Behavioral Ecology

The official journal of the ISBE
International Society for Behavioral Ecology

Behavioral Ecology (2016), 27(2), 669–676. doi:10.1093/beheco/arv204

Original Article

Vocal responses to noise reveal the presence of the Lombard effect in a frog

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Received 14 June 2015; revised 29 October 2015; accepted 30 October 2015; Advance Access publication 6 December 2015.

Many animal communication systems have evolved signal flexibility depending on environmental conditions. A common strategy of vocal communication is to increase amplitude in response to increasing noise levels. This phenomenon, known as the Lombard effect, is a widespread trait among mammals and birds. Anurans are a major group with many species that rely heavily on acoustic signals for sexual communication. Although these species often communicate in noisy environments, the presence of the Lombard effect in frogs remains unclear. We exposed male túngara frogs (*Physalaemus pustulosus*) to different noises with and without playback of conspecific calls. Males increased call amplitude, call rate, and call complexity in response to low-frequency noise (overlapping the species' call range) but not to high-frequency (nonoverlapping) noise. Vocal amplitude increased linearly with noise level demonstrating that túngara frogs exhibit the Lombard effect, and we discuss why different frog species may differ in their control over vocal amplitudes. Furthermore, we found the overall effect of noise to be similar to the effect of conspecific call playback. We speculate that vocal amplitude control may have evolved primarily as a response to increased competition at the cocktail party, similar to the way humans raise their voice when in a heated debate, and subsequently as a strategy to deal with background noise more generally.

Key words: **ambient noise, eavesdropping, Lombard effect, sexual selection, signal plasticity.**

INTRODUCTION

Acoustic signals can transmit over long distances through varied habitats and are used throughout much of the animal kingdom to attract mates and to defend resources (Tyack 1998; Zelick et al. 1999; Marler and Slabbekoorn 2004; Bradbury and Vehrencamp 2011). Selection for optimal detection has led to the evolution of signals such as bird songs or frog calls with spectral and temporal traits that often match acoustic properties of their habitats (Morton 1975; Wiley and Richards 1978; Dubois and Martens 1984; Feng et al. 2006; but see Kime et al. 2000). Many habitats, however, show large fluctuations in acoustic noise levels due to biotic and abiotic factors such as wind, rain, insect choruses, as well as human activities, and the risk of masking by noise favors an animal's ability to adjust their signals in a real time (Lengagne and Slater 2002; Brumm and Slabbekoorn 2005; Barber et al. 2009; Halfwerk and Slabbekoorn 2015). Strategies for flexible signal adjustment can range from timing shifts (e.g., avoiding temporal overlap; Zelick and Narins 1985; Brumm 2006; Arroyo-Solis et al. 2013) to spatial shifts (Halfwerk et al. 2012) to structural shifts (e.g., increasing signal element length or using different element types that reduce masking impacts; Halfwerk and Slabbekoorn 2009; Pohl et al. 2009). One major signaling strategy involves increasing amplitude in response to increased noise levels, a phenomenon known as the Lombard effect and described as early as 1911 for humans (Lombard 1911; Brumm and Zollinger 2011). The Lombard effect allows real-time signal-to-noise ratio adjustment, thereby providing optimal signal detection, localization, and discrimination (Lohr et al. 2003; Pohl et al. 2009). Interestingly, the Lombard effect seems to be widespread among animals that occupy very different habitats (e.g., whales, monkeys, bats, fowl, and songbirds; Potash 1972; Sinnott et al. 1975; Cynx et al. 1998; Brumm et al. 2004; Hage et al. 2013; Dunlop et al. 2014), suggesting that it is a common solution to a common problem.

