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Interactions between complex multisensory signal components result in unexpected mate choice responses

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Multimodal (multisensory) signalling is common in many species and often facilitates communication. How receivers integrate individual signal components of multisensory displays, especially with regard to variance in signal complexity, has received relatively little attention. In nature, male túngara frogs, *Physalaemus pustulosus*, produce multisensory courtship signals by vocalizing and presenting their inflating and deflating vocal sac as a visual cue. Males can produce a simple call (whine only) or a complex call (whine + one or more chucks). In a series of two-choice experiments, we tested female preferences for variation in acoustic call complexity and amplitude (unimodal signals). We then tested preferences for the same calls when a dynamic robotic frog was added to one call, generating a multimodal stimulus. Females preferred a complex call to a simple call; when both calls contained at least one chuck, additional numbers of chucks did not further increase attractiveness. When calls contained zero or one chuck, the visual stimulus of the robofrog increased call attractiveness. When calls contained multiple chucks, however, the visual component failed to enhance call attractiveness. Females also preferred higher amplitude calls and the addition of the visual component to a lower amplitude call did not alter this preference. At relatively small amplitude differences, however, the visual signal increased overall discrimination between the calls. These results indicate that the visual signal component does not provide simple enhancement of call attractiveness. Instead, females integrate multisensory components in a nonlinear fashion. The resulting perception and behavioural response to complex signals probably evolved in response to animals that communicate in noisy environments.

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Communication in both human and nonhuman animals typically incorporates multiple sensory systems (e.g. visual plus acoustic) that may facilitate signal transmission and reception (Hebets & Papaj, 2005; McGurk & MacDonald, 1976; Narins, Hödl, & Grabul, 2003; Partan & Marler, 1999; Uetz, Roberts, Clark, Gibson, & Gordon, 2013). These multisensory (multimodal) signals are thought to improve detection, discrimination or memorability of the signals by receivers and are widespread in diverse taxa (for review see Hebets & Papaj, 2005; Partan & Marler, 2005; Rowe, 1999). For example, bird signals may consist of conspicuous plumage plus vocalizations (Patricelli & Krakauer, 2010), frogs produce acoustic and visual displays (Preininger, Boeckle, Freudmann, et al., 2013), spiders produce both visual and seismic displays (Hebets, Vink, Sullivan-Beckers, & Rosenthal, 2013), and some fish combine conspicuous visual courtship displays with acoustic signals (Maruska, Ung, & Fernald, 2012).

How signals are detected and perceived by receivers (typically females) is important because this influences mate choice and dictates which males in a population gain matings. Recent studies have demonstrated substantial variation in how females evaluate individual signal components within complex courtship displays. For example, females may evaluate individual components of complex courtship signals in different contexts, at different temporal or spatial scales (Uy & Safran, 2013), and may vary their attention towards different signal components (Hebets et al., 2013); furthermore, interaction among signal components may generate unexpected patterns of mate preference (Hebets & Papaj, 2005; Taylor & Ryan, 2013).

Frogs are an excellent model system to investigate multimodal communication. They use acoustic signals during reproduction to

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convey information about reproductive state and territorial boundaries. Their stereotyped acoustic advertisements are easily synthesized, females respond readily to synthetic signals (Gerhardt & Huber, 2002; Ryan, 2001), and there is considerable information about the mechanisms that underlie signal production (Ryan & Guerra, 2014; Zornik & Kelley, 2011) and perception (Bee, 2015; Wilczynski & Ryan, 2010). In addition, a variety of species incorporate visual cues into their reproductive displays and these visual cues can be reproduced in playback experiments (Starnberger, Preininger, & Hödl, 2014; Taylor, Klein, Stein, & Ryan, 2008).

The túngara frog, *Physalaemus pustulosus*, has been extensively studied in the context of sexual selection and communication (Ryan, 1985; 2011). It is a small (ca. 30 mm), brown frog, common in Panama and throughout much of Middle America. The animals reproduce at night during the wet season from May to November. Males congregate in small ponds or puddles and vocalize. The male's vocalizations can be a simple call consisting of a whine (W) only, or they can include up to seven additional notes called chucks (C), to the whine, creating a complex call (see Fig. 1). Complex calls are five times more attractive than simple calls (Gridi-Papp, Rand, & Ryan, 2006). The vocalizations are accompanied by a conspicuous and synchronous inflation of an elastic vocal sac, and females are known to assess both the vocalization and movement of the vocal sac (Taylor, Klein, & Ryan, 2011; Taylor et al., 2008; Fig. 1). The vocal sac typically reaches close to its maximum volume approximately 250 ms after the onset of the whine (typical call is 350 ms). The vocal sac volume increases a small additional amount when chucks are produced. After assessing potential mates, females will approach a male; he then clasps her in amplexus. When the female begins to oviposit her eggs, the pair builds a foam nest and deposits the eggs in the nest (Ryan, 1985).

