Biological Journal of the Linnean Society, 2024, **XX**, 1–12
https://doi.org/10.1093/biolinnean/blae060
Advance access publication 29 June 2024
Original Article

Original Article

Visual cues do not function in a multimodal signalling context for mate attraction in eastern gray treefrogs

Guilherme Augusto-Alves[1,2,3,*], Olivia S. Feagles[3], Luís Felipe Toledo[1], Gerlinde Höbel[3]

[1]Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Unicamp, Campinas, São Paulo, 13083-862, Brazil

[2]Programa de Pós-Graduação em Ecologia, Instituto de Biologia, Unicamp, Campinas, São Paulo, 13083-862, Brazil

[3]Department of Biological Sciences, Behavioral and Molecular Ecology Group, University of Wisconsin-Milwaukee, Lapham Hall, 3209 North Maryland Avenue, Milwaukee, WI 53201-0413, USA

[*]Corresponding author. Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Unicamp, Campinas, São Paulo, 13083-862, Brazil. E-mail: alves.guilherme.augusto@gmail.com

ABSTRACT

Anurans use different sensory modalities to communicate and interact socially, including acoustic, chemical, tactile, and visual signals. In a multimodal context, these sensory modes can transmit different information to the receiver or even reinforce the same message. In this study, we hypothesized that body colour traits and vocal sac movements of male eastern gray treefrogs [*Dryophytes versicolor* (= *Hyla versicolor*)] serve as secondary visual cues that increase their attractiveness while calling to attract mates. We tested this hypothesis using playback trials combining synthetic advertisement calls with a variety of visual cues, including differently coloured resin frog models, simulated vocal sacs, and live frogs. None of the visual stimuli tested impacted female choice, and there was no difference in response rate, response time, choice angle, or distance covered in the arena between multimodal and unimodal stimuli. However, females showed a strong preference for longer calls even if the less attractive calls were paired with a visual stimulus. The study provides comprehensive insights into the role of visual stimuli in the mate attraction of eastern gray treefrogs and suggests that call traits play a dominant role in the male-selection process of this species, contrary to what was observed for visual stimuli.

Keywords: body coloration; *Hyla versicolor*; multicomponent signal; sexual selection; two-alternative trials; vocal sac

INTRODUCTION

In the last few decades, multimodal communication, also termed multisensory communication/signalling, has received significant attention from behavioural ecologists (Møller and Pomiankowski 1993, Candolin 2003, Coss *et al.* 2022), in no small part because of the intriguing potential of providing receivers with either redundant or different information in different sensory modalities (e.g. 'back-up signals' vs. 'multiple messages'; Johnstone 1996). Animals can use a range of sensory modalities to mediate social interactions (i.e. acoustic, chemical, electric, tactile, seismic/vibrational, and visual), but the combination of acoustic and visual cues is particularly common. It involves the emission of vocalizations coupled or alternated with stereotyped gestures/movements, in addition to the transmission of visual information through body colour or postures (Grafe and Wanger 2007, Ligon *et al.* 2018, Ferrer *et al.* 2021, Augusto-Alves *et al.* 2024).

Anurans are a valuable model system for conducting research on multimodal communication. Frogs communicate mainly by sounds, using their vocal repertoire to attract females and repel rival males (Toledo *et al.* 2015, Köhler *et al.* 2017), but many species also display visual signals (Hartmann *et al.* 2005, Giasson and Haddad 2006, Hirschmann and Hödl 2006). The emission of calls coupled or alternated with visual displays is commonly associated with diurnal species (Haddad and Giaretta 1999, Narins *et al.* 2005, Furtado *et al.* 2019). However, frogs possess well-adapted vision for low-light conditions, including the ability for colour discrimination (Kelber *et al.* 2017, Yovanovich *et al.* 2017, Robertson *et al.* 2022), and visual displays are also well documented for nocturnal species (Toledo *et al.* 2007, Barros and Feio 2011, Augusto-Alves *et al.* 2018). For example, females of *Scaphiopus couchii* Baird, 1854 can assess size and body condition of males based on dorsal colour traits, and they tend to prefer brighter males (Vásquez and Pfennig 2007). Furthermore,

---

Received 25 March 2024; revised 10 June 2024; accepted 10 June 2024

most frogs inflate and deflate their vocal sac during call emission, *de facto* generating a 'fixed' multimodal (audiovisual) signal that might provide additional visual and even olfactory stimulation (Smith 1977, Starnberger *et al.* 2014, Augusto-Alves and Toledo 2022).

Dryophytes versicolor (Cope, 1880), the eastern gray treefrog, is a common nocturnal species widely distributed in the eastern USA and Southern Canada (Frost 2023). During the breeding season, males aggregate and form choruses around small ponds, where females search for mates (Schwartz *et al.* 2001, 2002, Reichert *et al.* 2014). Females attend more to longer calls, i.e. calls with more pulses (Gerhardt *et al.* 2000, Reichert and Höbel 2015, Feagles and Höbel 2022). In this species, males have darker throats than females (i.e. they are sexually dimorphic). The colour of the vocal sac (measured deflated) is related to male body condition, and the vocal sac colour of mated males is darker when compared with unmated males (Höbel *et al.* 2022). Furthermore, males emit more advertisement calls when they are able to see an approaching female, indicating that they attend to visual cues (Reichert 2013). In contrast, both males and females exhibit sexually monomorphic colour traits, such as yellow coloration in the femoral and flank regions. Additionally, males and females have a high capacity for colour change (Edgren 1954), and individuals can change their dorsal colour between grey, green, and brown (G. Höbel, pers. obs.).

In the present study, we have investigated the role of various visual cues in the mate-choice process of *D. versicolor*. We used frog-like models with various colour traits (dorsal colour, throat colour, and thigh colour) and synthetic advertisement calls in two-choice trials with females. We predicted that *D. versicolor* females would prefer multimodal signals (average call + visual signals) over a unimodal signal (average call alone). We also tested whether females showed a preference for dorsal colour (grey, green, or brown), vocal sac coloration (black and white patterns), and thigh coloration (presence of yellow patches or painted with the same brown as a continuation of the dorsal pattern). Lastly, we tested whether visual stimulation (presence of a model) would increase acceptance by females of shorter, less attractive calls.

MATERIALS AND METHODS

Study site and data sampling

We conducted the study at a pond located at the University of Wisconsin-Milwaukee's (UWM) field station in Saukville, WI, USA (43°23′10″N, 88°01′57″W). Sampling was performed from 10 May to 9 June 2022. We collected 72 *D. versicolor* females, all of which were in amplexus, to ensure that they were sexually receptive. The collected females were transported to the laboratory and kept individually in boxes (~10 cm × 10 cm × 7 cm), which were placed inside coolers with melting ice to decrease metabolism and postpone oviposition. Females that laid eggs before testing were excluded from the trials. Females were tested within 3 days after capture, then released in the collection site.

Preparation of stimuli

We used frog-like models (except for experiment 4) and synthetic calls to standardize the traits of the stimuli, including body size and colour, and the spectral and temporal properties of calls. Using live individuals for visual stimulation might introduce confounding factors, such as chemical cues and uncontrolled movements, potentially biasing the results.

Synthetic calls

Male *D. versicolor* produce pulsed advertisement calls comprising a series of short pulses with a duration of ~25 ms (at 20°C) that are repeated after a pause of 25 ms. At a given temperature, the duration of the call can be expressed in milliseconds or as the number of pulses (used here). Females prefer calls of longer duration (Reichert and Höbel 2015, Feagles and Höbel 2022). To create synthetic *D. versicolor* advertisement calls, we used the SEEWAVE package (Sueur *et al.* 2008) in R v.3.1.0 (R Core Team 2014). We created four calls varying in duration, hence in attractiveness (6, 15, 18, and 21 pulses). These values cover much of the range of call durations observed in our study population (Reichert and Höbel 2015). All other call characteristics were maintained at the population average: length of pulse = 25 ms, pulse period = 25 ms, call period = 7750 ms, low-frequency peak (first visible harmonic band) = 1071 Hz, and high-frequency peak (second visible harmonic band) = 2142 Hz (which is 10 dB louder in low frequency) (Reichert and Höbel 2015).