The majority of data on the Lombard effect has come from mammals and birds (Brumm and Zollinger 2011; Schuster et al. 2012). Anurans are a major group in which many species rely heavily on acoustic signals to attract females and to defend resources against rivals (Gerhardt and Huber 2002). Several species of frogs have been shown to alter call amplitudes during noise exposure (Penna et al. 2005; Cunnington and Fahrig 2010), but the degree to which they demonstrate the Lombard effect is debated (see

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Love and Bee 2010; Schwartz and Bee 2013). Furthermore, previous studies may also have ignored important technical issues that affect signal amplitude measurements under fluctuating levels of background noise, such as orientation of the signaler in relation to the microphone and interference between signals of interest and background noise, which requires a subtraction method to avoid overestimating signal amplitude (see Brumm and Zollinger 2011 for a more detailed account of these methodological issues). The only study that accounted for the before mentioned issues showed no increase in call amplitudes in relation to noise levels (Love and Bee 2010), thus questioning whether the Lombard effect is present in frogs (Brumm and Zollinger 2011; Schuster et al. 2012).

The sexual advertisement behavior of the túngara frog (*Physalaemus pustulosus*) provides an ideal situation to test for signal flexibility in response to changing conditions. Males of this species can call alone or in groups of different sizes to attract females during the Panamanian rainy season (May–December). Males occasionally call under very high noise levels, in particular when conspecifics are calling nearby. Individual male calls can be as loud as 82 dB sound pressure level (SPL) at a distance of 50 cm (Ryan 1985), and in a high-density chorus, the background sound levels may reach well above 94 dB SPL (measured at 10 cm or the average intermale distance). Furthermore, calling males are under heavy risk of predation and parasitism from a large range of animals, including fringe-lipped bats (*Trachops cirrhosus*; Tuttle and Ryan 1981; Akre et al. 2011; Halfwerk, Jones, et al. 2014) and blood-sucking flies (*Corethrella* sp.; Bernal et al. 2006). Perhaps as a consequence of opposing selection pressures, males have evolved vocal plasticity (Page et al. 2013). Males always produce a simple call consisting of a frequency-modulated harmonic, known as a “whine” (Figure 1a). When males are visited by fertile females or challenged by rival males; however, they will add short amplitude-modulated pulses known as “chucks” (Ryan 1980, 1985; Bernal, Akre, et al. 2009; Akre and Ryan 2011; Halfwerk, Page Rachel, et al. 2014). Males can add up to 7 chucks to their whines, and these are known to increase attractiveness to females as well as aggressiveness to males (Bernal, Akre, et al. 2009; Akre et al. 2011). However, eavesdroppers are also increasingly attracted to these complex calls, in much the same way as the intended receivers (Bernal et al. 2006; Akre et al. 2011).

Here, we determine if vocal plasticity of túngara frogs also involves signal amplitude. This would further allow males to optimize their signals depending on the relative chances of attracting mates or predators. Furthermore, amplitude plasticity in response to increased noise levels would reveal the Lombard effect to be present in at least 1 species of frog, which would be interesting given that most frogs rely heavily on acoustic communication. We quantified natural variation in background sound levels at various different breeding sites throughout the breeding season and used our recordings to design several different sound treatments. These treatments assessed the importance of intensity, spectral content, and masking of different background sounds on change in vocal response by calling frogs, most importantly signal amplitude.

MATERIALS AND METHODS

We recorded ambient sounds at 12 different breeding sites in Soberanía National Park, Panama, between June and December 2012 using automated recorders (SM2, Wildlife Acoustics, Inc.). Two recorders sampled 30 s of sound at 15-min intervals, starting half an hour before sunset and ending 4 h later every evening. Recorders were switched randomly between sites every 3–5 days.

We conducted experiments 1 and 2 between June and September 2014 at the Smithsonian Tropical Research Institute lab in Gamboa, Panama. An additional experiment (experiment 3) was conducted between March and April 2012 in the laboratory at the University of Texas at Austin. In Panama, male frogs were collected between 1 and 3 h after sunset and toe-clipped for individual recognition (following guidelines of the American Society of Ichthyologists and Herpetologists). Males were released at their respective capture sites after testing. In Texas, we tested males from a captive colony at the University of Texas at Austin. All research conducted in Panama complied with IACUC protocols from the Smithsonian Tropical Research Institute (2014-0805-2017). We obtained all required permits from the Government of Panama. Research conducted in Austin, TX, was approved by IACUC at the University of Texas at Austin (AUP-2011-00023, AUP-2010-00014).