The call (acoustic modality) is the dominant feature of this signalling system, as it is for most frog species (Gerhardt & Huber, 2002; Kelley, 2004; Ryan, 2001). In several species the visual stimulus of the vocal sac inflation–deflation also serves as a cue or a signal component (Narins et al., 2003; Starnberger et al., 2014; Taylor, Buchanan, & Doherty, 2007). Female túngara frogs prefer a call that is accompanied by a vocal sac inflating synchronously with the call, but strongly reject a call that is accompanied by an asynchronously inflating vocal sac (Taylor et al., 2008; Taylor, Klein, Stein, et al., 2011). In dense choruses, significant call overlap occurs among male calls and generates discrimination challenges for female receivers (Bee & Micheyl, 2008; Gerhardt & Huber, 2002; Ryan, 2001; Schwartz, Buchanan, & Gerhardt, 2001; Vélez, Schwartz, & Bee, 2013). Frogs have evolved auditory mechanisms to improve discrimination in these noisy conditions. For example,

directional hearing allows females to identify callers when they are spatially separated and some species appear to rely on 'dip listening,' that is, identifying callers during periodic, brief windows when the background chorus noise subsides (Nityananda & Bee, 2012; Vélez & Bee, 2011). The addition of a visual component probably further improves detection and discrimination (Preininger, Boeckle, Freudmann, et al., 2013; Preininger, Boeckle, Sztatecsny, & Hödl, 2013; Starnberger et al., 2014; Taylor, Klein, Stein, et al., 2011).

Although studies of multimodal signalling are common, very little is still known about how signal components interact to influence receiver responses (Higham & Hebets, 2013; Partan, 2013). In this study, we were interested in better understanding audiovisual integration. Although superficially it appears to be simple, assigning sounds to their sources is not always an easy task, especially for animals such as frogs that communicate vocally in noisy environments. One mechanism for assigning sounds to their source is to integrate the timing of the sound with an associated visual cue. This audiovisual integration has been well studied in humans, cats, macaques and barn owls (Knudsen & Knudsen, 1989; Stein, 2012; Stein & Meredith, 1993), but very little is known about audiovisual integration outside of relatively large-brained vertebrates. In túngara frogs, preference for call variation is distance, and thus amplitude, dependent (Akre & Ryan, 2010). At farther assessment distances (e.g. >50 cm) the addition of more chucks to a whine does not make the call more attractive; that is, two chucks or three chucks are no more attractive than one. At higher amplitudes, such as those that females experience at close listening distances, more chucks do increase call attractiveness (Akre & Ryan, 2010). All else being equal, higher amplitude calls are also more attractive (Ryan & Keddy-Hector, 1992), but it is unknown how variance in amplitude or chuck number influences female choice in a multimodal context. Although it is often not explicitly stated, studies of multimodal signalling often treat each signal modality is as if they are cognitively independent. For example, researchers often study signal components as independent entities that influence behaviour (sensu Partan & Marler, 1999). We should note, however, that Partan and Marler (1999) did not assume that signals are always cognitively independent. Regardless, behavioural and/or neuroanatomical data in humans (Gerdes, Wieser, & Alpers, 2014; Ghazanfar & Schroeder, 2006; McGurk & MacDonald, 1976; Shore & Dehmel, 2012), birds (Patricelli, Uy, Walsh, & Borgia, 2002) and cats (Stein & Meredith, 1993) provide strong evidence that the senses are truly integrated, such that the perception of one component influences perception of another (Stein, 2012). In light of this, we examined how different levels of audio signal complexity interact with a visual component to influence female choice. Specifically, we asked: (1) how do different numbers of chucks (call complexity) influence female mate choice decisions at relatively low playback amplitudes? (2) how do different amplitudes of the same call influence female choice? and (3) how does the addition of a visual component alter the relative value of these same acoustic signals?

METHODS

Test Arena

We performed all experiments at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Republic of Panama. We collected mated pairs of túngara frogs at choruses between 1930 and 2100 hours. After collection, we placed pairs in a light-safe cooler in the laboratory in total darkness for a minimum of 1 h prior to testing to ensure that the female's eyes were dark-adapted.

Figure 1. Calling male túngara frog in a pond. The conspicuous inflated vocal sac is clearly visible below the male's mouth.

We did not test females that oviposited prior to or during the experiments. We tested females between 2200 and 0400 hours.