Frog-like model

Using living and preserved frogs for reference, we constructed a clay model (Sculpey Oven-Bake Clay) of a calling male *D. versicolor*. Subsequently, we used the clay model to create a silicone mould, from which we produced all resin frog models used in the experiments. Given that eastern gray treefrogs exhibit substantial ability to change colour, we selected numerous paint colours reflecting the most common dorsal displays. Dorsal coloration can vary from light grey to different shades of brown and green, with brown being the most common and grey the least common nocturnal colour (G. Höbel, pers. obs). Both sexes have yellow groins and thighs (i.e. they are sexually monomorphic), and the vocal sac of mature males darkens and can range from light grey to almost black (Höbel *et al.* 2022). To select the paint colours for the frog models, we used an Ocean Optics USB 2000+ spectrometer to measure reflectance spectra of the dorsal surface of live male frogs. We measured 10 males each that were grey, brown, or green, in addition to the thigh and throat coloration of 10 of these frogs. Then we sampled a large number of colour swatches from a Sherwin Williams Paint booklet until we found paint colours that matched the frog colours: green (Sherwin Williams #146-C3), brown (#209-C5), grey (#239-C2), yellow (#131-C4), and black (#237-C7) (Supporting Information, Fig. S1). To avoid model biases, we produced two models for each stimulus tested and randomly exchanged them during the trials.

Vocal sac device

To test whether vocal sac movement is used as a visual cue, we built a device that simulated male vocal sac inflation and deflation during calling activity. The device consisted of a square wooden base painted with black paint. A small inflatable white balloon was attached to this base, and this balloon was linked to a clear silicone tube. On the other side of this tube, we coupled a plastic syringe. During the trials (experiment 12, see below), we manually pressed and depressed the syringe, inflating and deflating the balloon upwards whenever the speaker broadcast a call. The volume of the inflated balloon was visually comparable to that of the vocal sacs of males. Given that the models could present several different colour stimuli to females (i.e. dorsal and thigh colour patterns), we opted not to include frog models associated with the balloon in this trial. In this way, we could isolate the response that only the vocal sac movement, as a visual cue, would provoke in female preference. For the same reason, we used a white balloon instead of a black one, testing only the influence of movement as a visual cue and not including the black colour as a possible visual signal. The black wooden base had the same colour as the arena background, not generating any stimulus to females.

Live males

We ran one experiment using live males (experiment 4) to observe whether the females responded in a similar manner to experiments with resin models and real males. To prevent these males from emitting calls, we used cold males (from the same ice-filled coolers we used to store females awaiting testing). We took the males out of the cooler 3 min before the trials, meaning that they could assume a natural sitting posture but not yet emit calls. In the arena, the males were placed inside a transparent container (~5 cm × 5 cm × 5 cm). An equal but empty container was placed on the opposite side of the test arena to prevent biases. These transparent containers were used to prevent movement of the live frogs; consequently, they were not used in trials that involved resin models.

Experimental design

Experiments took place in a semi-anechoic room containing a circular arena 2 m in diameter (Supporting Information, Fig. S2). This arena was made of wire fencing covered by a black fabric, with dark grey foam exercise mats on the floor. A circular wire container (9 cm in diameter) was placed in the centre of the arena as the release point, where females were placed at the beginning of each trial. The temperature in the arena was maintained at 20°C ± 1°C. The arena was dimly illuminated with an incandescent light bulb dimmed to a light intensity of 1 lux; light level was monitored using a Extech EasyView EA31 Digital Light Meter. Owing to variations in moon phase, cloud cover, vegetation around calling perches, etc., nocturnal light levels are characterized by significant fluctuations (Li *et al.* 2022). This makes it challenging to pinpoint the 'most appropriate' light level for experimental trials. Previously, studies with *D. versicolor* (i.e. same study species and same population) have shown that call preferences, including preference functions and choosiness for longer calls, are unaffected by variation in nocturnal light levels (Underhill and Höbel 2017) or even anthropogenic light pollution (Underhill and Höbel 2018). In light of the robustness of acoustic preferences to variation in light levels, we opted to supply somewhat increased light rather than risk the elimination of potential effects of visual cues owing to insufficient light.

The two alternative stimuli were presented with an angle of 90° between them. For acoustic stimulation, we placed two speakers outside the arena wall, and for visual stimulation the models, live males, or the vocal sac device, were placed inside the arena in front of the speaker (Supporting Information, Fig. S2). Where applicable, the models were positioned at a 45° angle (from the perspective of the release point), allowing females to access dorsal, flank, and vocal sac colour traits visually. In all trials, we offered two different stimuli for females (Table 1). During the trials, the calls were broadcast alternately between the stimuli. All acoustic stimuli had the same spectral properties and were presented at an amplitude of 85 dB SPL, measured from the release point of the females, at the centre of the arena (verified using a sound level meter; Extech Instruments; C-weighting, fast RMS). The call duration (i.e. number of pulses) was mostly set to 18 pulses (average of the study population), except for trials that tested for an interaction between call attractiveness and the presence of a multimodal cue (experiments 5 and 6) (Table 1).

Before each trial and after temperature acclimation (20°C), the female was placed at the release point and presented with three calls in each speaker (always randomizing the start of the stimulus), before the start of each trial. The same female had a 5 min resting period between trials. Rest periods of 2–5 min duration between trials 'erases' memory of the prior trial in treefrogs (Boyd and Gordon 2021). The order of the trials (experiments 1–12) and the position of the stimuli were established randomly and changed with each female tested. In all trials, we scored a choice when the female arrived in a demarcated area in front of the model/speaker (Supporting Information, Fig. S2). Each trial had a maximum duration of 5 min. Females that did not respond in a trial were tested again after a 5 min rest period. Females that did not respond during a second attempt were considered unreceptive [nine females (12.5%) stopped responding during the trials; additional information can be found in Supporting Information, Fig. S2, which contains the datasets generated by this study], and we stopped testing them in subsequent trials. On average (±SD) we tested 43.3 ± 2.7 (range 35–45) females per experiment.

Our study included 12 different experiments (Table 1), in which we explored the function of visual stimuli (presence/absence of males/frog-like models; dorsal, vocal sac, and thigh colour; and vocal sac movement) and acoustic stimuli (advertisement call with different numbers of pulses) in a unimodal or multimodal context. We did this by using frog-like models with different colour traits in two-choice trials with females, exploring differences in dorsal colours (grey, green, and brown), vocal sac coloration (black and white), and the presence of yellow patches on the thigh (yellow and painted with the same brown as a continuation of the dorsal pattern). The experiments were organized into five sets (Table 1).

Set 1: influence of the frog-like model/male presence as visual cue

In this set of experiments, we explored whether a multimodal signal elicits a stronger response than a unimodal one. For this, we paired frog-like models with different body colours (green, grey, or brown models; Fig. 1), with an average (18-pulse) call (multimodal stimulus), and presented it against an average (18-pulse) call (unimodal stimulus) (experiments 1–3). We also executed a trial in which the visual stimulus in the multimodal condition was a live male (experiment 4).

Set 2: attractive calls vs. unattractive calls in multimodal context

We tested whether visual stimulation (i.e. presence of the model) would increase the attraction of females to a less attractive (shorter) call. We tested this at two different levels of

Table 1. Description of the stimuli offered in each experiment, which were organized into sets according to the tested aims, with the number of females that responded to each stimulus in all experiments. The bold value represents a significant difference in the two-tailed binomial test between offered stimuli. As described in the main text, brown models were used for experiments 5, 6, and 10–12, because males with this colour are more common when they are in calling activity. Despite the stimuli being identified as 'a' and 'b', the sides were randomized during the trials to avoid any biases.

Experiment	Stimulus (context)	Female response (*N*)	*P*-value
1	a, green frog-like model + call 18 pulses (multimodal)	25	.451
	b, call 18 pulses (unimodal)	19	
2	a, grey frog-like model + call 18 pulses (multimodal)	27	.174
	b, call 18 pulses (unimodal)	17	
3	a, brown frog-like model + call 18 pulses (multimodal)	17	.222
	b, call 18 pulses (unimodal)	26	
4	a, live male + call 18 pulses (multimodal)	15	.761
	b, call 18 pulses (unimodal)	20	
5	a, call 18 pulses (unimodal)	43	**<.001**
	b, brown frog-like model + call 6 pulses (multimodal)	1	
6	a, call 21 pulses (unimodal)	28	.066
	b, brown frog-like model + call 15 pulses (multimodal)	15	
7	a, green frog-like model + call 18 pulses (multimodal)	23	1
	b, grey frog-like model + call 18 pulses (multimodal)	22	
8	a, grey frog-like model + call 18 pulses (multimodal)	24	.766
	b, brown frog-like model + call 18 pulses (multimodal)	21	
9	a, green frog-like model + call 18 pulses (multimodal)	20	.761
	b, brown frog-like model + call 18 pulses (multimodal)	23	
10	a, brown frog-like model with yellow thighs + call 18 pulses (multimodal)	16	.073
	b, brown frog-like model + call 18 pulses (multimodal)	29	
11	a, brown frog-like model with black throat + call 18 pulses (multimodal)	21	.88
	b, brown frog-like model with white throat + call 18 pulses (multimodal)	23	
12	a, vocal sac movement + call 18 pulses (multimodal)	21	.88
	b, call 18 pulses (unimodal)	23	

