac, to test interactions among visual and acoustic signal components. Females preferred vocalizations with faster call rates (i.e., high energy cost) and lower call frequencies (i.e., larger males). When call properties were held equal, females discriminated against an acoustic only stimulus in favor of the combined acoustic/visual multimodal signal. A visual component did not, however, increase the attractiveness of an otherwise unattractive (high-frequency) acoustic signal. Thus, female green tree frogs integrate the visual display into the acoustic communication system and males that are visually accessible can increase their probability of mating success. Visual accessibility, however, is unlikely to improve mating success for small males (high-frequency callers).

Significance statement
Animal communication signals are often complex and communicated in multiple sensory channels (e.g., auditory + visual). Female choice is known to be an important mechanism driving signal evolution. Thus, for complex mating signals, a first step in understanding their evolution is to test how females respond to various combinations of components. Here, we tested female mate choice in the green tree frog, *H. cinerea*, using a combination of audio playbacks and a robotic frog as the visual component. When the audio signal was standardized, females preferred a signal enhanced by a robotic frog. The robotic frog did not increase female responses to an unattractive call (indicative of a small male), however. These results suggest that visual accessibility can improve a male's chance of mating, but this advantage is context dependent and does not extend to smaller males.

Keywords *Hyla cinerea* · Mate choice · Multimodal signaling · Robotic frog

Introduction

A critical component of animal communication is the detection of signals by conspecific receivers (Guilford and Dawkins 1991; Rowe 1999); without detection or recognition, receivers cannot exert selection on the signal. This detection is often hindered, however, by environmental noise or degradation of the signal (Wiley 2015). In many species, overlapping acoustic signals generated by conspecifics present especially difficult detection problems for receivers, often referred to as

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the "cocktail party problem" (Cherry 1953; Bronkhorst 2000; Bee and Micheyl 2008). Distinguishing individuals in this environment requires that listeners form auditory "objects" or coherent auditory streams that correspond to the location of individual signalers. Acoustically communicating animals have evolved a number of mechanisms that improve auditory object formation in noisy environments. For example, animals may group spectral cues (Gerhardt and Höbel 2005; Nityananda and Bee 2011), they may group sounds in space or time (Wilczynski et al. 1999; Farris et al. 2002; Lombardo et al. 2008; Farris and Ryan 2011), and they may exploit periods when background noise temporarily declines, to catch acoustic "glimpses," known as dip listening (Vélez and Bee 2011). Finally, the integration of multiple sensory modalities may improve a receiver's ability to detect and discriminate signals (Rowe 1999; Hebets and Papaj 2005).

Multimodal signals are widespread in the animal kingdom (Partan and Marler 1999) and signals with various combinations of olfactory, seismic, visual, and acoustic components have been identified in both vertebrate and invertebrate taxa (McLennan 2003; Hebets et al. 2006; Baldwin and Johnsen 2009; Partan et al. 2009). For instance, male satin bowerbirds, *Ptilonorhynchus violaceus*, produce vocalizations, visual stimuli in the form of bower decorations (Coleman et al. 2004), and "paint" the inside of their bowers which may also act as a chemical stimulus for females (Hicks et al. 2013). Likewise, many anuran amphibians have been shown to augment their acoustic signals with visual components during courtship (Hödl and Amézquita 2001; Preininger et al. 2013).

In typical frog mating systems, males produce vocalizations that are used as courtship signals (Ryan 2001; Bee 2015). In many species, males advertise in large numbers, generating loud choruses. Gravid females evaluate calling males and choose a mate based on specific properties of these calls (Gerhardt and Huber 2002). In experimental studies, researchers can exploit female anurans' natural tendency to approach calling males and test preferences in controlled playback studies (Bee 2015). This behavior, along with the application of robotics, enables researchers to manipulate signal components that receivers perceive, thus empirically addressing questions in acoustic perception and multimodal communication. Using robotics to generate courtship signals avoids presenting live stimuli that are unpredictable, uncontrollable, and may bias receiver responses (Klein et al. 2012). Robotic models can deliver multimodal signals that stimulate anti-predator, courting, territorial, or mate choice behaviors (Narins et al. 2003; Rundus et al. 2007; Partan et al. 2009; Patricelli and Krakauer 2009; Taylor et al. 2011; Macedonia et al. 2013).

Understanding how animals detect and respond to basic components of complex signals is a prerequisite to understanding how receivers integrate different signal components in complex acoustic environments. Emerging data indicate that increasingly complex signaling environments and interactions among signal components can lead to unexpected receiver responses (Hebets et al. 2011; Taylor and Ryan 2013; Lea and Ryan 2015). Thus, female responses to complex male courtship signals are likely to be context dependent, rather than fixed values. For this study, we focused on the green tree frog, *Hyla cinerea*, a species where female mate preferences to acoustic signals have been thoroughly studied (Gerhardt 1987; Gerhardt and Höbel 2005). We investigated for the first time in this species, the role of vision in courtship with the use of a robotic frog. Specifically, we presented female green tree frogs with unimodal and multimodal signals to (1) characterize the acoustic mate preference functions of a population in Maryland, USA; (2) test if females evaluate a visual component of male courtship displays; and (3) test the relative mate attraction value of visual vs. acoustic components.