Males were tested in an experimental pool (Ø 50 cm) filled with 4 L of rainwater in a hemi-anechoic chamber under infrared lighting (in Panama in an AC-controlled room held at constant temperature of 27 °C) or in sound-attenuated boxes (dimensions: $46 \times 33 \times 30.5\,\mathrm{cm}^3$) in dechlorinated tap water–filled bowls (4-cm diameter, 1.5-cm water depth). In Panama, males were placed in the center of a pool inside a small cage (Ø 7 cm; 12-cm height). The cage consisted of nylon threads (0.05 mm) evenly spaced every 0.5 mm. A camera (mini 1/4″ CCTV Camera; 2.8-mm lens) was mounted on top of the cage to monitor male behavior. Males were stimulated prior to testing with chorus sounds until they started calling. A 5-min silent period preceded the first experimental trial and 1-min silent periods were used in between trials. In Texas, males could move voluntarily into the water and start calling. When a male was calling for 2–5 min in response to a synthetic whine–chuck stimulus, we would start the noise exposure.

We conducted 3 different experiments during which males were exposed to different types of sounds for 1 min. For experiments 1 and 2 (conducted in Panama), male calls were recorded at 45° (from the water surface) at a distance of 50 cm with a microphone setup (G.R.A.S. microphone amplified by 20 dB by G.R.A.S. amplifiers connected to a Avisoft sound gate [sampling rate of 50 kHz] and desktop PC). The microphone was calibrated prior to each experiment using a G.R.A.S. 42 AB tone generator. For experiment 3 (conducted in Texas), males were recorded on a desktop pc (with a Creative SoundBlaster, type sb1290, sampling rate of 44.1 kHz) with a miniature condenser-type bullet microphone (MG Electronics, 20 Hz–16 kHz frequency response) positioned 90° from the water surface and 13 cm from the frog. These recordings were not calibrated and were therefore analyzed using relative amplitude levels. This approach allows comparison of amplitude levels within but not across individuals. Males were recorded during spontaneous calling (absence of conspecific sound playback) or during evoked calling. We evoked calling by broadcasting either chorus sounds at different sound levels or a single call from a speaker (Peerless TG9FD10-08, 3.5 inch, 8 Ω, which has less than 4 dB difference in spectral profile between 0.1 and 10 kHz) placed 20 cm behind the focal frog and 65 cm from the microphone. For the single call, we used a synthetic signal consisting of a whine plus one chuck broadcast at 0.5 calls/s and 82 dB SPL (re. 20 µPa at 50 cm, measured with Extech SPL-meter type 407764, set to C-weighted, fast and max). Multiple chorus recordings from different breeding sites around Gamboa were combined to create a chorus sound file with only small fluctuations in the amplitude envelop (and therefore similar to the amplitude envelop of white noise).