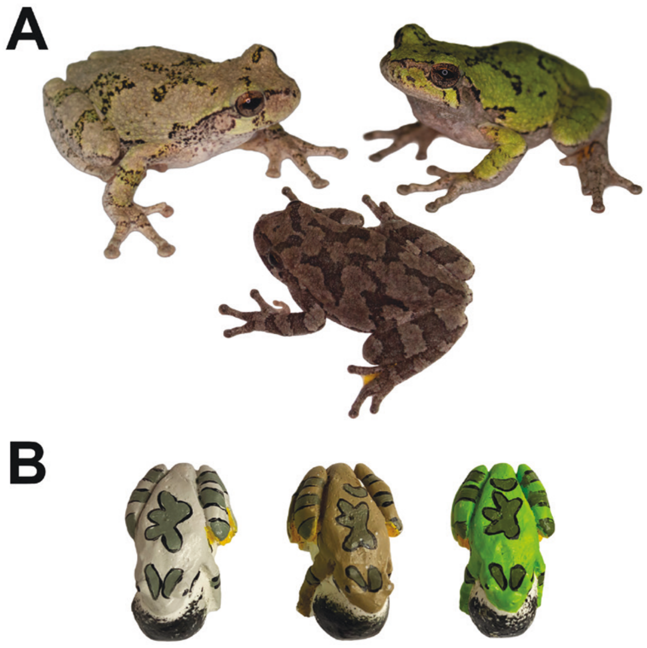

Figure 1. A, *Dryophytes versicolor* body colour patterns. All individuals were collected from the same pond at the University of Wisconsin-Milwaukee's (UWM) field station in Saukville, WI, USA. B, models painted based on the colour traits of the males.

variation: (i) unimodal 18 pulses vs. multimodal six pulses + model (experiment 5: 66.6% of variation between the acoustic stimuli); (ii) unimodal 21 pulses vs. multimodal 15 pulses + model (experiment 6: 28.6% of variation between the acoustic stimuli). In these test, we used only brown models, because brown males represent 60%–80% of the males found chorusing at the pond (G. Höbel, pers. obs.).

Set 3: female preference for dorsal colour

In this set of experiments, we tested whether the females show preferences for any specific dorsal colour among those observed in nature (green, grey, and brown; Fig. 1A), by offering the different colour pattern combinations in the two-choice trials (experiments 7–9; Table 1). In these tests, models were always presented in multimodal conditions, i.e. paired with a speaker broadcasting an 18-pulse call.

Set 4: thigh colour and vocal sac colour as visual cues

Other body colour portions might transmit information to conspecifics as secondary cues; therefore, we tested whether the yellow thigh or black throat, respectively, increased the response of females (experiments 10 and 11; Table 1). The dorsal colour of these frog models was always brown, and presented in multimodal conditions, i.e. paired with a speaker broadcasting an 18-pulse call.

Set 5: vocal sac movement as visual cue

The dynamic aspect of an inflating vocal sac might represent a particularly salient visual cue; therefore, we tested whether the presence of a simulated moving vocal sac would make the stimulus more attractive than a call alone (experiments 12; Table 1). Vocal sac models were presented in multimodal conditions, i.e. paired with a speaker broadcasting an 18-pulse call.

Statistical analyses

We used a two-tailed binomial test (computed online: https://www.graphpad.com/quickcalcs/binomial1/) to calculate whether the proportions of females choosing the two alternatives were significantly different. Additionally, for all experiments, given that the data were non-parametric, we used the Mann–Whitney *U* test to calculate whether the response latency (i.e. in seconds, from the moment we released the females up to the time of choice) differed between the alternatives for each experiment.

Our overall analyses were based on population averages (above). Because we had tested most females in a number of experiments, we also collated their colour choices and used logistic regressions to test whether a colour choice in one experiment was correlated with a colour choice in another experiment. This allowed us to examine whether a lack of population colour preference might hide individual but diverging colour preferences (i.e. if some individuals consistently preferred green, brown, or grey models, the population analysis would average out to no preferences). These tests were run using JMP PRO v.11.1.0.

For the first set of experiments (1–4), we video-recorded the trials with a camera placed directly above the release point of the females and with coverage of the entire arena. Following Bonachea and Ryan (2011), we extracted the frames from the videos, and from those image stacks we produced a single image using the ImageJ software (Schneider *et al.* 2012) with the Extended Depth of Field plugin (Forster *et al.* 2004). From this image, we measured the length of the approach path of the female. We used the Mann–Whitney *U* test to test for differences in path length. All Mann–Whitney *U* tests were run using R v.3.6.3 (R Core Team 2019).

From the stacked images, we also measured the angle at which the female left the release area. The multimodal stimulus was established as 0°, and the unimodal one was at 90° in the four experiments. We used the software Oriana v.4.02 (Kovach Computing Services, Pentraeth, Anglesey, UK) to calculate descriptive circular statistics. We provide the mean vector angle (μ) and the length of the mean vector (r) for each test. The length of the mean vector ranges from zero to one, where $r = 0$ indicates total uniform dispersion and $r = 1$ indicates concentrated dispersion in one direction.

Using the exit angles, we also tested whether the visual cue could generate a more directional component in the response of females. For this, we calculated the angular deviation from the straight path towards the chosen stimulus (multimodal or unimodal), then used *t*-tests to compare whether movement was more directional (i.e. smaller angular deviation) towards the multimodal stimuli. This approach accounts for the fact that 30 and 330 are different angles, but equally 'directional'.

By using both path length and angle analyses, we investigated whether the visual signal enhances sound source localization. This enhancement would be evident if females exhibit a more directional angle towards the multimodal option compared with the unimodal one and if they cover shorter distances in the arena when selecting the multimodal option.

Ethical note

Experiments were approved by the University of Wisconsin-Milwaukee's Institutional Animal Care and Use Committee (no. 19-20 #26) and adhered to the ASAB/ABS Guidelines for the ethical treatment of animals. We obtained permission from the Wisconsin Department of Natural Resources (Scientific Research License #SRLR-22-62) and from the landowners to enter their property. All frogs were released unharmed at the collection site.

RESULTS

Set 1: influence of the frog-like model/male presence as visual cue

Females did not prefer the multimodal stimulus. The presence of the models did not increase female response (two-tailed binomial: all $P > .174$; Fig. 2; Table 1). Furthermore, neither response latency (Mann–Whitney *U* test: all $P > .067$; Fig. 2) nor path length (Mann–Whitney *U* test: all $P > .214$; Fig. 2) differed between unimodal and multimodal alternatives. Analysis of approach angles showed that females jumped out of the release point in the direction of the chosen stimulus, independently of whether it was the multimodal stimulus or the unimodal one (i.e. the exit angle was directed towards 0° when females chose the multimodal stimulus and towards 90° when they chose the unimodal one; Fig. 2; Table 2). The analysis of angular deviation from the straight path showed that females were not more directional when approaching a multimodal cue than when approaching a unimodal one (angular deviation: all $P > .21$; Table 2).

The trial that used a live male instead of a frog model as the visual cue showed similar results (experiment 4). The presence of the male did not increase the proportion of females responding to the multimodal alternative (two-tailed binomial: $P > .761$; Fig. 2; Table 1), and response latency did not differ between the two alternatives (Mann–Whitney *U* test: $P > .278$; Fig. 2). The path length was slightly shorter when females approached the live male alternative (Mann–Whitney *U* test: $P = .051$; Fig. 2). Again, the same pattern of directional response was observed regardless of the chosen stimulus (Fig. 2; Table 2; for Mann–Whitney *U* test detailed results in all experiments, see Supporting Information, Table S1).