Methods

Study organism

The green tree frog, *Hyla cinerea*, is an arboreal frog inhabiting the southeastern USA, from central Texas to Florida and as far north as Maryland and southern Illinois in the Mississippi River drainage. This species is primarily a vibrant green, with a white labial and lateral stripe (these stripes are often continuous; Fig. 1a). The stripe is variable in size and even non-existent in some individuals. Males produce short advertisement calls, typically at a rate of 0.8 call per second (Höbel 2010). The calls are biphasic containing a prominent low-frequency component (0.64–0.96 kHz) and high-frequency component (2.34–3.45 kHz) (Oldham and Gerhardt 1975). Additional harmonics are also present creating a spectrally complex call.

Call recording and analyses

We recorded advertisement calls from 64 male green tree frogs at a field site in Vienna, Maryland (38° 28′ 30.75″ N, 75° 50′ 19.79″ W) from 1–8 June 2014. All males were distant enough from each other to obtain clear recordings without interference from other calling males and were recorded in situ. Once a male began to call, we recorded the calls using a Marantz Professional Solid State Recorder PMD620 (Denon Professional Fort Lauderdale, FL) or a Tascam DR-05 Linear PCM Recorder (TEAC America, Inc. Montebello, CA) and a SME-ATR55 shot-gun microphone (Saul Mineroff Electronics, Inc. Elmont, NY). The microphone was positioned approximately 30 cm away from the snout of the vocalizing male. After recording, we measured the mass and snout vent length (SVL) of each frog. We then took photographs of the males to calculate the average lateral stripe area

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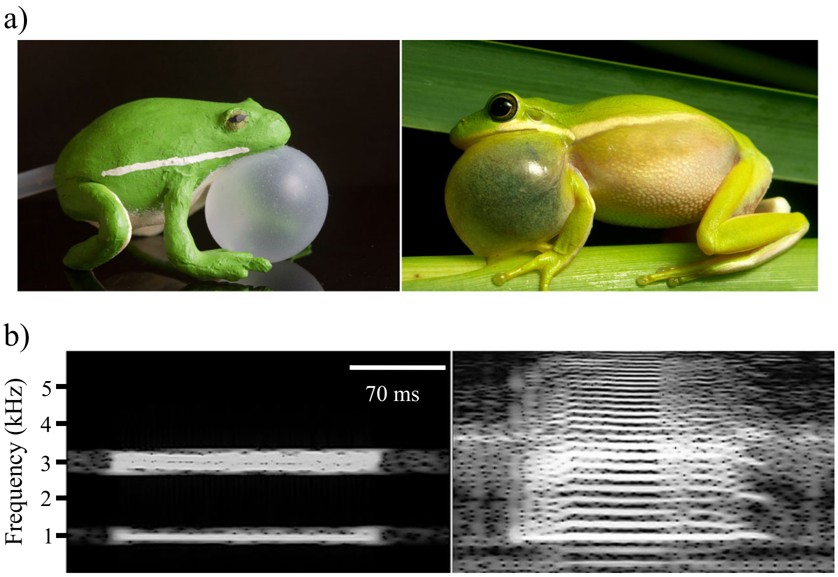

Fig. 1 **a** Robofrog *Hyla cinerea* (*left*) and calling *H. cinerea* male (*right*). **b** Sonograms of synthesized (*left*) and natural (*right*) *H. cinerea* advertisement calls

for this population. A unique toe clip was taken to prevent resampling and to collect genetic material. Male vocalization sound files (.WAV files) were analyzed using SoundRuler (SourceForge.net) and AviSoft SASLab Pro (Avisoft Bioacoustics Berlin, Germany). We synthesized advertisement calls using Adobe Audition 2.0 (San Jose, CA) and Avisoft SASLab Pro software. The synthetic calls were constructed by using a natural call, close to the centroid of the population, as a template. This call was based on six call parameters, from 64 unique individuals, known to be important for female choice in this species (see Table 2). In Avisoft, we erased harmonic bands lower than 0.9 kHz, bands between 1 and 2.9 kHz, and all harmonic energy above 3.2 kHz. This left energy in the two primary spectral bands. The amplitudes of individual pulses within the call were then adjusted to equalize pulse amplitude across the call. Overall, call frequencies were then adjusted by shifting the frequency in Adobe Audition. We generated one synthetic exemplar each of three call frequencies: low, medium, and high, spanning a substantial proportion of spectral range produced by frogs in this population (Table 1).

Robotic frog

To explore the importance of multiple modalities, we constructed a robotic frog system (robofrog) to test female preferences (Fig. 1a). We cast the body of the model green tree frog in polyurethane resin from a preserved specimen mold and painted it with acrylic paints to resemble a live frog (sensu Klein et al. 2012). We painted a white lateral stripe on the frog models that represented the average stripe size for the local population (mean length = 28.4 mm, mean width = 1.4 mm). We fabricated synthetic, translucent vocal sacs (hereafter referred to as “fauxcal sacs”) by repeatedly immersing a Teflon ball in a suspension of platinum-catalyzed silicon rubber and allowed it to dry. After curing, the Teflon ball was removed, leaving a silicon fauxcal sac that could be inflated to mimic a calling male’s vocal sac.