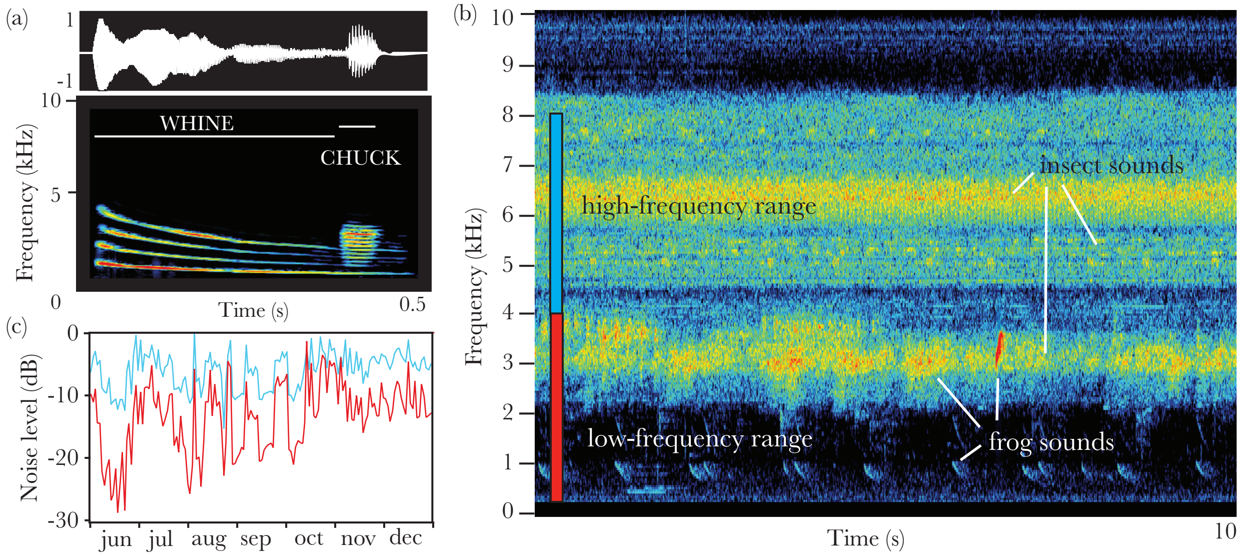

Figure 1

Ambient noise levels within and outside túngara call frequency range. (a) Example recording of a túngara frog call, upper panel: amplitude envelop; lower panel: spectrogram. Male túngara frogs always produce a downward frequency-modulated harmonic element known as the whine. Males can add 1–7 amplitude-modulated pulses known as chucks to the whine. (b) Example of sound recorded at a breeding site (7:45 PM on 6 July 2012). The low-frequency range (0.1–4.0 kHz) overlaps with the main frequency range of the túngara frog's call; the high-frequency range (4.0–8.0 kHz) does not. Noise from both frequency ranges was used for noise analyses and experiments. Noise levels in the high-frequency range are mainly influenced by rain and insect sounds. Noise levels in the low-frequency range are also mainly influenced by rain and insect sounds, but in addition by conspecific and heterospecific frog sounds. (c) Noise levels in the high- (blue) and low- (red) frequency range recorded over the breeding season at 12 different sites along a 20-km transect. Data points on each night are the average of 32 recordings of 30 s each, starting half an hour before sunset and recorded in 15-min intervals for 4 h, totaling 4050 recordings across the breeding season.

Noise stimuli

Noise stimuli consisted either of band-pass–filtered white noise in a low-frequency range (0.1–4.0 kHz; overlapping with the frequency range containing most of the acoustic energy of the túngara frog's call, here after referred to as "low-frequency noise"), a band-pass–filtered high-frequency range (4.0–8.0 kHz, here after referred to as "high-frequency noise"), or white noise filtered with the spectral shape of a real chorus recording (here after referred to as "chorus noise"). Noise was played through a full-range speaker (Peerless TG9FD10-08, 3.5 inch, 8 Ω) at different levels of intensity, ranging from 54 to 94 dB SPL at the position of the focal male (Extech SPL-meter, same settings as call playback). Noise playback had a 5-s ramp on.

In experiment 1, we exposed males to low- and high-frequency noise set to 82 dB SPL and a control condition of no background noise. Furthermore, males were tested either on spontaneous calling or on evoked calling by starting conspecific call playback 5 s after noise onset. In experiment 2, we tested male evoked calling during exposure to low-frequency noise at 54, 70, 76, and 82 dB SPL. In experiment 3, males were exposed to either chorus sounds or chorus noise at 8 different levels ranging from 70 to 94 dB SPL. A chorus sound file was created by concatenating several high-intensity chorus recordings, thus creating a file with a temporal envelope similar to white noise. Chorus noise was created by filtering white noise with the spectral envelope of a real chorus recording (following Schwartz and Gerhardt 1998). In experiments 1 and 2, experimental conditions were randomly applied, whereas in experiment 3, sound levels were increased in steps of 3 dB, starting at 70 dB SPL, or decreased in steps of 3 dB, starting at 94 dB SPL.