Set 2: attractive calls vs. unattractive calls in multimodal context

Females preferred attractive calls (i.e. calls with more pulses) over unattractive ones, even when these unattractive calls were associated with a visual stimulus. In experiment 5, with high variation between calls (18 pulses vs. six pulses + visual cue), only one female chose the multimodal (but shorter) stimulus (two-tailed binomial: $P < .001$; Fig. 3; Table 1). In experiment 6,

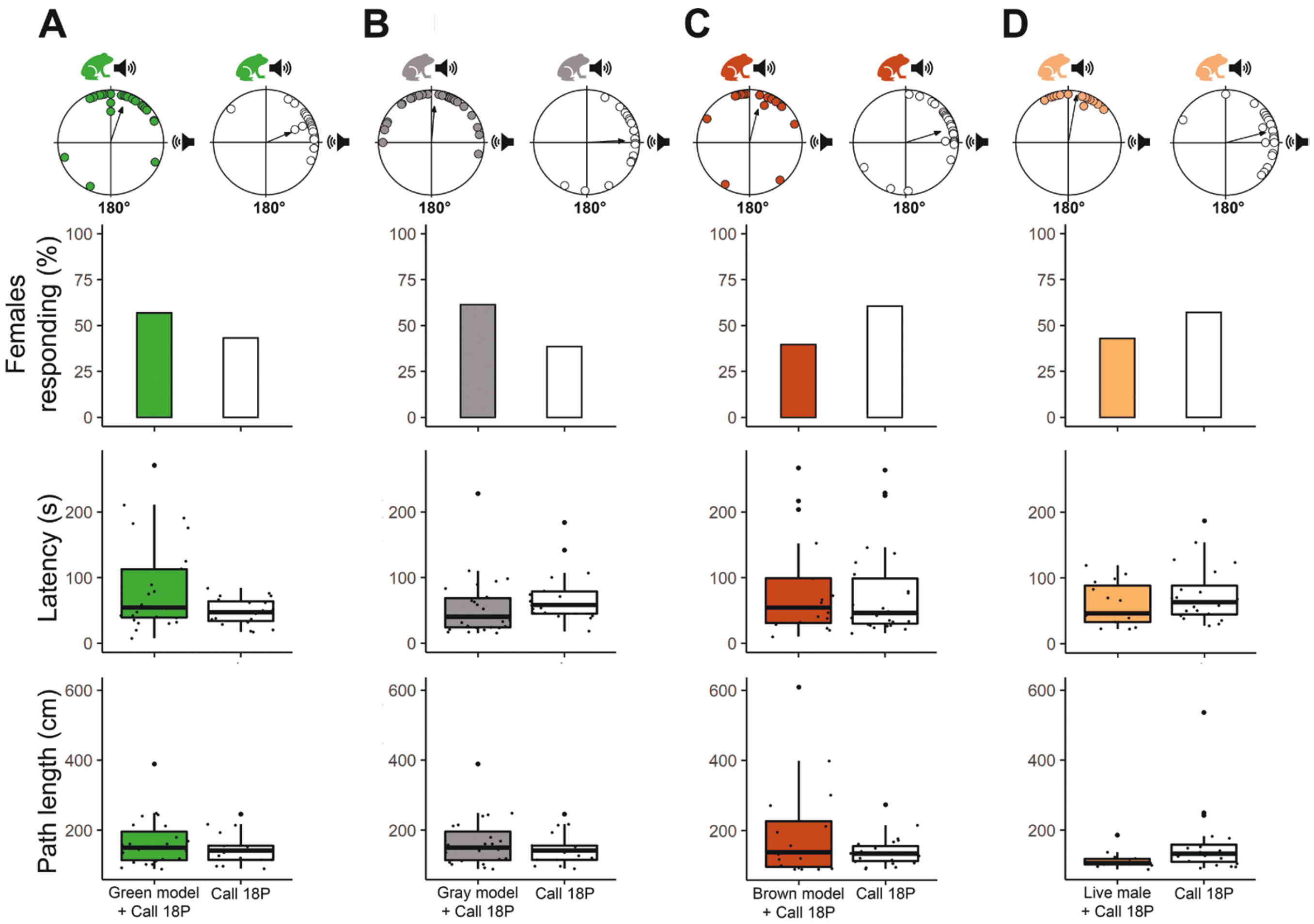

Figure 2. Responses of *Dryophytes versicolor* females to two-alternative choice trials. The results are presented by angle direction; percentage of females responding; response latency; and path length. A, green model + average call vs. average call. B, grey model + average call vs. average call. C, brown model + average call vs. average call. D, live male + average call vs. average call. Coloured (green, grey, brown, and light pink) graphics represent the results when females chose the multimodal stimulus in each experiment, and white graphics represent when females chose the unimodal stimulus. In the circular graphics, each point represents the angle of the first jump when females left the release area, and the arrows represent the mean angle direction (all females together). Boxplots indicate the median, upper and lower quartiles, and upper and lower whiskers. See Table 1 for detailed results of the two-tailed binomial test and Supporting Information, Table S1 for the Mann–Whitney *U* test.

with less variation between calls (21 pulses vs. 15 pulses + visual cue), a greater number of females also showed a preference for the longer call (Fig. 3), but the difference was not significant (Table 1; two-tailed binomial: $P = .066$). There were no differences in the response latency between stimuli (Fig. 3; Supporting Information, Table S1).

Set 3: female preference for dorsal colour

Females did not show a preference for any dorsal colour (two-tailed binomial: all $P > .761$; Fig. 4; Table 1). There were no differences in the response latency between green and grey and between brown and grey models (Fig. 4; Supporting Information, Table S1), but the response latencies of females choosing the green model over the brown model were significantly shorter (Mann–Whitney *U* test: $P < .001$; Fig. 4).

Set 4: thigh colour and vocal sac colour as visual cue

The presence of yellow thighs or a black vocal sac in the models did not increase the preference of females (two-tailed binomial: $P > .073$; Fig. 5; Table 1). Also, there were no differences in the response latency between stimuli (Mann–Whitney *U* test: $P > .213$; Fig. 5; Supporting Information, Table S1).

Set 5: vocal sac movement as visual cue

The vocal sac movement coupled with the call (multimodal) did not increase female response (two-tailed binomial: $P = .88$; Fig. 6; Table 1). Also, there were no differences in the response latency between stimuli (Mann–Whitney *U* test: $P = .503$; Fig. 6; Supporting Information, Table S1).

Individual colour preferences

Individual females did not show consistent preferences for specific colours, nor was their choice of a colour in one experiment related to the choice in another experiment (Table 3). This suggests a lack of colour preferences on an individual level and a population level.

DISCUSSION

It is well established that the auditory signal component of anuran advertisement displays is necessary and sufficient to elicit

Table 2. Mean vector angle (μ) and length of the mean vector (*r*) for each experiment. The multimodal stimulus (call + frog-like model/live male) was established at 0°, while the unimodal one (call) was at 90° in all four experiments. The length of the mean vector ranges from zero to one, where *r* = 0 indicates total uniform dispersion and *r* = 1 indicates concentrated dispersion in one direction.

Experiment	1		2		3		4	
Stimulus	**Green model + call**	**Call**	**Grey model + call**	**Call**	**Brown model + call**	**Call**	**Live male + call**	**Call**
Mean vector (μ)	17.74°	68.16°	4.84°	87.38°	13.97°	72.45°	9.91°	75.78°
Length of mean vector (*r*)	.708	.938	.69	.71	.656	.698	.92	.778
Angular deviation	*T*-ratio = −58, d.f. = 37.8, *P* = .56		*T*-ratio = −38, d.f. = 31.26, *P* = .71		*T*-ratio = −24, d.f. = 29.6, *P* = .81		*T*-ratio = 1.24, d.f. = 24.7, *P* = .21	

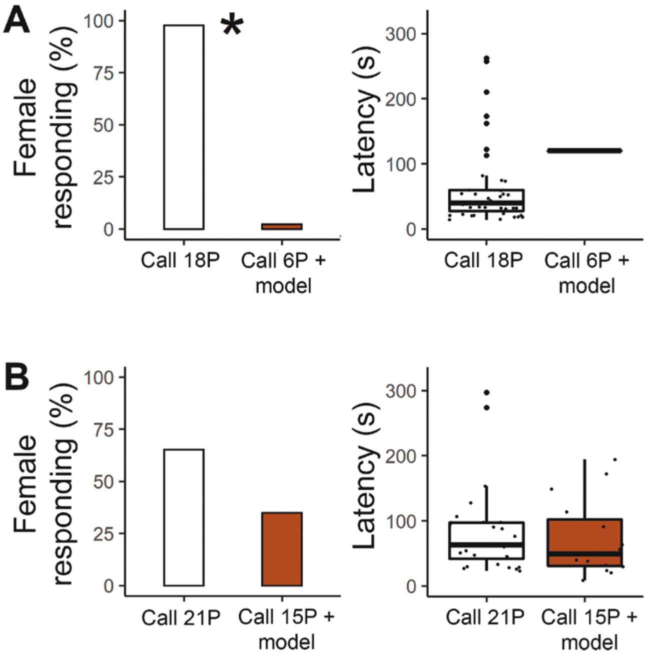

Figure 3. Responses of *Dryophytes versicolor* females to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. A, attractive calls [18 pulses (P); unimodal] vs. unattractive calls (six pulses + frog model; multimodal). B, attractive calls (21 pulses; unimodal) vs. unattractive call (15 pulses + frog model; multimodal). Boxplots indicate the median, upper and lower quartiles, and upper and lower whiskers. An asterisk indicates significant *P*-values. See Table 1 for detailed results of the two-tailed binomial test and Supporting Information, Table S1 for the Mann–Whitney *U* test.

mate-choice behaviour and that females prefer certain call features over others (Gerhardt and Huber 2002). The combination of bright coloration, contrasting colour patches, and the 'fixed' multimodal cue generated by the inflating vocal sac during call emission make it intuitively plausible that frogs should also incorporate visual cues in mediating social interactions. To date, the function of acoustic and visual signals in a multimodal context has been tested in eight species of nocturnal anurans. A detailed examination of the published literature suggests that this phenomenon is less widespread than anticipated (see also discussion by Li *et al.* 2022). Below, we summarize the results of those studies and show that our study, documenting a lack of multimodal communication in *D. versicolor*, is not unusual.