We conducted experiments in an Acoustic Systems (ETS-Lindgren, Austin, TX, U.S.A.) sound attenuation chamber (2.7 × 1.8 m and 2 m high). We used a plastic funnel to restrain the female in the centre of the sound chamber prior to initiating each playback trial. The female could move freely about under the funnel, but was confined to the area under the funnel, ensuring an equidistant start position at the beginning of the playback experiment. Sections of the plastic funnel were removed and the remaining ribs were covered with acoustically transparent, polyethylene food wrap, ensuring that the female could receive both visual and acoustic stimuli prior to being released. We positioned the funnel 80 cm from two Mirage Nanosat speakers (frequency range 110 Hz−20 000 Hz; Klipsch Group Inc. Indianapolis, IN, U.S.A.) and placed a robotic frog (see Klein, Stein, & Taylor, 2012 for details) with an inflatable vocal sac in front of both speakers. The distance between the speakers was also 80 cm, creating a 60° angle between speakers and funnel (Martin, Guy, & Taylor, 2011). The speakers broadcast the acoustic signal and the robofrog provided the visual stimulus. The arena was illuminated by a single GE nightlight (ca. 5.8×10^{-10} W/cm^2, model no. 55507; Fairfield, CT, U.S.A.). The spectrum and intensity were similar to the irradiance at a typical nocturnal breeding site (Cummings, Bernal, Reynaga, Rand, & Ryan, 2008; Taylor et al., 2008).
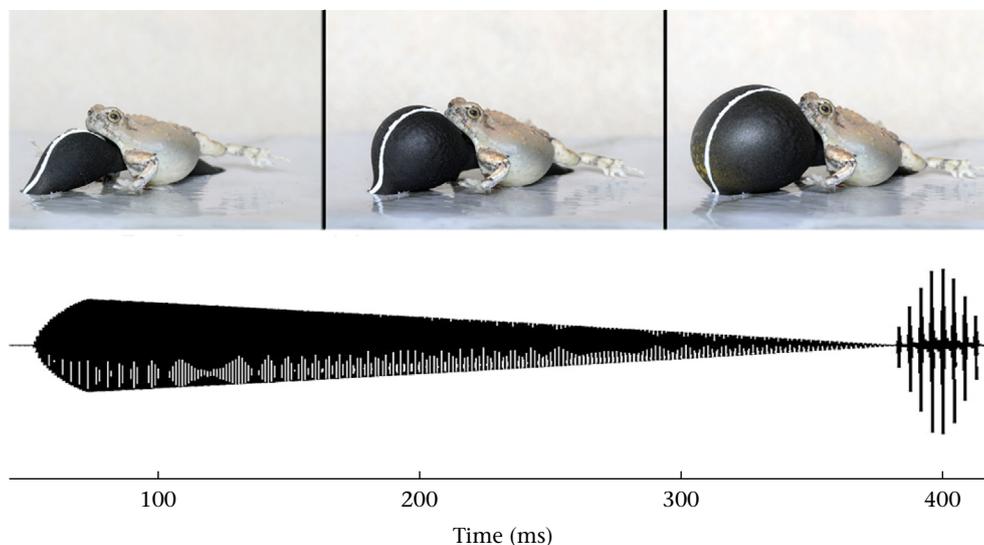
Signal Design and Presentation

For all experiments the acoustic signal was a synthetic, computer-generated call that was either simple (whine) or complex (whine + chucks). The synthetic whine and chuck were generated (by M.J.R.), using the program Signal (Engineering Design, Berkley, CA, U.S.A.). This synthetic call was created digitally as a centroid of 15 parameters derived from recordings of 300 calls from 50 unique males (Ryan and Rand, 2003). All trials used the same whine and had the same chuck digitally appended to the whine (0−3 depending on treatment). The use of identical synthetic calls standardizes the experiments because the call does not contain variable upper harmonics or unique waveforms as do natural calls. Further, synthetic calls have been shown to be as attractive as an average natural call (Rand, Ryan, & Wilczynski, 1992). Calls were played using the loop function in Adobe Audition (Adobe Systems Inc., San Jose, CA, U.S.A.) and amplified through an NAD 40x2 W amplifier (NAD Electronics, Ontario, Canada). The call amplitudes were measured at the position of the female's release point (fast, C weighting, sensu Taylor et al., 2008). Hereafter we refer to the calls by the sequence of whines and chucks presented: W = whine, WC = whine + one chuck, WCC = whine + two chucks, WCCC = whine + three chucks; any call that is followed by 'robo' had that acoustic signal enhanced with a dynamic robofrog whose vocal sac inflated synchronously with the call, creating a multimodal signal (Fig. 2).

In all experiments, each speaker had a robofrog placed in front where it could be seen by a female. The robofrog possessed an inflatable vocal sac that was powered by a piston-driven pneumatic pump and actuated by the computer producing the acoustic stimulus (Taylor et al., 2008 for details). One speaker was chosen to broadcast the acoustic component of the multimodal stimulus and the inflation/deflation cycle of that robofrog vocal sac was timed to occur synchronously with the call playback from the speaker (hereafter referred to as dynamic; Fig. 2). The dynamic robofrog's vocal sac shape, pattern and inflation timing provided a realistic representation of a calling male, as shown in previous studies (Klein et al., 2012; Taylor et al., 2008). The vocal sac on the robofrog at the other speaker remained uninflated (static) during the playbacks. Taylor et al. (2008) showed that the vocal sac movement (inflation/deflation cycle) is the salient visual component of the male's courtship display; females do not respond to immobile objects, even when they look like a frog. Both robofrogs were cast from the same mould and painted with the same batch of paint ensuring colour consistency. Thus, the use of the static robofrog at the opposing speaker provided a control for the visual component of the signal.

Playback Procedures

We conducted a total of 12 experiments (Table 1). Females were tested only once in every experiment and thus all experiments consisted of independent samples (*N* = 30 for each experiment). For each trial we placed a female under the funnel, broadcast a

Figure 2. Robofrog inflation sequence. Each successive panel illustrates the inflation sequence of the robotic frog vocal sac that was coupled with an exemplar call (WC) to generate the multimodal stimuli (acoustic + visual). Both the waveform and robofrog inflation correspond with the time scale. The *y*-axis for the waveform (amplitude) is an arbitrary voltage scale. The robofrog deflation (not shown) requires ca. 50 ms, which corresponds to the temporal aspects of natural male calling behaviour.