Analyses

We analyzed ambient sound recordings in the low (0.1–4.0 kHz; containing most of the energy of the frog's mating call) and high (4.0–8.0 kHz) frequency range. Sound recordings were band-pass filtered in Matlab 8.0 (the Mathworks, Inc.) and the acoustic energy in each band was calculated using the root-mean-square (RMS) method. The RMS values were averaged over each recording night and plotted over the season to assess variation in sound levels. We visually and acoustically inspected sound sources on nights with high RMS levels for each of the 12 sites.

Male calls were analyzed in SASLab Pro (Avisoft Bioacoustics). The call of a male túngara frog consists of relatively long-frequency–modulated harmonic tones, known as the "whine," and an amplitude-modulate harmonic short pulse, known as "chuck," which can be facultative added to the whine (see Figure 1). The number of whines and chucks produced during trials was counted manually by observing videos and listening to recordings. The total number of whines counted over the 1-min trial period was used to calculate call rate (calls/second), and number of chucks was used to calculate call complexity (number of chucks/number of whines). For experiment 1 and 2, we tested 25 and 24 males in total, respectively. For both experiments, 5 males did not respond after repeated stimulation and were omitted from the analyses. For experiment 3, we tested 30 males and omitted 18 unresponsive males from the analyses (typical success rate for colony frogs).

Amplitude measurements were calibrated using a G.R.A.S. tone generator that played a 1 kHz tone at 114 dB SPL. Peak and RMS amplitude values (in Volts) were obtained on a linear scale by the program Avisoft (see Specht 2004 for details), and we divided these values by the value recorded for the calibration tone (recorded using a gain of 0 dB for each individual separately). Values were transformed to log scale after calculations and statistics, and we added 94 dB to get SPL values. A major issue concerning amplitude measurements under noisy conditions is that signal and noise add when both sounds overlap in frequency. We dealt with this issue in 2 ways. First, for experiments 1 and 2, we subtracted (linear) amplitude values of noise from (linear) amplitude values of the signal using the RMS method (see for more details below). Furthermore, we carried out playback experiments with a reference signal set at 82 dB SPL at 50 cm under various levels of noise exposure to assess the reliability of the noise subtraction method. A noise exposure level of 82.0 dB SPL at the position of the frog resulted in exposure levels of 72.2 dB at our microphone. Under these conditions, an 82.0 dB reference signal, for example, was recorded at 82.3 dB and after noise subtraction was estimated to be 81.8 dB, thus showing that the noise-subtracting method was quite reliable. We could not reliably measure the signals above noise treatment level of 82 dB for 3 different reasons. First, we could not detect all calls on the spectrogram; second, noise level was occasionally higher compared with signal level, leading to irrational numbers after subtraction; and third, at low signal-to-noise ratios the noise subtraction underestimated signal level. For experiment 3, we dealt with this limitation using a second approach: We selected the first call produced by a male within 5 s after noise was switched off (thus avoiding an additive effect of noise on the recorded signal levels). All males produced calls within 1 s after noise offset.

Túngara frogs call for tens to hundreds of seconds, and only the first 10 calls are typically lower in amplitude. We assume calls recorded within a second after noise offset to represent similar amplitude values produced shortly before.

For experiment 1, we selected complete calls from the spectrogram using a selection window with a length of 500 ms. We selected 3 calls at random from the midsection of the noise exposure treatment and verified male calling on videos to ensure we did not bias our sample by omitting very low-amplitude calls. For experiment 2 we selected the whine and chuck part of the call separately using a window length of 400 ms and selected the 1st, 5th, and 10th call from a recording, again using videos for reference.

We used an automated parameter setup in SASLab Pro (fast Fourier transform = 512, overlap = 98%, element detection threshold set to −20 dB below peak amplitude, gap length set to 20 ms) to measure peak frequency, peak and RMS amplitude of calls or call elements. Peak and RMS amplitude values were taken from the amplitude envelope using a linear scale by the program and peak frequency was taken from the power spectrum. For each recording, we measured peak and RMS amplitude of a small portion of noise (~500 ms, starting before call onset) and subtracted noise amplitudes from signal amplitude using the RMS method ($\sqrt{[\text{signal}^2 - \text{noise}^2]}$), on a linear scale. For experiment 1, we only analyzed peak amplitudes as RMS amplitude covaried with overall call duration and noise may have affected the amount of signal to be analyzed.