To mimic a live calling frog, we glued the fauxcal sac to a silicone pneumatic tube that was threaded through the body of the robotic frog. This left only the fauxcal sac itself emerging from the gular region of the model frog, closely matching the external anatomy of live frogs. The opposite end of the silicone tube was attached to a computer-activated pneumatic piston, allowing the fauxcal sac to be inflated and deflated. We fabricated multiple fauxcal sacs as they periodically ruptured and had to be replaced. In this manner, we also had two additional robofrogs, pre-prepared with fauxcal sacs, available to switch out when necessary. The piston motion, and hence fauxcal sac inflation/deflation, was triggered by a 19 kHz tone (inflate) and 16 kHz tone (deflate). The tones were placed at the beginning and end of the synthetic call in Adobe Audition to synchronize the inflation/deflation sequence with the call produced at the speaker. These tones are beyond the range of hearing of the frogs and therefore should not influence female choice (Buerkle et al. 2014). Additionally, to ensure that the tones did not bias preference, we conducted a two-choice experiment (identical call with and without tones). Females did not exhibit a bias for or against calls with the trigger tones (two-tailed binomial test, $p = 0.332$, $n = 16$).

Collection of green tree frogs

We collected amplectant pairs from Vienna, MD, from 10 June to 3 August 2014. We placed pairs in individual plastic bags and placed these into a cooler for transport to Salisbury University. Frogs were housed in a cooler for a minimum of 1 h prior to testing, allowing the eyes of the

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Table 1 Synthesized green tree frog call parameters used for phonotaxis experiments

Call parameter		Low/Slow	Medium/Average	High/Fast
Frequency (kHz)	Low-frequency peak	0.88	1.03	1.20
	High-frequency peak	2.85	3.18	3.44
Call rate (calls/min)		58	68	78

frogs to undergo dark adaptation (Fain et al. 2001). We tested females only once in each experiment and on the same night of capture. Females that responded in phonotaxis had morphometric data collected and received a unique toe clip. Likewise, each male mating partner was measured and toe clipped. We returned all pairs to their original site of capture after trials were complete. A small amount of dechlorinated water was provided for pairs during testing. We also returned any deposited eggs to the field site, thereby minimizing any influence of collection on the population size.

Phonotaxis experiments

Female mate choice was tested using two-speaker phonotaxis tests in a 1.8 × 2.7-m hemi-anechoic chamber (ETS-Lindgren Acoustic Systems, Austin, TX). The ambient temperature in the chamber averaged 26 °C for all experiments. We set the speakers at a 60° angle relative to the female, with each speaker 1.2 m from the female release point. A series of synthesized male calls were played antiphonally from two Mirage Nanosat speakers at an amplitude of 86 dB (re. 20 μPa, fast-C weighting) as measured at the females' release point. We switched the acoustic signals between the two speakers across trials to avoid side bias. The trials were observed via an IR video camera (EverFocus EHD 500IR) mounted on the ceiling of the acoustic chamber, allowing a viewer outside to score the female's behavior in real time. All video trials were also recorded using Media Recorder (Noldus Information Technology Inc. Leesburg, VA). The data were not scored blindly as the experimenter (KLL) could observe the trials live. Videos were, however, confirmed independently after the experiments by KLH and RCT.

For each trial, we separated the female from her male and placed her at one end of the chamber under an acoustically and visually transparent funnel at the release location (sensu Taylor et al. 2008). The male was placed back in the plastic bag and returned to the cooler. Calls were broadcast from the speakers and females were acclimated for 2 min under the funnel to eliminate leader bias. After this period, we lifted the funnel by a nylon string from outside the chamber, freeing the female. We scored a choice when the female moved to within 10 cm of the front or sides of the speaker and remained there for at least 2 s. Females that did not leave the release location, were static for 2 min, or exceeded 10 min without making a choice were discarded from the analysis. Reproductively proceptive females reliably exhibit phonotaxis behaviors when exposed to conspecific male vocalizations (Bee 2015). Motivated females will respond multiple times and even intervals of a few minutes between trials are typically sufficient to extinguish bias from previous experience (Akre and Ryan 2010). Thus, our experimental protocol provides an excellent system to address questions in anuran mate choice.

Unimodal phonotaxis

We conducted a series of two-choice phonotaxis experiments to test female preferences for variations of frequency and call rate in this population. We determined preference for call frequency by providing females with three different acoustic stimulus pairs: low vs. medium, medium vs. high, and low vs. high frequencies (Table 1). Preference for call rate was determined by presenting different stimuli: slow vs. average, average vs. fast, and slow vs. fast call rates (Table 1). For experiments where we manipulated call rate, the average frequency was used (high-frequency peak = 3.18 kHz, low-frequency peak = 1.03 kHz). When we manipulated frequency, the rate was set to a constant rate of 68 calls per minute for both speakers.

Multimodal phonotaxis

We tested females in two-choice stimulus tests to determine if they prefer multimodal signals or unimodal signals. We placed a robofrog in front of one of the speakers in the sound chamber; the fauxcal sac was inflated synchronously with a call produced from the corresponding speaker, thereby mimicking a live calling male (sensu Taylor et al. 2008). The alternative choice was solely the acoustic playback of the same signal. The acoustic stimulus played from each speaker was the average frequency call, broadcasted at 68 calls per minute.

Signal weighting phonotaxis

Finally, we performed a signal weighting experiment to determine if a visual display alters the attraction value of calls. We gave females a choice between an attractive

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average-frequency call and an unattractive high-frequency call. We placed the robofrog in front of the speaker broadcasting the unattractive high-frequency signal, and the fauxcal sac was inflated synchronously with the call. The opposite speaker broadcasts the average frequency with no robofrog. Call attractiveness was determined from our previous unimodal phonotaxis tests.