Are multimodal stimuli preferred over unimodal stimuli?

A lack of female preference for a multimodal stimulus has been reported in two species (Li *et al.* 2022, Reichert *et al.* 2014; this study), whereas females from five species have been reported to prefer a multimodal alternative over a unimodal one (Taylor *et al.* 2007, Taylor and Ryan 2013, Laird *et al.* 2016, Zhao *et al.* 2021, Zhu *et al.* 2021). The proportion of females that did approach the multimodal alternative in those studies ranged from 70% to 80%. In *Engystomops pustulosus* (Cope, 1864), the only species for which multiple replicates of the same test are available, the proportion of females approaching a dynamic multimodal alternative ranged from 60% to 80%, i.e. from no preference

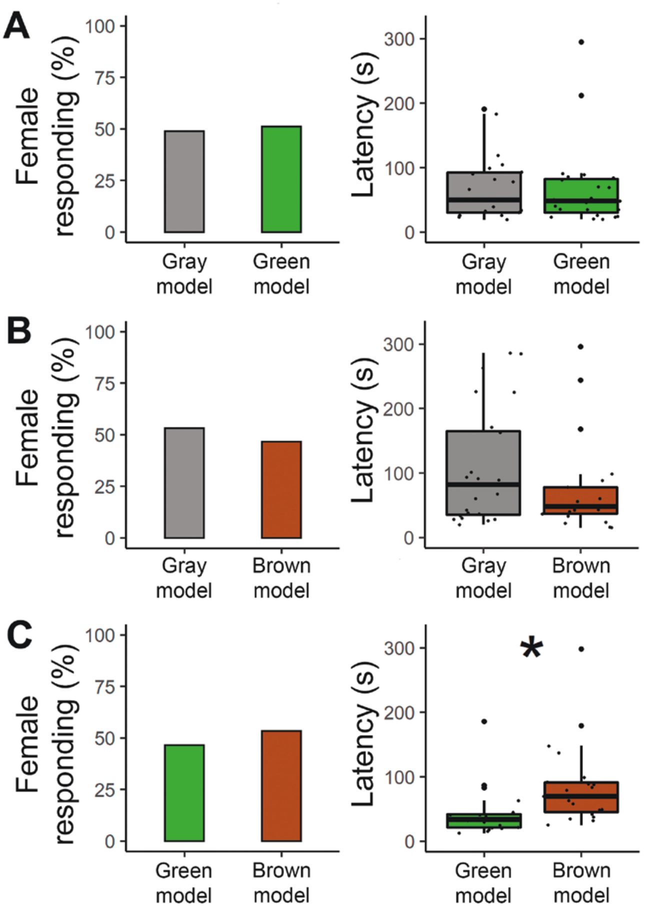

Figure 4. Responses of *Dryophytes versicolor* females to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. In this set of experiments, for acoustic stimulation we broadcast the average call with 18 pulses for all options, changing only the types of visual cues. A, grey model vs. green model. B, grey model vs. brown model. C, green model vs. brown model. Boxplots indicate the median, upper and lower quartiles, and upper and lower whiskers. An asterisk indicates significant *P*-values. See Table 1 for detailed results of the two-tailed binomial test and Supporting Information, Table S1 for the Mann–Whitney *U* test.

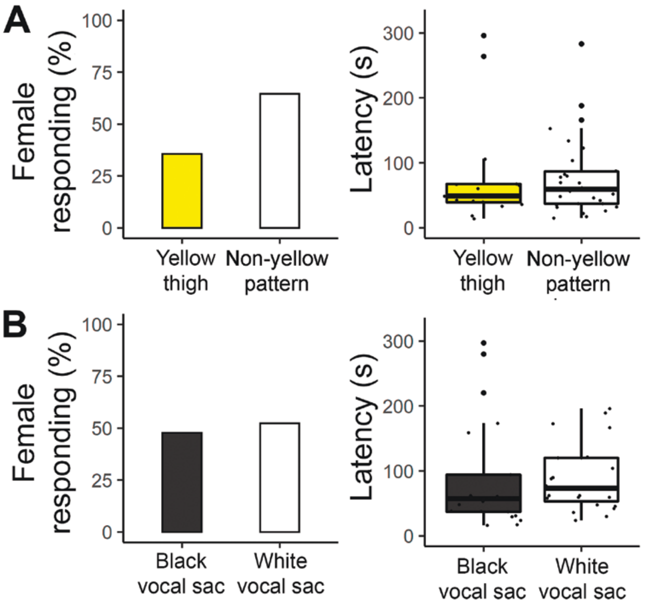

Figure 5. Responses of *Dryophytes versicolor* females to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. In this set of experiments, for acoustic stimulation we broadcast the average call with 18 pulses for all options, changing only the types of visual cues. A, frog models with yellow thighs vs. frog models with brown thighs. B, frog models with black vocal sac vs. frog models with white vocal sac. Boxplots indicate the median, upper and lower quartiles, and upper and lower whiskers. See Table 1 for detailed results of the two-tailed binomial test and Supporting Information, Table S1 for the Mann–Whitney U test.

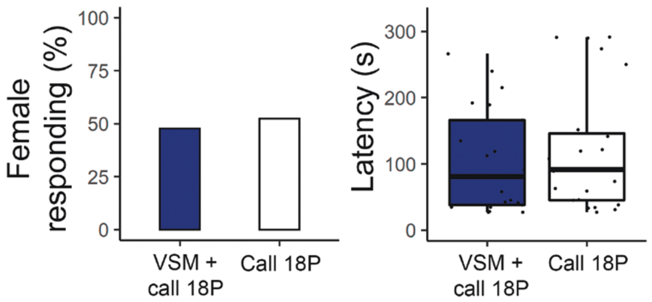

Figure 6. Responses of *Dryophytes versicolor* females to two-alternative choice trials of the experiment that evaluated whether the vocal sac movement (VSM) increases the preference of females. The graphs display the percentage of females that chose each stimulus and their response latency. In this experiment, the average call with 18 pulses (P) was broadcast for both options. Boxplots indicate the median, upper and lower quartiles, and upper and lower whiskers. See Table 1 for detailed results of the two-tailed binomial test and Supporting Information, Table S1 for the Mann–Whitney U test.

over non-significant trends favouring multimodal to statistically significant preferences for the multimodal alternative (Taylor *et al.* 2008, 2011b, Taylor and Ryan 2013, Stange *et al.* 2017). Furthermore, only dynamic visual cues were effective; females did not prefer a static multimodal stimulus to a unimodal stimulus (Taylor *et al.* 2008). Taken together, this suggests that a preference for a multimodal stimulus is not shared by all females or that it is easily influenced by small variations in the stimuli tested or the experimental design.

The type of visual stimulus used in the experiments did not appear to influence whether a preference was observed. Multimodal stimuli that did not elicit a positive response included static resin models (this study), dynamic models (Li *et al.* 2022, this study), and video playbacks (Reichert *et al.* 2014). Those that did elicit a positive response included dynamic models (Taylor *et al.* 2007, Taylor and Ryan 2013, Laird *et al.* 2016) and video playbacks (Zhao *et al.* 2021, Zhu *et al.* 2021). However, it is important to note that studies on our focus species, *D. versicolor*, have shown that females exhibit strong positive phototaxis. When exposed to video playbacks (Reichert *et al.* 2016) or light-emitting diodes (Reichert and Höbel 2015), females responded more robustly and exhibited more directional behaviour towards multimodal stimuli in which sound was paired with a light source. See Reichert *et al.* (2014) for a broad discussion about phototaxis of *D. versicolor* females towards a variety of stimuli on the computer screen (including non-biological stimuli, such as a white square).

Table 3. Nominal logistic regressions comparing colour choices of the same females across different trials. In none of the comparisons did the colour choice of a given female in one trial predict her colour choice in another trial.