Table 1
Outline of experiments

	Stimuli
Part 1	
Unimodal complexity	W vs WC
	WC vs WCC
	WCC vs WCCC
Multimodal complexity	W/robo vs WC
	WC/robo vs WCC
	WCC/robo vs WCCC
Part 2	
Unimodal amplitude	76 vs 77.5 dB
	76 vs 79 dB
	76 vs 82 dB
Multimodal amplitude	76/robo vs 77.5 dB
	76/robo vs 79 dB
	76/robo vs 82 dB

Stimulus pairs tested in this study are shown. W = whine, WC = whine + one chuck, WCC = whine + two chucks, WCCC = whine + three chucks; any call followed by 'robo' had that acoustic signal enhanced with a dynamic robofrog. Tests of calls only (unimodal) had a static robofrog placed in front of the speaker as a visual control.

digitally synthesized call from the speakers and, in experiments with a dynamic robofrog, simultaneously activated the inflation–deflation of that robofrog's vocal sac for a 2 min acclimation period. In all experiments, the speakers broadcast calls antiphonally, as males do in nature, so that they did not overlap. In trials testing call preferences only, both speakers possessed a static robofrog. In trials testing multimodal preferences, one speaker had a dynamic robofrog, inflating in time with the call, and the other had a static robofrog control. After the acclimation period, the funnel was lifted and the female was free to move around the test arena while the stimuli were broadcast. A choice was recorded when she approached within 5 cm of a speaker–robofrog combination and remained there for 5 s. We alternated the side on which the dynamic robofrog was presented between trials. For all experiments, we only scored responsive females. If a female failed to move for 2 min after the funnel was raised or failed to make a choice after 10 min, we interpreted this as a lack of motivation and discarded the trial from the data set. For detailed methods see Taylor et al. (2008). We recorded female behaviour using an infrared sensitive camera mounted on the ceiling of the chamber (Everfocus EHD500IR, Everfocus Electronics, Duarte, CA, U.S.A.). A video feed allowed simultaneous viewing of the female from outside the sound chamber. We recorded final choice and latency (time to choice). The latency was extracted from digital-videos for the frogs' responses with the recording program Ethovision (Noldus Information Technology, Wageningen, The Netherlands).

To test for side bias, we performed a two-speaker test with a static robofrog placed in front of each speaker; each speaker also broadcast the same acoustic signal (WC at 76 dB SPL). We tested 20 túngara frog females. Ten chose the right speaker and ten chose the left, demonstrating no side bias. The latency was also not significantly different (left side: 105.3 ± 90.9 s, right side: 75.2 ± 79.9 s; unpaired t test: $P = 0.443$).

In part 1 of this study, we tested female frogs in two-choice experiments in which we varied the complexity and therefore potential attractiveness of the calls. Call complexity was increased by adding one or more chucks to the whine. We first tested whether females expressed a preference for increased chuck number. Here females could choose between two speakers, each possessing a static robofrog. Both speakers broadcast a whine and one speaker broadcast a signal with one more chuck than the other (Table 1). Both speakers broadcast calls at the same amplitude, 76 dB SPL.

We then tested the same combination of acoustic stimuli (Table 1, part I, multimodal complexity) with a dynamic robofrog

(vocal sac inflating synchronously with the call) placed in front of the speaker broadcasting one chuck less than the alternative speaker. The opposing speaker had a static robofrog. We determined the change in strength of preference of the test population of females with and without the dynamic robofrog as an estimate of the value of adding the visual cue. This allowed us to measure how the added value of the visual cue is dependent on the differences between the two calls available to females.

In part 2 of this study, we tested female frogs in two-choice experiments where the amplitude of the calls was varied. In this set of experiments, we broadcast the identical call (WC) from each speaker, but varied the amplitude. We tested three different combinations of amplitudes (Table 1, part 2), ranging from 76 to 82 dB SPL. As previously, we placed a static robofrog in front of each speaker. We then repeated these experiments where we placed a dynamic inflating robofrog in front of the speaker broadcast at the lower amplitude. The other speaker retained the noninflating robofrog as a visual control.

Statistical Analysis

Statistics were conducted with Minitab (Version 15.1.30.0, Minitab Inc., State College, PA, U.S.A.) and the online statistical calculator SISA for binomial distributions (Uitenbroek, 1997). To test for differences in the two-speaker tests, we conducted binomial tests with an a priori equal probability value of 0.5 and we report two-tailed mid P values (Agresti, 2007). Latency differences were statistically quantified with a Mann–Whitney U test for nonnormally distributed data. Potential differences in overall preferences between acoustic only and multimodal experiments were calculated with a Fisher's exact test using a Bonferroni adjusted alpha = 0.025 to account for reuse of data. Additionally, we calculated a multimodal value index (V_{multi}) for the multimodal preference/weighting in comparison to the unimodal preference. V_{multi} = proportion of females choosing multimodal–proportion of females choosing the same unimodal stimulus.