Statistical analyses

We analyzed six different call properties from live males using AviSoft software. Frequencies (low peak, high peak, fundamental, and dominant) were automatically analyzed by the software package for each call recording. We measured call and pulse durations manually. Call analyses and morphometric statistics were calculated using the descriptive statistics function in the Microsoft Excel Data package. We conducted regression analyses (size vs. frequency) using SigmaPlot 13.0 (Systat Software, Inc. San Jose, CA). In all cases, females were tested only once per experiment. Female mate preferences were tested for significance with a binomial test using an expected probability distribution of 0.5. Calculations were made using the SISA-Binomial calculator (Uitenbroek 1997).

Results

Call analyses

Male advertisement calls (Fig. 1b) had a mean duration of 153 ms ± 35 SD (*n* = 64). Calls were composed of 38.11 (±6.08 SD) pulses and each pulse had a mean duration of 3.50 ms ± 0.52 SD (*n* = 64). As typical for the species, there were two primary energy bands with stacks of lower-level harmonic energy between the bands. The dominant frequency was typically the higher spectral band (93.75 % of the calls), and the lower energy band was 11.34 dB lower than the dominant on average. We correlated the low-frequency peak with both SVL and mass and found significant negative relationships (SVL: $r^2 = 0.182$, $p < 0.001$, $n = 64$; mass: $r^2 = 0.238$, $p < 0.001$, $n = 64$; Fig. 2). Larger males had lower-frequency advertisement calls with respect to the low-frequency component of the call. The dominant frequency peaks (high-frequency band) were not significantly influenced by increasing frog size (SVL: $r^2 = 0.007$, $p = 0.501$, $n = 64$; mass: $r^2 = 0.012$, $p = 0.382$, $n = 64$). The average call rate was 68.40 calls per minute ±16.83 SD (*n* = 64) (Table 2).

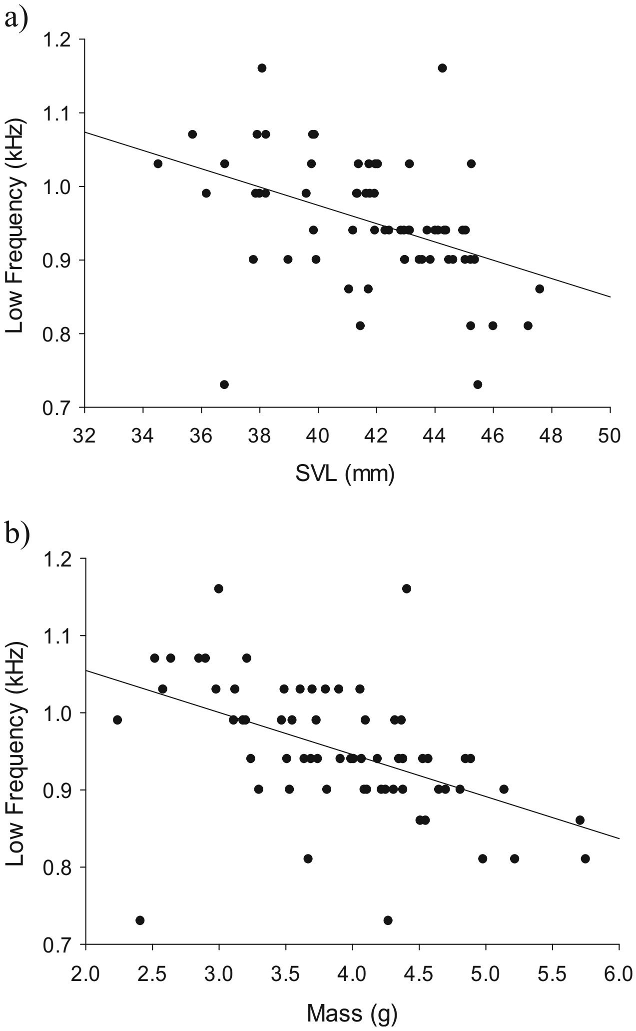

Fig. 2 Correlation between low-frequency band of male advertisement calls, **a** SVL ($p < 0.001$) and **b** mass ($p < 0.001$)

Unimodal phonotaxis

Females preferred faster call rates in two-choice tests. The fast call rate was chosen by 23 females as opposed to the slow call rate, selected by seven females ($p = 0.005$, $n = 30$). Similarly, the average call rate was preferred over the slow call rate 22:8 ($p = 0.016$, $n = 30$). There was no preference for a medium vs. fast call rate ($p = 0.473$, $n = 30$; Fig. 3a).

Table 2 Call data recorded from males at the Vienna, MD, field site

Parameter	Mean	±1 SD	Range	N
Lower-frequency peak (kHz)	0.95	0.09	0.73–1.16	64
Higher-frequency peak (kHz)	3.05	0.33	1.16–3.48	64
Call rate (calls/min)	68.40	16.83	33.15–108.60	64
Call duration (ms)	153.0	35.0	100.0–260.0	64
Number of pulses	38.11	6.08	21–49	64
Pulse duration (ms)	3.50	0.52	2.70–5.40	64