Response in one trial	Response in other trial	Likelihood ratio test
Prefer **brown** model in Exp. 3 (model vs. call alone)	Prefer **green** model in Exp. 1 (model vs. call alone)	$\chi^2_1 = 0.873$; $P = .350$
Prefer **brown** model in Exp. 3 (model vs. call alone)	Prefer **grey** model in Exp. 2 (model vs. call alone)	$\chi^2_1 = 0.927$, $P = .336$
Prefer **grey** model in Exp. 7 (grey vs. green)	Prefer **grey** model in Exp. 8 (grey vs. brown)	$\chi^2_1 = 0$, $P = 1$
Prefer **green** model in Exp. 7 (grey vs. green)	Prefer **green** model in Exp. 9 (brown vs. green)	$\chi^2_1 = 0.836$, $P = .360$
Prefer **brown** model in Exp. 9 (brown vs. green)	Prefer **brown** model in Exp. 8 (grey vs. brown)	$\chi^2_1 = 0.569$, $P = .451$
Prefer **brown** model in Exp. 3 (model vs. call alone)	Prefer **brown + yellow thigh** in Exp. 10	$\chi^2_1 = 0.108$, $P = .742$
Prefer **green** model in Exp. 1 (model vs. call alone)	Prefer **brown + yellow thigh** in Exp. 10	$\chi^2_1 = 0.078$, $P = .780$
Prefer **grey** model in Exp. 2 (model vs. call alone)	Prefer **brown + yellow thigh** in Exp. 10	$\chi^2_1 = 1.327$, $P = .249$
Prefer **brown** model in Exp. 3 (model vs. call alone)	Prefer **brown + black throat** in Exp. 11	$\chi^2_1 = 0.042$, $P = .837$
Prefer **green** model in Exp. 1 (model vs. call alone)	Prefer **brown + black throat** in Exp. 11	$\chi^2_1 = 0.242$, $P = .623$
Prefer **grey** model in Exp. 2 (model vs. call alone)	Prefer **brown + black throat** in Exp. 11	$\chi^2_1 = 3.288$, $P = .070$

Are dynamic visual cues more effective than static visual cues?

The inflation and deflation of the vocal sac during call production generates a dynamic visual cue that might be particularly salient in eliciting responses. But there is surprisingly little support for this hypothesis. Three species do not show a preference for a dynamic visual component. *Dryophytes chrysoscelis* (Cope, 1880) and *Dryophytes squirellus* (Daudin, 1800) did not prefer a dynamic model over a static one (Taylor *et al.* 2007, Li *et al.* 2022). Likewise, *D. versicolor* did not prefer a video with a dynamic vocal sac over a static one (Reichert *et al.* 2014), and they also did not prefer a multimodal presentation of a simulated vocal sac (this study).

For two species, some data are consistent with a preference for the dynamic component. In a video playback study with *E. pustulosus*, 14 females approached a video of a male with an inflating vocal sac, whereas six approached a video of a male whose vocal sac did not inflate (Rosenthal *et al.* 2004). However, this difference is not statistically significant when using a two-tailed binomial test to determine whether the proportions of subjects choosing the two alternatives significantly deviate from 0.5 (two-tailed $P = .12$). In a study with the same species that used three-dimensional models, females preferred a multimodal alternative over a unimodal one only if it was a dynamic model (16:4; two-tailed $P = .01$), but not if it was a static model (7:13; two tailed $P = .26$) (Taylor *et al.* 2008). In *Hyla arborea* (Linnaeus, 1758), a dynamic model with attractive features did improve the attractiveness of an unattractive call (Richardson *et al.* 2010), whereas a similar test using static models did not (Troïanowski *et al.* 2014). This could also be interpreted as females attending more to the dynamic visual component of a multimodal signal.

Do nocturnal frogs have colour preferences?

We conducted a comprehensive exploration of visual preferences, particularly focusing on colour, in a nocturnal anuran. This was motivated by the exceptional ability of *D. versicolor* to change colour (Edgren 1954), in addition to the presence of their contrasting body colour patches (yellow thigh and dark throat). We found that variation in male dorsal coloration did not influence female mate-choice behaviour, which suggests that body colour change is not a component of a visual signal but might be related to thermoregulation, light intensity, or a defensive strategy involved in camouflage.

Female mate-choice behaviour was also not influenced by yellow thigh coloration of a model. Yellow (in addition to orange and red) colour in vertebrates, including frogs (Suga and Munesada 1988, Umbers *et al.* 2016, Brenes-Soto *et al.* 2017), is usually formed by pigments belonging to the carotenoid category. These pigments are acquired from the diet (Feltl *et al.* 2005, Umbers *et al.* 2016) and are commonly associated with the foraging ability of an individual and/or food availability, which, in turn, might reflect their health and body condition. Therefore, they have the potential to provide honest information to conspecifics. Then again, bright coloration is also often associated with aposematism/unpalatability, and colourful patches on the thighs and flanks of otherwise cryptic frogs might represent an intermediate state between crypsis and aposematism (Loeffler-Henry *et al.* 2023). Conspicuous hidden coloration is common among hylids (Loeffler-Henry *et al.* 2023), and Cannizzaro IV and Höbel (2023) recently documented that the black and yellow thigh colour of *D. versicolor* does function as an aposematic signal. Although this does not preclude an additional function in conspecific communication, our data suggest that female *D. versicolor* do not attend to it during mate choice.

In *D. versicolor*, male body condition is associated with vocal sac pigmentation, and the vocal sac colour of mated males is darker when compared with unmated ones (Höbel *et al.* 2022). Although this suggests a role of vocal sac colour in mate choice, our experiments demonstrated that dark vocal sac colour did not increase the preference of females. This is in line with a previous study that used video playbacks to test for preferences for vocal sac colour in this species (Reichert *et al.* 2014). Only one other species (*H. arborea*) has been tested for vocal sac colour preferences, with mixed results. In a study using video playbacks, 17 of 24 females (71%) approached the alternative with a more colourful vocal sac, a difference that is not significantly different from the expected proportion of 0.5 in a two-tailed binomial test ($P = .06$; Gomez *et al.* 2009). In a study using painted static models, only 55% approached a light red over a dark orange vocal sac (11:9; two-tailed $P = .82$), but 75% of females approached a dark red over a light orange vocal sac (15:5; two-tailed $P = .04$). Gomez *et al.* (2010) suggested that this difference might be attributable to females evaluating brightness and hue separately. Given the pronounced phototaxis exhibited by many anurans (Hailman and Jaeger 1974), this idea warrants further examination.

Can addition of a visual component modify preferences for the auditory component?

Assuming that visual components are attractive, adding a visual cue might improve a call with unattractive features. In contrast, this is not the case for *Dryophytes cinereus* (Schneider, 1799), for which the addition of a visual component did not change the preference for the low-frequency call (Laird *et al.* 2016). In our study with *D. versicolor*, females strongly discriminated against an unattractive short call that was paired with a visual cue if the difference between the two call alternatives was large (difference of 12 pulses; two-tailed test: $P < .001$), but not if the difference was smaller (difference of six pulses; two-tailed test: $P = .066$). However, we do not interpret the latter result as evidence for a positive effect of adding a visual cue to the unattractive call, but suggest that preferences for longer calls diminish as alternatives become more similar and/or are closer to the population average; a study in which the stimuli tested were only unimodal calls found that females did not significantly differentiate between calls with 14 pulses and those with 19 pulses, a 26.3% variation between the acoustic stimuli (Stratman and Höbel 2019). This variation is similar to our test of 15 vs. 21 pulses, a 28.6% variation.

In one species (*D. squirellus*), adding a visual cue increased the attractiveness of an unattractive call, but never made it more attractive than the unimodal attractive call stimulus (Taylor *et al.* 2011a). However, different studies obtained opposing results, such that only some data are consistent with an 'improving' function of adding a visual cue. In *E. pustulosus*, adding a visual cue rendered an unattractive/simple call less unattractive than the attractive complex call in one study (Stange *et al.* 2017), but in another study the addition of a visual cue did not modulate female responses, and the less attractive/simple call remained less attractive when the alternative was a complex call (Taylor *et al.* 2011a). In *H. arborea*, when females had to choose between an all-attractive audiovisual stimulus and an all-unattractive audiovisual stimulus, they strongly preferred the all-attractive alternative. But when the attractive call was associated with unattractive vocal sac coloration and opposed to an unattractive call combined with attractive coloration, half of the females now approached the alternative with the unattractive call (Richardson *et al.* 2010). However, in another study that also tested a combination of attractive call + unattractive vocal sac coloration against unattractive call + attractive coloration, females discriminated significantly against the alternative with the unattractive call (Troïanowski *et al.* 2014). The latter comparison is complicated by differences in experimental design, however, because the former study used dynamic video playbacks and the latter used static resin models. Hence, the addition of a visual cue does not always improve an unattractive call. Even in the cases where there is an improvement, the visual cue never renders the (bimodal) unattractive call more attractive than an (unimodal) attractive call, underscoring the dominance of the acoustic communication channel.