Ethical Note

We toe-clipped all animals to avoid testing recaptures on following nights (hence avoiding pseudoreplication). This procedure entailed clipping the distal end of up to one toe per foot (maximum), generating a unique numbering combination for each animal. The toe-clipping procedure followed guidelines of the American Association of Ichthyologists and Herpetologists, and the ASAB/ABS guidelines for treatment of animals in behavioural research. We also consulted the ARRIVE guidelines checklist for animal research reporting. Further, we routinely recaptured frogs, both male and female, at the breeding sites, indicating that the toe-clipping procedure had minimal impact on their survivorship or reproductive behaviour. Male/female pairs were released within 3 h after testing, allowing them the opportunity to reproduce in the wild. The work was conducted under STRI IACUC protocol no. 2011-0825-2014-02 and collecting permit no. SE/A-30-12 from Panama's Autoridad Nacional del Ambiente (ANAM).
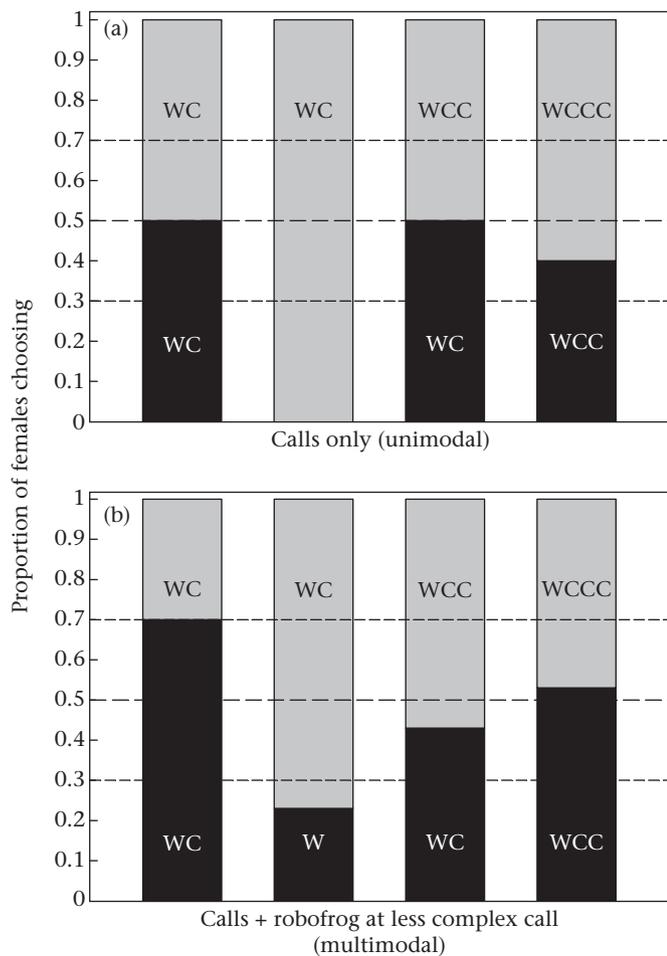
RESULTS

Part 1: How Does Chuck Number Influence Female Mate Choice?

As an overall control, we repeated a previous experiment (Taylor et al., 2008) where we tested female preference for a multimodal signal (robofrog inflating synchronously with a WC) versus the same WC alone. Here we confirmed those results; females again showed a significant preference for the multimodal signal over the
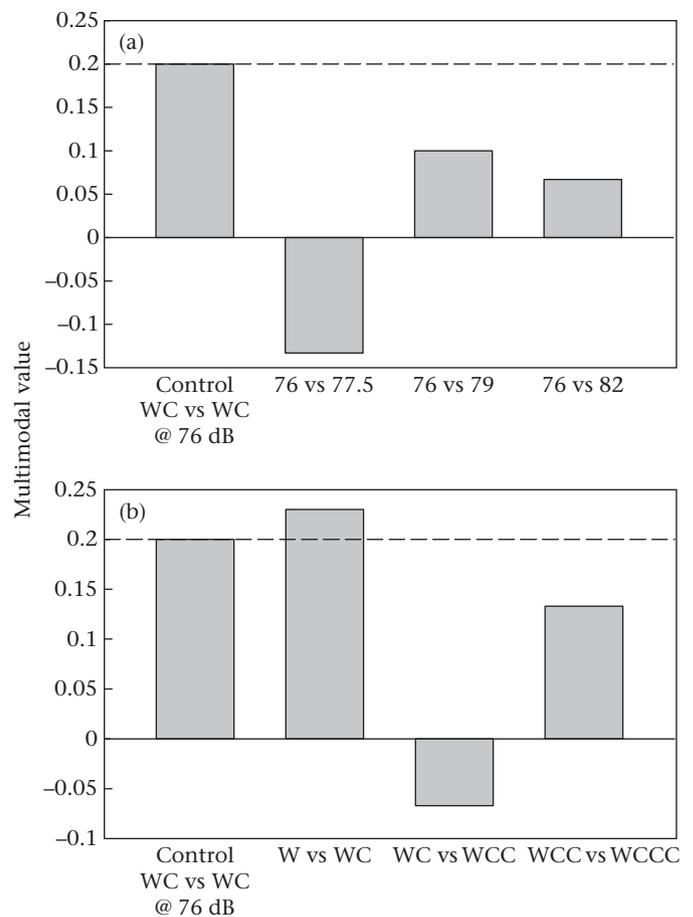
unimodal signal (21:9; two-tailed binomial test: $P = 0.023$). This represents a 20% increase in preference when the visual stimulus of an inflating robofrog was added to the acoustic signal ($V_{multi} = 0.20$).

In the acoustic only tests, 100% of the tested females ($N = 30$) preferred the speaker emitting the WC over the speaker playing the W only (Fig. 3a). Females failed to express any preference for a speaker broadcasting a WCC when the alternative was a speaker broadcasting a WC (15:15; two-tailed binomial test: $P = 0.927$). Similarly, there was no significant preference for the more complex call when we compared WCCC versus WCC (18:12; $P = 0.321$). In sum, appending one chuck to a whine dramatically increased attractiveness of the call; when at least one chuck was present on each call, the addition of more chucks did not further increase attractiveness (e.g. two chucks are not more attractive than one; Figs. 3a and 4a).