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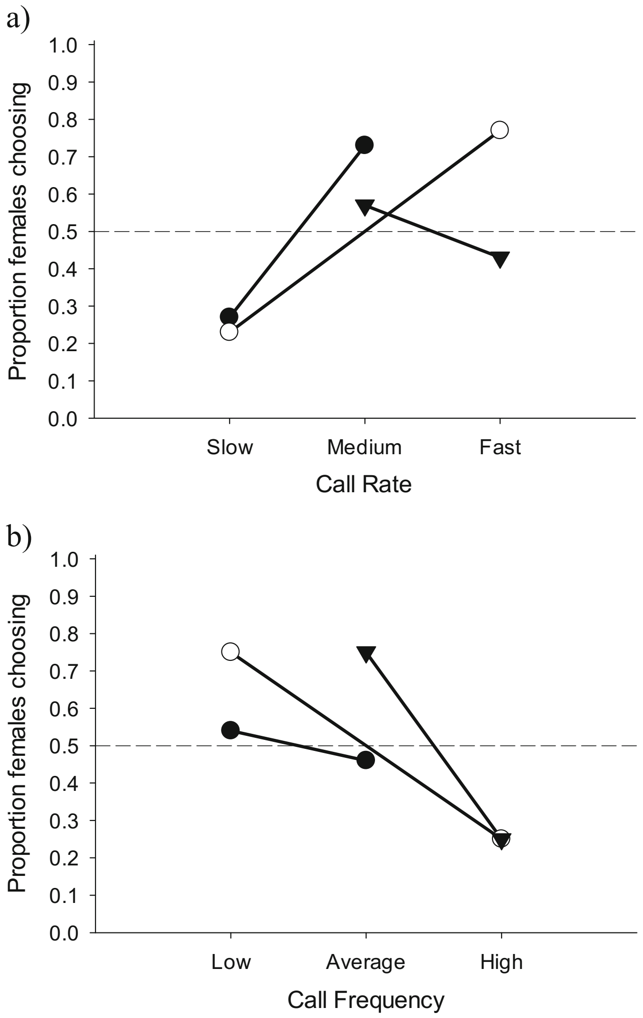

Fig. 3 Preference function of female choice (proportion choosing) of unimodal stimuli, **a** call rate and **b** call frequency. The *line connecting two points* represents choice between a particular stimulus pair; *steeper lines* indicate stronger preferences. The *horizontal dashed line* indicates random choice

In both average- vs. high-frequency and low- vs. high-frequency experiments, females preferred the lower frequency 18:6 ($p = 0.023$, $n = 24$). No preference was detected for low- vs. average-frequency calls ($p = 0.839$, $n = 24$; Fig. 3b).

Multimodal phonotaxis

Females expressed a significant preference for the multimodal stimulus compared to the same vocalization alone. The robofrog/acoustic signal was preferred by 19 females, whereas only five females chose the unimodal stimulus ($p = 0.007$, $n = 24$; Fig. 4a).

When the robofrog was paired with an unattractive high-frequency call, females preferred the unimodal, average-frequency call over the multimodal stimulus 16:4 ($p = 0.012$, $n = 20$; Fig. 4b). Thus, the addition of the robotic frog failed to increase the relative attractiveness of a high-frequency call.

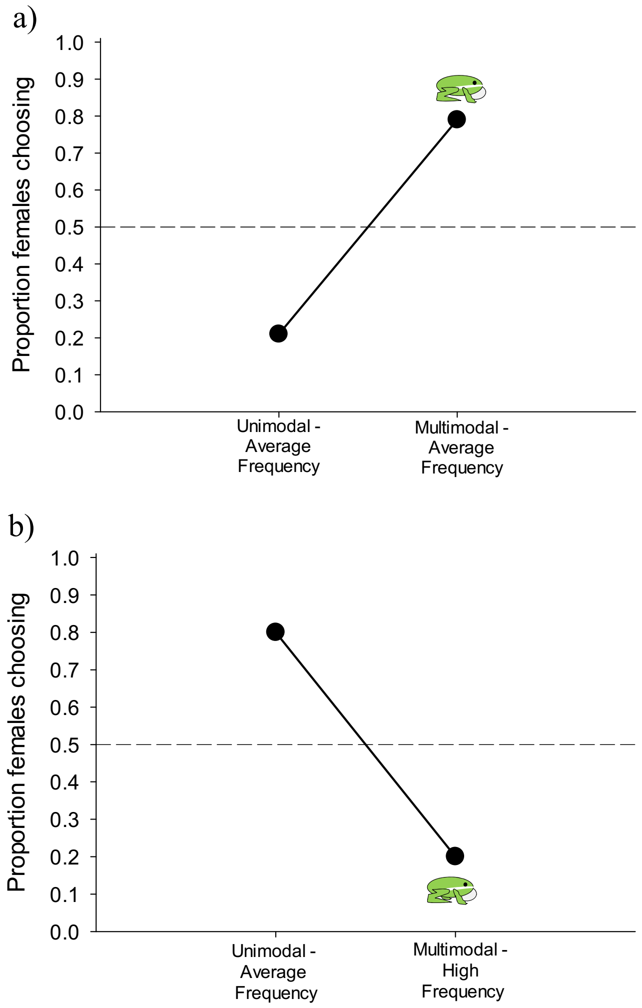

Fig. 4 Preference functions of female choice (proportion choosing) of multimodal stimuli, **a** modality and **b** signal weighting (average vs. high frequency). The *line connecting two points* represents choice between a stimulus pair. The *horizontal dashed line* represents random choice and *frog image* denotes robofrog stimulus