CONCLUSION

In frogs, a multimodal stimulus is sometimes more effective than a call alone, but the particular feature of the visual cue is secondary. Adding visual information to a call sometimes changes how females respond to it; however, that is not always the case. Moreover, replicate studies involving the same species frequently yield opposing results (which might be attributable, in part, to differences in the visual cue used). Hence, multimodal communication might affect mate-choice decisions in some nocturnal anurans, but it is neither universally present nor are preferences particularly strong.

In addition to mediating mate-choice decisions, visual cues might facilitate communication by improving sound source localization or signal reliability. In our study, we tested whether a visual component improved sound source localization but failed to find support for this hypothesis. Moreover, the three studies that have tested whether females shift to greater reliance on the visual component if the reliability of the auditory component is compromised, for example because it is temporally degraded or masked by background noise, found no evidence for it (Coss *et al.* 2022, Li *et al.* 2022, Zhu *et al.* 2022). Overall, visual cues might play some role in the social behaviour of some species, but acoustic communication is the dominant modality mediating mate choice in nocturnal anurans (Gerhardt 2001, Li *et al.* 2022).

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

ACKNOWLEDGEMENTS

We thank Joseph S. Cannizzaro, Hannah Warner, and Kane Stratman for assistance during data collection, Jacqueline Joyce for initial model construction, and staff at the University of Wisconsin-Milwaukee's Field Station for logistical support.

AUTHOR CONTRIBUTIONS

Guilherme Augusto-Alves (Conceptualization, Methodology, Data analysis, Writing—Original draft, Review & editing), Olivia S. Feagles (Methodology, Review & editing), Luís Felipe Toledo (Review & editing), Gerlinde Höbel (Conceptualization, Methodology, Data analysis, Review & editing).

CONFLICT OF INTEREST

The authors declare no conflicting interests.

FUNDING

This work was supported by São Paulo Research Foundation (FAPESP #2016/25358-3; #2019/18335-5; #2019/03170-0; #2019/25109-1) and the National Council for Scientific and Technological Development (CNPq #302834/2020-6).

DATA AVAILABILITY

Additional information and datasets generated during the study are available as Supporting Information.

REFERENCES

Augusto-Alves G, Dena SA, Toledo LF. Visual communication and aggressive behaviour in a giant mute torrent-frog, *Megaelosia apuana* (Anura; Hylodidae). *Amphibia-Reptilia* 2018;**39**:260–4. https://doi.org/10.1163/15685381-20181000

Augusto-Alves G, Höbel G, Toledo LF. Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, *Boana albomarginata*. *Behavioral Ecology and Sociobiology* 2024;**78**:48. https://doi.org/10.1007/s00265-024-03462-7

Augusto-Alves G, Toledo LF. Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog. *Behaviour* 2022;**159**:351–75. https://doi.org/10.1163/1568539x-bja10133

Barros AB, Feio RN. Visual communication in *Scinax maracaya* (Cardoso & Sazima, 1980) (Anura: Hylidae) at the Serra da Canastra National Park, southeastern Brazil. *Herpetology Notes* 2011;**4**:103–4.

Bonachea LA, Ryan MJ. Localization error and search costs during mate choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 2011;**117**:56–62. https://doi.org/10.1111/j.1439-0310.2010.01843.x

Boyd SK, Gordon NM. Auditory and distance cues interact to modulate female gray treefrog preferences for male advertisement calls. *Behavioral Ecology and Sociobiology* 2021;**75**:95.

Brenes-Soto A, Dierenfeld ES, Janssens GP. Colouration in amphibians as a reflection of nutritional status: the case of tree frogs in Costa Rica. *PLoS One* 2017;**12**:e0182020.

Candolin U. The use of multiple cues in mate choice. *Biological Reviews of the Cambridge Philosophical Society* 2003;**78**:575–95. https://doi.org/10.1017/s1464793103006158

Cannizzaro JS IV, Höbel G. Hidden black and yellow thigh color acts as an aposematic signal in the Eastern Gray Treefrog (*Hyla versicolor*). *Ethology* 2023;**129**:679–85. https://doi-org.ez88.periodicos.capes.gov.br/10.1111/eth.13404

Coss DA, Ryan MJ, Page RA *et al.* Can you hear/see me? Multisensory integration of signals does not always facilitate mate choice. *Behavioral Ecology* 2022;**33**:903–11. https://doi.org/10.1093/beheco/arac061

Edgren RA. Factors controlling color change in the tree frog, *Hyla versicolor* Wiech. *Experimental Biology and Medicine* 1954;**87**:20–3. https://doi.org/10.3181/00379727-87-21272

Feagles OS, Höbel G. Female gray treefrogs maintain mate choice decisions under predation threat, but adjust movements to reduce conspicuousness during mate approach. *Behavioral Ecology and Sociobiology* 2022;**76**:17. https://doi.org/10.1007/s00265-021-03124-y

Feltl L, Pacakova V, Stulik K *et al.* Reliability of carotenoid analyses: a review. *Current Analytical Chemistry* 2005;**1**:93–102. https://doi.org/10.2174/1573411052948424

Ferrer ES, Uribe F, Sanz JJ *et al.* Are plumage colour and song redundant ornaments in great tits (*Parus major*)? It depends on the colour. *Biological Journal of the Linnean Society* 2021;**133**:1077–83. https://doi.org/10.1093/biolinnean/blab063

Forster B, Van De Ville D, Berent J *et al.* Complex wavelets for extended depth-of-field: a new method for the fusion of multichannel microscopy images. *Microscopy Research and Technique* 2004;**65**:33–42. https://doi.org/10.1002/jemt.20092

Frost DR. *Amphibian Species of the World: an Online Reference. Version 6.2 (27 September 2023, date last accessed)*. New York: American Museum of Natural History, 2023. https://amphibiansoftheworld.amnh.org/index.php

Furtado R, Lermen LN, Márquez R *et al.* Neotropical dancing frog: the rich repertoire of visual displays in a hylodine species. *Journal of Ethology* 2019;**37**:291–300. https://doi.org/10.1007/s10164-019-00600-x

Gerhardt HC. Acoustic communication in two groups of closely related treefrogs. *Advances in the Study of Behavior* 2001;**30**:99–167.

Gerhardt HC, Huber F. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press, 2002.

Gerhardt HC, Tanner SD, Corrigan CM *et al.* Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behavioral Ecology* 2000;**11**:663–9. https://doi.org/10.1093/beheco/11.6.663

Giasson LO, Haddad CFB. Social interactions in *Hypsiboas albomarginatus* (Anura: Hylidae) and the significance of acoustic and visual signals. *Journal of Herpetology* 2006;**40**:171–80.

Gomez D, Richardson C, Lengagne T *et al.* The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proceedings Biological Sciences* 2009;**276**:2351–8. https://doi.org/10.1098/rspb.2009.0168

Gomez D, Richardson C, Lengagne T *et al.* Support for a role of colour vision in mate choice in the nocturnal European treefrog (*Hyla arborea*). *Behaviour* 2010;**147**:1753–68.

Grafe TU, Wanger TC. Multimodal signaling in male and female foot-flagging frogs *Staurois guttatus* (Ranidae): an alerting function of calling. *Ethology* 2007;**113**:772–81. https://doi.org/10.1111/j.1439-0310.2007.01378.x

Haddad CFB, Giaretta AA. Visual and acoustic communication in the Brazilian torrent frog, *Hylodes asper* (Anura: Leptodactylidae). *Herpetologica* 1999;**55**:324–33.

Hailman JP, Jaeger RG. Phototactic responses to spectrally dominant stimuli and use of colour vision by adult anuran amphibians: a comparative survey. *Animal Behaviour* 1974;**22**:757–95. https://doi.org/10.1016/0003-3472(74)90002-5

Hartmann MT, Giasson LO, Hartmann PA *et al.* Visual communication in Brazilian species of anurans from the Atlantic forest. *Journal of Natural History* 2005;**39**:1675–85.

Hirschmann W, Hödl W. Visual signaling in *Phrynobatrachus krefftii* Boulenger, 1909 (Anura: Ranidae). *Herpetologica* 2006;**62**:18–27. https://doi.org/10.1655/04-06.1

Höbel G, Feagles O, Ruder E. Diversity and sexual dichromatism in treefrog throat coloration: potential signal function? *Journal of Herpetology* 2022;**56**:294–301.

Johnstone RA. Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 1996;**351**:329–38.