Statistics

Call parameters were analyzed with generalized linear mixed models in R. All models were analyzed using an identity link-function with normal error distribution (in the package lme4), except for call complexity models, which were analyzed with a Poisson distribution and log-link function. We visually checked the assumptions of the linear mixed model by inspecting a quantile–quantile plot of the residuals to check for normality and by plotting the residuals against the fitted values and each of the explanatory variables. For experiment 1, the mixed models included noise treatment (low, high, and control) and call playback as fixed factors and trial number and male identity as random intercepts. We tested for significant fixed effects and their interaction using likelihood-ratio tests. Nonsignificant effects were excluded from final models. We further explored significant effects using independent contrast between the 3 noise treatments. For significant interactions, we reran models and used independent contrast between the 6 different treatment groups. For experiment 2, the mixed models included noise level as fixed factor and trial number and male identity as random intercept. Furthermore, we added male identity as random slope to assess individual responses to noise. For experiment 3, models included sound treatment (chorus sound or noise) and noise level as fixed factor and male identity as well as trial number as random intercepts. Amplitude values were transformed to log scale after statistical analyses for illustrative purpose.

RESULTS

Ambient sound levels showed substantial fluctuations in both low-frequency (0.1–4.0 kHz, overlapping call frequency) and high-frequency ranges (4.0–8.0 kHz, nonoverlapping range; Figure 1b,c). Sound levels could differ as much as 30 dB between nights. Rain and water dripping from leaves were a common source of noise with acoustic energy present across both frequency ranges. In the high-frequency range, insect sounds were by far the most abundant source of biotic noise. In the low-frequency range, biotic noise was mostly generated by insects, as well as conspecific and heterospecific frog species (see Figure 1b for an example of ambient sound recording).

In the first experiment, we tested whether the noise present in either of these frequency ranges influenced calling behavior of túngara frogs during spontaneous and evoked calling. We exposed males to no noise and low- and high-frequency–filtered white noise, all with and without conspecific signal playback (thus resulting in 6 experimental treatments). We found strong support for an effect of noise treatment, signal playback, and their interaction on models containing peak amplitude and call complexity as response variable ($N = 20$; degrees of freedom [df] = 2; peak amplitude model; $\chi^2 = 10.26$, $P = 0.0042$; complexity model; $\chi^2 = 13.30$, $P = 0.0013$; Figure 2a–c). We used post hoc independent contrast to follow up on main effect of noise treatment as well as the interaction with signal playback. Males increased amplitude in response to low-frequency noise (z value = 9.53; $P < 0.001$) but not to high-frequency noise (z value = 0.528, $P = 0.86$). Furthermore, call complexity increased during low frequency (z value = 3.03, $P = 0.005$) and not during high-frequency noise exposure (z value = −0.71, $P = 0.75$). Call rate showed an effect of noise treatment ($\chi^2 = 16.66$, df = 2, $P = 0.0023$) and signal playback ($\chi^2 = 53.31$, df = 1, $P < 0.001$). We did not find an interaction effect between these 2 treatments (call rate model: $\chi^2 = 1.92$, df = 2, $P = 0.38$). Again, only low-frequency noise led to an increase in call rate (z value = 4.09; $P < 0.001$), whereas high-frequency noise exposure had no effect (z value = 0.43, $P = 0.90$). Signal playback led to an increase in peak amplitude, call complexity, and rate during both control and high-frequency noise exposure (Figure 2) but had no additive effect during low-frequency noise, except for call complexity (Figure 2). Peak frequency did not change in response to noise ($\chi^2 = 4.17$, df = 2, $P = 0.12$) or call playback ($\chi^2 = 1.31$, df = 1, $P = 0.25$). Further post hoc exploration showed that under control conditions peak amplitude was highly correlated with other call parameters (call rate: $r = 0.43$, $P < 0.001$; call complexity: $r = 0.53$, $P < 0.001$; peak frequency: $r = 0.40$, $P < 0.001$).