Discussion

In this study, we characterized the advertisement call properties of a northern population of green tree frog and investigated female preference for both unimodal and multimodal signals. We show for the first time in this species that the visual display is an important component of the courtship signal and also delineate some of the limits to its integration in the acoustic signal. In animal communication, a distinction is often made between cues (stimuli that did not evolve for the purpose of communication) and signals (stimuli that did evolve for the purpose of communication) (Bradbury and Vehrencamp 1998). In frogs, the vocal sac evolved originally to increase calling efficiency, not for visual communication (Pauly et al. 2006). A body of evidence now indicates that in many species, the vocal sac movement has been perceptually incorporated into the courtship system of frogs as a visual component

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(Narins et al. 2003; Hirschman and Hödl 2006; Taylor and Ryan 2013; Starnberger et al. 2014a, b). In addition, the relatively large size of the anuran vocal sac has been hypothesized to act as a cavity resonator, amplifying the call or lowering frequency. In at least one species, the vocal sac was shown not to act as a cavity resonator; thus, the large size of the vocal sac does not explain acoustic signal output (Rand and Dudley 1993; Ryan and Guerra 2014). In light of this evidence, we refer to the visual aspect as a "component" of the signaling system, or more simply a multimodal signal. This reflects the current utility of the visual stimulus to modulate a receiver response, irrespective of the evolutionary origin or intent (sensu Wiley 1994).

In phonotaxis tests, females expressed a preference for call frequencies near the population average or lower. Females did not express a preference for the lower-frequency call over the average, but did discriminate against the high-frequency call when any lower frequency was available. This suggests that females do not make fine-scale discriminations among male size; instead, only the smallest males in the population may be at a selective disadvantage. A similar pattern was also seen in the squirrel tree frog (*Hyla squirella*), a closely related hylid (Taylor et al. 2007). In our unimodal tests, green tree frog females also preferred call rates at and above the population mean. This pattern is consistent with a southern population of green tree frogs (Gerhardt 1987). The preference was also similar to other frog and insect species (Gerhardt and Huber 2002; Greenfield and Schul 2008).

When a robofrog was employed as a visual display, the multimodal signal was favored over the acoustic, unimodal signal. All else being equal, this suggests that calling males that are visually accessible increase their probability of attracting a mate. At our Vienna field site, males often ascend cattail (*Typha* sp.) stems and call from these visually exposed sites. Some males call from more protected sites within dense vegetation; thus, choice of call site influences visual accessibility and probability of mate attraction. Females did not, however, prefer the multimodal stimulus of the robofrog/high-frequency call, when a lower-frequency call was available. In green tree frogs, male chorus attendance and the concomitant level of acoustic interference is variable. On some nights, only a small number of males attend the chorus; on others, attendance is high. Our data show that in relatively simple listening environments where call overlap is minimal, the visual component does not increase the relative attraction value of a high-frequency call (small male). This suggests that the visual component of the signal is not a traditional sexually selected ornament. Instead, the visual cue has likely been integrated into the acoustic communication system as an additional mechanism to improve signal detection or localization, analogous to human lip reading in noisy environments (Sumby and Pollack 1954; Rowe 1999; Taylor and Ryan 2013).

Multimodal robofrog signal weighting experiments have been conducted on two other frog species, the closely related squirrel tree frog (*H. squirella*) and the distantly related túngara frog (*Physalaemus pustulosus*); both of these frogs prefer the multimodal signal to the acoustic-only signal. When túngara frog females were presented with a robofrog coupled to a relatively unattractive call, the robofrog did not enhance the attractiveness of the unattractive call (Taylor et al. 2011). Interestingly, this contrasts with what Taylor et al. (2007) found in the more closely related squirrel tree frog. In squirrel tree frogs, coupling a model male frog (visual stimulus) to an unattractive call increased preference for the unattractive call. This suggests that female squirrel tree frogs are flexible in their mate preferences with regard to weighting acoustic vs. visual signal components. The data presented in this study suggest that female green tree frogs may not be as flexible with respect to multimodal signal components. It is important to note, however, that these studies are not directly comparable. Taylor et al. (2007) coupled the visual stimulus to a slower call rate, with frequency held constant. In the present study, the robofrog was coupled to a higher-frequency call, while rate was held constant. Whether or not female green tree frogs exhibit flexibility across call rates remains to be tested.

Another interesting question is what specific feature(s) of the visual display are salient in this communication system. A large body of data suggests that the inflating vocal sac is the relevant component. In frogs, motion is necessary for eliciting behaviors ranging from foraging to courtship. For example, Lettvin et al. (1959) reviewed the importance of motion and visual physiology with respect to foraging and escape behavior. Later work has detailed the importance of the vocal sac in eliciting appropriate courtship responses in a variety of species (Taylor et al. 2008; Preininger et al. 2013; Starnberger et al. 2014a, b). These data suggest that the vocal sac is also likely an important feature of the green tree frog visual display. The strongly contrasting lateral stripe, however, could also play an important role and remains to be tested in future work.