Kelber A, Yovanovich C, Olsson P. Thresholds and noise limitations of colour vision in dim light. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 2017;**372**:20160065. https://doi.org/10.1098/rstb.2016.0065

Köhler J, Jansen M, Rodriguez A *et al.* The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 2017;**4251**:1–124. https://doi.org/10.11646/zootaxa.4251.1.1

Laird KL, Clements P, Hunter KL *et al.* Multimodal signaling improves mating success in the green tree frog (*Hyla cinerea*), but may not help small males. *Behavioral Ecology and Sociobiology* 2016;**70**:1517–25. https://doi.org/10.1007/s00265-016-2160-9

Li H, Schrode KM, Bee MA. Vocal sacs do not function in multimodal mate attraction under nocturnal illumination in Cope's grey treefrog. *Animal Behaviour* 2022;**189**:127–46. https://doi.org/10.1016/j.anbehav.2022.05.003

Ligon RA, Diaz CD, Morano JL *et al.* Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. *PLoS Biology* 2018;**16**:e2006962. https://doi.org/10.1371/journal.pbio.2006962

Loeffler-Henry K, Kang C, Sherratt TN. Evolutionary transitions from camouflage to aposematism: hidden signals play a pivotal role. *Science* 2023;**379**:1136–40. https://doi.org/10.1126/science.ade5156

Møller A, Pomiankowski A. Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology* 1993;**32**:167–76.

Narins PM, Grabul DS, Soma KK *et al.* Cross-modal integration in a dart-poison frog. *Proceedings of the National Academy of Sciences of the United States of America* 2005;**102**:2425–9. https://doi.org/10.1073/pnas.0406407102

R Core Team. *R: A Language and Environment for Statistical Computing. Version 3.1.0*. Vienna: R Foundation for Statistical Computing, 2014. http://www.R-project.org/

R Core Team. *R: A Language and Environment for Statistical Computing. Version 3.6.3*. Vienna: R Foundation for Statistical Computing, 2019. http://www.R-project.org/

Reichert MS. Visual cues elicit courtship signals in a nocturnal anuran. *Behavioral Ecology and Sociobiology* 2013;**67**:265–71. https://doi.org/10.1007/s00265-012-1446-9

Reichert MS, Galante H, Höbel G. Female gray treefrogs, *Hyla versicolor*, are responsive to visual stimuli but unselective of stimulus characteristics. *The Journal of Experimental Biology* 2014;**217**:3254–62. https://doi.org/10.1242/jeb.106666

Richardson C, Gomez D, Durieux R *et al.* Hearing is not necessarily believing in nocturnal anurans. *Biology Letters* 2010;**6**:633–5. https://doi.org/10.1098/rsbl.2010.0038

Reichert MS, Höbel G. Modality interactions alter the shape of acoustic mate preference functions in gray treefrogs. *Evolution* 2015;**69**:2384–98. https://doi.org/10.1111/evo.12750

Reichert MS, Symes LB, Höbel G. Lighting up sound preferences: cross-modal influences on the precedence effect in treefrogs. *Animal Behaviour* 2016;**119**:151–9. https://doi.org/10.1016/j.anbehav.2016.07.003

Robertson JM, Bell RC, Loew ER. Vision in dim light and the evolution of color pattern in a crepuscular/nocturnal frog. *Evolutionary Ecology* 2022;**36**:355–71. https://doi.org/10.1007/s10682-022-10173-w

Rosenthal GG, Rand AS, Ryan MJ. The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. *Animal Behaviour* 2004;**68**:55–8. https://doi.org/10.1016/j.anbehav.2003.07.013

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 2012;**9**:671–5. https://doi.org/10.1038/nmeth.2089

Schwartz JJ, Buchanan BW, Gerhardt HC. Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behavioral Ecology and Sociobiology* 2001;**49**:443–55. https://doi.org/10.1007/s002650100317

Schwartz JJ, Buchanan BW, Gerhardt H. Acoustic interactions among male gray treefrogs, *Hyla versicolor*, in a chorus setting. *Behavioral Ecology and Sociobiology* 2002;**53**:9–19.

Smith WJ. The behavior of communicating: an ethological approach. Cambridge (MA): Harvard University Press,1977.

Stange N, Page RA, Ryan MJ *et al.* Interactions between complex multisensory signal components result in unexpected mate choice responses. *Animal Behaviour* 2017;**134**:239–47. https://doi.org/10.1016/j.anbehav.2016.07.005

Starnberger I, Preininger D, Hödl W. From uni- to multimodality: towards an integrative view on anuran communication. *Journal of Comparative Physiology A* 2014;**200**:777–87. https://doi.org/10.1007/s00359-014-0923-1

Stratman KD, Höbel G. Opportunity for female choice in the wild is frequently curtailed by low male trait variation in *Hyla versicolor*. *Behavioral Ecology and Sociobiology* 2019;**73**:1–12.

Sueur J, Aubin T, Simonis C. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 2008;**18**:213–26. https://doi.org/10.1080/09524622.2008.9753600

Suga T, Munesada K. The pigments in the dorsal skin of frogs. *Journal of Natural Products* 1988;**51**:713–8. https://doi.org/10.1021/np50058a008

Taylor RC, Buchanan BW, Doherty JL. Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. *Animal Behaviour* 2007;**74**:1753–63. https://doi.org/10.1016/j.anbehav.2007.03.010

Taylor RC, Klein BA, Ryan MJ. Inter-signal interaction and uncertain information in anuran multimodal signals. *Current Zoology* 2011a;**57**:153–61. https://doi.org/10.1093/czoolo/57.2.153

Taylor RC, Klein BA, Stein J *et al.* Faux frogs: multimodal signalling and the value of robotics in animal behaviour. *Animal Behaviour* 2008;**76**:1089–97. https://doi.org/10.1016/j.anbehav.2008.01.031

Taylor RC, Klein BA, Stein J *et al.* Multimodal signal variation in space and time: how important is matching a signal with its signaler? *The Journal of Experimental Biology* 2011b;**214**:815–20. https://doi.org/10.1242/jeb.043638

Taylor RC, Ryan MJ. Interactions of multisensory components perceptually rescue túngara frog mating signals. *Science* 2013;**341**:273–4.

Toledo LF, Araújo OG, Guimarães LD *et al.* Visual and acoustic signaling in three species of Brazilian nocturnal tree frogs (Anura, Hylidae). *Phyllomedusa: Journal of Herpetology* 2007;**6**:61–8.

Toledo LF, Martins IA, Bruschi DP *et al.* The anuran calling repertoire in the light of social context. *Acta Ethologica* 2015;**18**:87–99. https://doi.org/10.1007/s10211-014-0194-4

Troïanowski M, Melot G, Lengagne T. Multimodality: a way to cope with road traffic noise? The case of European treefrog (*Hyla arborea*). *Behavioural Processes* 2014;**107**:88–93. https://doi.org/10.1016/j.beproc.2014.08.007

Umbers KD, Silla AJ, Bailey JA *et al.* Dietary carotenoids change the colour of Southern corroboree frogs. *Biological Journal of the Linnean Society* 2016;**119**:436–44.

Underhill VA, Höbel G. Variation in nocturnal light levels does not alter mate choice behavior in female eastern gray treefrogs (*Hyla versicolor*). *Behavioral Ecology and Sociobiology* 2017;**71**:15.

Underhill VA, Höbel G. Mate choice behavior of female Eastern gray treefrogs (*Hyla versicolor*) is robust to anthropogenic light pollution. *Ethology* 2018;**124**:537–48. https://doi.org/10.1111/eth.12759

Vásquez T, Pfennig KS. Looking on the bright side: females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, *Scaphiopus couchii*. *Behavioral Ecology and Sociobiology* 2007;**62**:127–35. https://doi.org/10.1007/s00265-007-0446-7

Yovanovich CA, Koskela SM, Nevala N *et al.* The dual rod system of amphibians supports colour discrimination at the absolute visual threshold. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 2017;**372**:20160066. https://doi.org/10.1098/rstb.2016.0066

Zhao L, Wang J, Cai Y *et al.* Behavioral and neurogenomic responses to acoustic and visual sexual cues are correlated in female torrent frogs. *Asian Herpetological Research* 2021;**12**:88–99B.

Zhu B, Zhang H, Chen Q *et al.* Noise affects mate choice based on visual information via cross-sensory interference. *Environmental pollution (Barking, Essex : 1987)* 2022;**308**:119680. https://doi.org/10.1016/j.envpol.2022.119680

Zhu B, Zhou Y, Yang Y *et al.* Multisensory modalities increase working memory for mating signals in a treefrog. *The Journal of Animal Ecology* 2021;**90**:1455–65. https://doi.org/10.1111/1365-2656.13465