When we presented females with a W-robo versus a WC, females still showed a significant preference for the WC (7:23; two-tailed binomial test: $P = 0.003$), as occurred for this stimulus set when only the calls were tested. Females did, however, show a significant increase in preference for the simple W, when it was

Figure 3. Proportion of females choosing a particular stimulus (stimuli indicated within bars). The 0.5 line represents equal choice between the two stimuli. The upper and lower bounds represent level of significance (binomial distribution). (a) Female choice for calls; in bar 2, the W is not shown because no female chose this stimulus. (b) Female choice for the same stimuli when the dynamic robofrog was coupled with the less complex call (black bars). The leftmost bars are the control experiments for acoustic stimuli only (a) and when the dynamic robofrog was then added to one of the identical stimuli in the multimodal test (b). The control tests consisted of a WC versus WC broadcast at 76 dB.
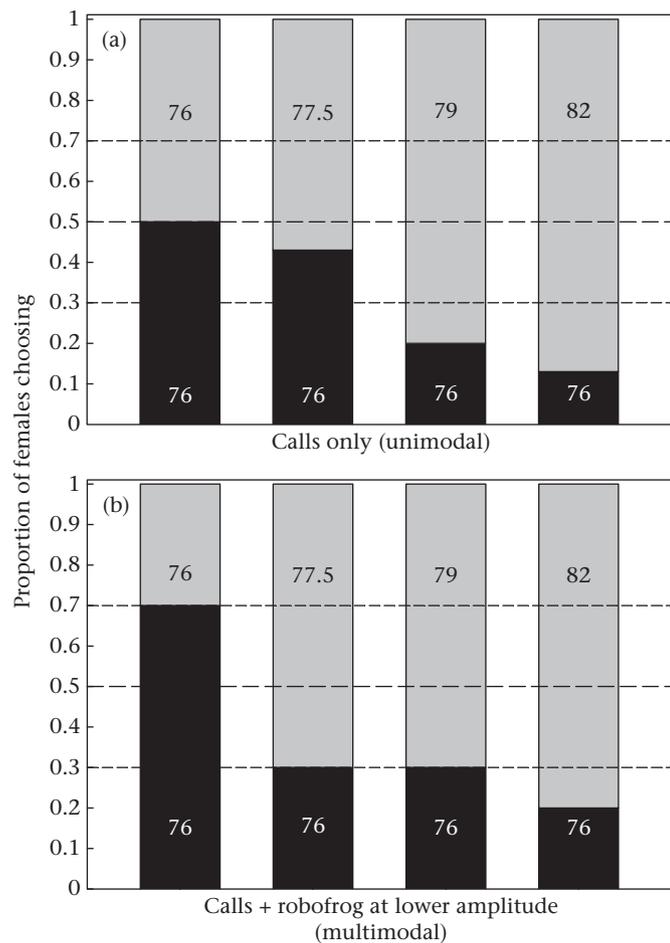

Figure 4. Multimodal value index. V_{multi} = difference between proportion of females choosing multimodal and unimodal stimuli, when the robofrog was coupled with the (a) lower amplitude or (b) less complex call. Values above the zero line indicate preference for multimodal signal. Values below the zero line indicate preference for the unimodal signal; the dashed line indicates the binomial level of significance.

coupled with the visual stimulus of the inflating robofrog ($V_{multi} = 0.23$; Fisher's exact test, unimodal to multimodal comparison: $P = 0.015$).

In the next experiment, we presented females with a WC-robo against an alternative of the more acoustically complex WCC. Females showed no preference for either stimulus (13:17; two-tailed binomial test: $P = 0.417$). For this test, the robofrog did not significantly increase the preference for the less complex call (Fisher's exact test: $P = 0.796$). The preference for the multimodal stimulus in this case was 6.7% lower ($V_{multi} = -0.067$) than the same call without the robofrog. Finally, we pitted a WCC-robo against an alternative call broadcasting a WCCC. Here females did not express a significant preference for either signal (16:14; two tailed-binomial test: $P = 0.72$) and the robofrog did not increase attractiveness for the less complex call (Fisher's exact test: $P = 0.438$). The robofrog increased the preference for the WCC by 13.3% ($V_{multi} = 0.133$). These experiments show that when calls contained zero or one chuck, the addition of the robofrog significantly increased preference for the multimodal signal. When the calls contained two or more chucks, however, the multimodal preference disappeared (Figs. 3b and 4a). Likewise, when we compared the latency of the female's decision with static versus dynamic robofrogs, there was no significant difference (multimodal: 112.4 ± 86.5 s; unimodal: 115.3 ± 101.1 s; Mann–Whitney U test: $P = 0.169$).

Part 2: How Does Amplitude Influence Female Mate Choice?