Lek-breeding anurans are often faced with the challenge of communicating in noisy environments. Several species of frog have been shown to exhibit significant reductions in signal discrimination ability when the playbacks are conducted in the presence of background noise (Gerhardt and Klump 1988; Wollerman 1999; Schwartz et al. 2001). In a series of detailed studies (Ward et al. 2013; Caldwell and Bee 2014; Caldwell et al. 2014; Schrode and Bee 2015), gray tree frogs (*Hyla chrysoscelis*) have been shown to employ a variety of mechanisms to improve auditory object formation in noisy choruses (e.g., spatial, temporal, spectral segregation, dip listening); green tree frogs and other hylids also employ similar techniques (see Bee 2012 for review). Unlike gray tree frogs, however, green tree frogs do not seem to employ dip listening as a mechanism of improving signal detection and discrimination (Vélez et al. 2012). One reason for this could be an

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increased reliance on visual cues. Gray tree frogs generally lack the conspicuous contrasting color pattern possessed by both green and squirrel tree frogs. Almost 74 % of female squirrel tree frogs preferred a model with a large labial and lateral stripe over an alternative small stripe model (Taylor et al. 2007). Green tree frogs possess an even more conspicuous lateral stripe than do squirrel tree frogs, indicating that this may be an important visual signal component. The difference in color pattern and auditory processing mechanisms between gray and green tree frogs strongly suggests that even these closely related species have evolved different mechanisms for solving cocktail party-like problems. However, very little is known about the use of vision in gray tree frog courtship; additional work is still needed (but see Reichert et al. 2014). The current data for multimodal preferences and auditory perception across four species (green tree frog, gray tree frog, squirrel tree frog, and túngara frog) indicate that there are multiple strategies for how anurans solve difficult acoustic communication tasks. This offers exciting possibilities for additional comparative studies and such comparisons will likely provide important insights into how evolution has solved signal detection problems across species.

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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 Salisbury University guidelines and approved by the SU Institutional Animal Care and Use Committee (Protocol # SU 0036).

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

References

Akre KL, Ryan MJ (2010) Complexity increases working memory for mating signals. Curr Biol 20:502–505

Baldwin J, Johnsen S (2009) The importance of color in mate choice of the blue crab *Callinectes sapidus*. J Exp Biol 212:3762–3768

Bee MA (2012) Sound source perception in anuran amphibians. Curr Opin Neurobiol 22:301–310

Bee MA (2015) Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. Int J Psychophysiol 95:216–237

Bee MA, Micheyl C (2008) The "cocktail party problem": what is it? How can it be solved? And why should animal behaviorists study it? J Comp Psychol 122:235–251

Bradbury JW, Vehrencamp SL (1998) Principles of animal communication. Sinauer, Sunderland, MA

Bronkhorst AW (2000) The cocktail party phenomenon: a review of research on speech intelligibility in multiple-talker conditions. Acustica 86:117–128

Buerkle NP, Schrode KM, Bee MA (2014) Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). Comp Biochem Physiol A 178:68–81

Caldwell MS, Bee MA (2014) Spatial hearing in Cope's gray treefrog: I. Open and closed loop experiments on sound localization in the presence and absence of noise. J Comp Physiol A 200:265–284

Caldwell MS, Lee N, Schrode KM, Johns AR, Christensen-Dalsgaard J, Bee MA (2014) Spatial hearing in Cope's gray treefrog: II. Frequency-dependent directionality of the amplitude and phase of tympanum vibrations. J Comp Physiol A 200:285–304

Cherry EC (1953) Some experiments on the recognition of speech, with one and with two ears. J Acoust Soc Am 25:975–979

Coleman SW, Patricelli GL, Borgia G (2004) Variable female preferences drive complex male displays. Nature 428:742–745

Fain GL, Matthews HR, Cornwall MC, Koutalos Y (2001) Adaptation in vertebrate photoreceptors. Physiol Rev 81:117–151

Farris HE, Ryan MJ (2011) Relative comparisons of call parameters enable auditory grouping in frogs. Nat Commun 2:410

Farris HE, Rand AS, Ryan MJ (2002) The effects of spatially separated call components on phonotaxis in túngara frogs: evidence for auditory grouping. Brain Behav Evol 60:181–188

Gerhardt HC (1987) Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. Anim Behav 35:1479–1489

Gerhardt HC, Höbel G (2005) Mid-frequency suppression in the green treefrog (*Hyla cinerea*): mechanisms and implications for the evolution of acoustic communication. J Comp Physiol A 191:707–714

Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans: common problems and diverse solutions. University of Chicago Press, Chicago

Gerhardt HC, Klump GM (1988) Masking of acoustic signals by the chorus background noise in the green tree frog: a limitation on mate choice. Anim Behav 36:1247–1249

Greenfield MD, Schul J (2008) Mechanisms and evolution of synchronous chorusing: emergent properties and adaptive functions in *Neoconocephalus* katydids (Orthoptera: Tettigoniidae). J Comp Psychol 122:289–297

Guilford T, Dawkins MS (1991) Receiver psychology and the evolution of animal signals. Anim Behav 42:1–14

Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol 57:197–214

Hebets EA, Cuasay K, Rivlin PK (2006) The role of visual ornamentation in female choice of a multimodal male courtship display. Ethology 112:1062–1070

Hebets EA, Stafstrom JA, Rodriguez RL, Wilgers DJ (2011) Enigmatic ornamentation eases male reliance on courtship performance for mating success. Anim Behav 81:963–972

Hicks RE, Larned A, Borgia G (2013) Bower paint removal leads to reduced female visits, suggesting bower paint functions as a chemical signal. Anim Behav 85:1209–1215

Hirschman W, Hödl W (2006) Visual signaling in *Phrynobatrachus krefftii* Boulenger, 1909 (Anura: Ranidae). Herpetologica 62:18–27

Höbel G (2010) Interaction between signal timing and signal feature preferences: causes and implications for sexual selection. Anim Behav 79:1257–1266

Hödl W, Amézquita A (2001) Visual signaling in anuran amphibians. In: Ryan MJ (ed) Anuran communication. Smithsonian Institution Press, Washington DC, pp. 121–141

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Klein BA, Stein J, Taylor RC (2012) Robots in the service of animal behavior. Commun Integr Biol 5:466–472

Lea AL, Ryan MJ (2015) Irrationality in mate choice revealed by túngara frogs. Science 349:964–966

Lettvin JY, Maturana HR, McCulloch WS, Pitts WH (1959) What the frog's eye tells the frog's brain. P IRE 47:1940–1951

Lombardo SR, Mackey E, Tang L, Smith BR, Blumstein DT (2008) Multimodal communication and spatial binding in pied currawongs (*Strepera graculina*). Anim Cogn 11:675–682

Macedonia JM, Clark DL, Riley RG, Kemp DJ (2013) Species recognition of color and motion signals in *Anolis grahami*: evidence from responses to lizard robots. Behav Ecol 24:846–852

McLennan DA (2003) The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. Biol J Linn Soc 80:555–572

Narins PM, Hödl W, Grabul DS (2003) Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. Proc Natl Acad Sci U S A 100:577–580

Nityananda V, Bee MA (2011) Finding your mate at a cocktail party: frequency separation promotes auditory stream segregation of concurrent voices in multi-species frog choruses. PLoS One 6:e21191

Oldham RS, Gerhardt HC (1975) Behavioral isolating mechanisms of the treefrogs *Hyla cinerea* and *H. gratiosa*. Copeia 1975:223–231

Partan S, Marler P (1999) Communication goes multimodal. Science 283: 1272–1273

Partan SR, Larco CP, Owens MJ (2009) Wild tree squirrels respond with multisensory enhancement to conspecific robot alarm behaviour. Anim Behav 77:1127–1135

Patricelli GL, Krakauer AH (2009) Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. Behav Ecol 21:97–106

Pauly GB, Bernal XE, Rand AS, Ryan MJ (2006) The vocal sac increases call rate in the túngara frog *Physalaemus pustulosus*. Physiol Biochem Zool 79:708–719

Preininger D, Boeckle M, Freudmann A, Starnberger I, Sztatecsny M, Hödl W (2013) Multimodal signaling in the small torrent frog (*Micrixalus saxicola*) in a complex acoustic environment. Behav Ecol Sociobiol 67:1449–1456

Rand AS, Dudley R (1993) Frogs in helium: the anuran vocal sac is not a cavity resonator. Physiol Zool 66:793–806

Reichert MS, Galante H, Höbel G (2014) Female gray treefrogs, *Hyla versicolor*, are responsive to visual stimuli but unselective of stimulus characteristics. J Exp Biol 217:3254–3262

Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. Anim Behav 58:921–931

Rundus AS, Owings DH, Joshi SS, Chinn E, Giannini N (2007) Ground squirrels use an infrared signal to deter rattlesnake predation. Proc Natl Acad Sci U S A 104:14372–14376

Ryan MJ (2001) Anuran communication. Smithsonian Institution Press, Washington DC

Ryan MJ, Guerra MA (2014) The mechanism of sound production in túngara frogs and its role in sexual selection and speciation. Curr Opin Neurobiol 28:54–59

Schrode KM, Bee MA (2015) Evolutionary adaptations for the temporal processing of natural sounds by the anuran peripheral auditory system. J Exp Biol 218:837–848

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

Starnberger I, Preininger D, Hödl W (2014a) The anuran vocal sac: a tool for multimodal signaling. Anim Behav 97:281–288

Starnberger I, Preininger D, Hödl W (2014b) From uni- to multimodality: towards an integrative view on anuran communication. J Comp Physiol A 200:777–787

Sumby WH, Pollack I (1954) Visual contribution to speech intelligibility in noise. J Acoust Soc Am 26:212–215

Taylor RC, Ryan MJ (2013) Interactions of multisensory components perceptually rescue túngara frog mating signals. Science 341: 273–274

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 BA, 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, Ryan MJ (2011) Inter-signal interaction and uncertain information in anuran multimodal signals. Curr Zool 57: 153–161

Uitenbroek DG (1997) SISA Binomial. D.G. Uitenbroek, Southampton, http://www.quantitativeskills.com/sisa/distributions/binomial.htm.

Vélez A, Bee MA (2011) Dip listening and the cocktail party problem in grey treefrogs: signal recognition in temporally fluctuating noise. Anim Behav 82:1319–1327

Vélez A, Höbel G, Gordon NM, Bee MA (2012) Dip listening or modulation masking? Call recognition by green treefrogs (*Hyla cinerea*) in temporally fluctuating noise. J Comp Physiol A 198:891–904

Ward JL, Buerkle NP, Bee MA (2013) Spatial release from masking in a biologically relevant temporal pattern discrimination task in gray treefrogs. Hear Res 306:63–75

Wilczynski W, Rand SA, Ryan MJ (1999) Female preferences for temporal order of call components in the túngara frog: a Bayesian analysis. Anim Behav 58:841–851

Wiley RH (1994) Errors, exaggeration, and deception in animal communication. In: Real L (ed) Behavioral mechanisms in evolutionary ecology. University of Chicago Press, Chicago, pp. 157–189

Wiley RH (2015) Noise matters: the evolution of communication. Harvard University Press, Cambridge, MA

Wollerman L (1999) Acoustic interference limits call detection in a neotropical frog *Hyla ebraccata*. Anim Behav 57:529–536