When females were presented with the identical WC call broadcast at 76 dB versus 77.5 dB, females failed to show a significant preference for the higher amplitude call (13:17; two-tailed binomial test: $P = 0.529$; Fig. 5a). Interestingly, when an inflating robofrog was coupled to the speaker broadcasting at 76 dB, females did express a significant preference for the higher amplitude unimodal stimulus (9:21; binomial test: $P = 0.023$; Fig. 5b) and the robofrog reduced the value of the lower amplitude call ($V_{multi} = -0.133$). A Fisher's exact test revealed no significant difference between the unimodal and multimodal distributions ($P = 0.42$). When we presented females with a 76 versus 79 dB call, females expressed a significant preference for the higher amplitude call (6:24; $P = 0.001$). When we coupled the robofrog to the lower amplitude call in this same acoustic pair, it did not change the overall pattern as females still expressed a significant preference for the louder call (9:21; $P = 0.023$; $V_{multi} = 0.10$). There was no significant difference between the unimodal and multimodal distributions (Fisher's exact test: $P = 0.552$). When we presented females with a 76 versus 82 dB call, the pattern was the same. Females expressed a significant preference for the higher

amplitude call both when the robofrog was absent (4:26; $P < 0.0001$; Fig. 5) and when it was present (6:24; $P = 0.001$; $V_{multi} = 0.067$) and there was no significant difference in the distributions (Fisher's exact test: $P = 0.730$). Most females preferred the higher amplitude signal and the addition of the robofrog did not increase the preference for lower amplitude calls ($V_{multi} < 0.20$; Figs. 4b and 5b).

DISCUSSION

Multimodal communication is common and a number of studies are beginning to elucidate the processes driving the evolution of these complex signals (Higham & Hebets, 2013; Partan & Marler, 1999). Environmental noise, environmental variance, species-specific behaviours, predation and receiver psychology have all been shown to influence the evolution of multisensory signals (see reviews by Bro-Jørgensen, 2010; Candolin, 2003; Hebets & Papaj, 2005; Partan & Marler, 2005; Rowe, 1999). An important remaining question though, is how do receivers weight/value each component of complex multisensory signals when assessing potential mates?

Here we investigated this question by manipulating the complexity of the courtship displays of male túngara frogs. We presented females with stimuli in a series of two-choice tests, where the calls differed in acoustic and visual complexity (number of chucks and robofrog). First, we showed that females prefer complex acoustic signals over simple signals (WC versus W). This corresponds to previous studies where female túngara frogs expressed a strong preference for a WC over a W (Bernal, Page, Argo, & Wilson, 2009; Gridi-Papp et al., 2006; Ryan, 1985). Next, we compared female preferences for acoustic signals of varying complexity in the absence of a moving visual stimulus (each call containing one to three chucks); this revealed no preference for more complex calls. This is also consistent with previous studies when playbacks were conducted at less than 90 dB SPL (Akre & Ryan, 2010; Akre, Farris, Lea, Page, & Ryan, 2011). Our studies were conducted at 76 dB SPL, and further confirm that preference for increasing chuck number is amplitude-dependent and thus in nature should vary with the female's distance from the source. At lower amplitudes, such as those we tested in the present study, females only discriminate between the presence and absence of chucks (Bernal et al., 2009; Ryan, 1985).

The lack of female preference for increasingly complex calls is likely to be due to the smaller relative difference between the two signals, corresponding to Weber's law of proportional differences. The whine and chuck each primarily stimulate a separate inner ear organ (the amphibian papilla and basilar papilla) as each is tuned to specific frequencies; the whine alone results in a substantial reduction in acoustic stimulation compared to when both whine and chuck are present (Ryan, Fox, Wilczynski, & Rand, 1990). When each call contains at least one chuck, the acoustic difference between the calls is relatively small. Thus, continued call elaboration decreases the relative stimulus ratio difference between the calls and has been shown to predict female preferences for calls of varying complexity (Akre et al., 2011).

The influence of multimodal signals on female túngara frog mate choice has been well documented; when presented with two identical calls, the presence of the visual cue, a dynamic vocal sac, increases the attractiveness of the call (Rosenthal, Rand, & Ryan, 2004; Taylor et al., 2008). Further, the salient portion of the visual component is the movement of the vocal sac (Taylor et al., 2008) and the temporal relationship between vocal sac movement and call production is also important for mate attraction (Taylor, Klein, Stein, et al., 2011; Taylor & Ryan, 2013). We confirmed these results again here. Our phonotaxis results show that at 76 dB

Figure 5. Proportion of females choosing a particular stimulus (stimuli indicated within bars). The 0.5 line represents equal choice between stimuli. The upper and lower bounds represent level of significance (two-tailed binomial distribution). (a) Female choice for calls only. (b) Female choice for the same stimuli when the dynamic robofrog was coupled with the lower amplitude (76 dB) call (black bars). The leftmost bars are the control experiments for amplitude only (a) and when the dynamic robofrog was added to one of the identical stimuli in the multimodal test (b). The control test consisted of a WC versus WC broadcast at 76 dB.

SPL, females do not find additional chucks more attractive when each call has at least one chuck present. When females were presented with the identical call at each speaker (WC), but a robofrog with a synchronously inflating vocal sac was placed at one speaker, the visual stimulus increased the overall attractiveness of the call at that speaker. So if females find a WC + robofrog more attractive than just a WC, then adding more chucks to complex calls renders them equally attractive (e.g. three versus two chucks or two versus one chuck). We thus predicted that adding a robofrog to an experiment with equally attractive calls would result in preference for the multimodal stimulus. This was not the case. In tests where competing acoustic signals contained two or three chucks, the robofrog failed to increase the relative attractiveness of the acoustic signal with fewer chucks. The addition of the visual cue to the vocalization only increased attractiveness of the call when it contained one or zero chucks (Fig. 4a). Thus female preference for the multimodal signal is not consistent across variations in call complexity. If females expressed a preference for the multisensory signal in all cases, it would indicate that the visual stimulus provides simple 'enhancement' of the acoustic signal (sensu Partan & Marler, 1999). The variable preference across signal complexity makes it clear that the audiovisual integration is not merely additive (e.g. providing simple enhancement).

Call amplitude is known to influence female mate choice in anurans; higher amplitude calls are almost always more attractive (Ryan & Keddy-Hector, 1992). We demonstrated this phenomenon here as well. It is not surprising that when one signal was 3 or 6 dB higher in amplitude, female túngara frogs preferred the louder signal even when an inflating robofrog was placed with the lower amplitude call. What is interesting, however, is that females initially did not express a significant preference for the signal that was 1.5 dB louder. When the robofrog was coupled with the lower amplitude signal, females then expressed a significant preference for the higher amplitude signal. Although the Fisher's exact test revealed no difference between the unimodal and multimodal distributions, it is important to note that categorical analyses have relatively lower power to detect differences. A power analysis revealed that our sample sizes of $N = 30$ yielded only 20% power to detect a difference between the tests. Further, our work in the túngara frog has shown that experimental results tend to be strongly repeatable (e.g. this study, Gridi-Papp et al., 2006; Taylor & Ryan, 2013). The significant preference that females expressed for the +1.5 dB call in the presence of the robofrog suggests that females may not easily make acoustic distinctions between signals that differ by only 1.5 dB, but that the addition of the visual cue increases females' ability to distinguish between these relatively small differences. In hearing threshold tests, a similar effect occurs in humans (Lovelace, Stein, & Wallace, 2003). When an irrelevant light source is flashed concurrently with a tone, human listeners improve hearing threshold detection (Lovelace et al., 2003). Thus, the addition of the robofrog to the lower amplitude signal may improve auditory signal discrimination.

Taylor and Ryan (2013) demonstrated that temporal rearrangements of acoustic and visual signal components alter the perception of the signal. Specifically, an unattractive signal combined with a neutral signal (minimally attractive) can restore attractiveness, even when the combined multisensory signal is one that females would normally not experience. That study demonstrated that the way in which female túngara frogs combine multisensory signals is nonlinear. The results we report here are surprising, particularly that visual signal components enhance acoustic signals with one or zero chucks, but this enhancement disappears with increasing call complexity. Importantly, our results provide further evidence that audiovisual integration occurring during multisensory signal processing does not necessarily generate a corresponding linear change in behaviour relative to unimodal signals.

Several important questions remain unsolved (Partan, 2013). First, what are the underlying neural processes governing multisensory integration and are these fundamentally different from unimodal signal integration? The work we report here, coupled with behavioural data in cats, suggests that multi- and unisensory integrations engage different computations, even in simple detection or discrimination tasks (Gingras, Rowland, & Stein, 2009). A second important question is, how do individual sensory systems respond to changes in stimuli, and how do these differ across taxa? Recent work in auditory and visual neurons shows that they respond in a nonlinear fashion to changes in stimuli (Kremkow et al., 2014; Nagel & Doupe, 2008). Perhaps this explains why neural processing of multiple nonlinear systems yield unpredictable behavioural results (termed 'emergence' Partan & Marler, 2005; and 'intersignal interaction' Hebets & Papaj, 2005). This nonlinear dynamic may be critically important for the evolution of signal diversity. It is well established that receivers of signals play an important role in shaping their evolution (Guilford & Dawkins, 1991; Ryan, 1990). If receivers recognize complex conspecific signals only within narrow parameters, then the evolution of signal characteristics will be constrained. On the other hand, if receivers are widely permissive about the signals they recognize, the signal may be free to evolve under sexual selection. Thus, understanding how animals across diverse taxa integrate complex signals will be critical for our understanding of signal evolution.

One question asked in this issue on multimodal signal complexity is, can similarities in cognitive skills underlying communicative complexity be explained by homology or convergent evolution? Although few would argue that frogs and humans possess similar cognitive skills, some of the behavioural correlates between the species (e.g. incorporating visual stimuli into acoustic discrimination tasks) suggest some basic similarities in audiovisual integration. Frogs and humans last shared a common ancestor about 359 MYA (Hedges, Dudley, & Kumar, 2006) and data show that hearing mechanisms in the two groups have arisen independently since the divergence (Christensen-Dalsgaard & Carr, 2008). Thus similarities in audiovisual integration have almost certainly resulted from convergent evolution, probably a result of evolution solving the problem of communicating in noise.

Available behavioural (Joshi et al., 2011; Klein et al., 2012) and neurobiological techniques (Hoke, Ryan, & Wilczynski, 2007; Wong & Cummings, 2014) can provide important insights into the processes by which receivers' brains integrate signal components and generate behavioural responses. Developing standardized empirical frameworks that can be used to fully investigate signal interaction, particularly in a comparative approach, hold the greatest promise for providing a comprehensive understanding of complex signal evolution (Hebets et al., 2016; Smith & Evans, 2013; Wilkins, Shizuka, Joseph, Hubbard, & Safran, 2015). As outlined in Miller and Bee (2012), it is time for research in receiver psychology to integrate neural (both peripheral and central) and behavioural techniques in a comparative framework.

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