In the second experiment, we further explored the relationship between low-frequency noise and call amplitude. We focused on the effect of different noise levels on the different call elements (whine and chuck), on different amplitude parameters (acoustic energy,

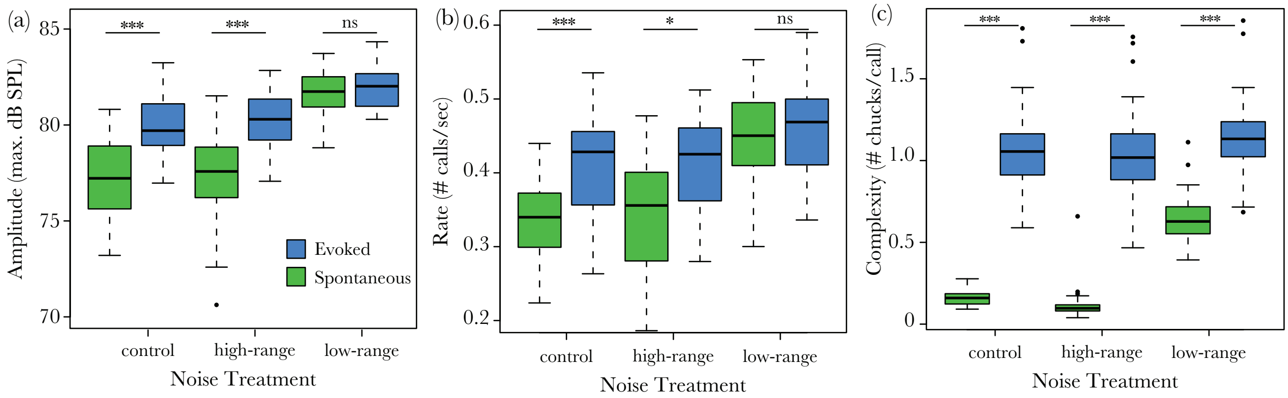

Figure 2

Effect of noise on spontaneous and evoked calling by male túngara frogs. (a) Males increase call amplitude in response to playback of low-frequency noise (overlapping in frequency with their own call) but not in response to high-frequency noise (outside call frequency range). Males also increase amplitude when their calling is evoked by the playback of a conspecific acoustic signal. Low-frequency noise has no additive effect on call amplitude during evoked calling. Males also increase other call parameters in response to low-frequency noise exposure, such as call rate (b) and call complexity (c). High-frequency noise has no effect on any of these parameters. Male call complexity (c) shows an additive effect of noise during evoked calling, demonstrating that males can still perceive the conspecific acoustic signal under high levels of masking noise (signal-to-noise ratio was set to 0 dB based on peak amplitude).

or RMS amplitude, as well as peak amplitude), and on call order. Call order had a clear effect on call amplitude of the whine (RMS amplitude: $N = 19$; df = 1, $\chi^2 = 139$, $P < 0.001$; peak amplitude: $\chi^2 = 112$, $P < 0.001$), with the first call typically being much lower in amplitude compared with subsequent calls (Figure 3a). Males did not always produce chucks during the first few calls and the analyses of chuck amplitude therefore did not include call order. Both RMS ($\chi^2 = 37.1$, $P < 0.001$) as well as peak amplitude of the whine part showed a linear increase with noise level ($\chi^2 = 119$, $P < 0.001$; Figure 3b). Furthermore, RMS ($\chi^2 = 29.1$, $P < 0.001$) as well as peak amplitude ($\chi^2 = 7.12$, $P = 0.008$; Figure 3c) of the chuck part increased linearly with noise level. Individuals showed the strongest response in peak amplitude of the whine and increased their calls on average 2.7 dB (0–10 dB range; Figure 3b) over the 24 dB increase in noise level.

In the third experiment, we compared male responses to chorus sounds (of a natural frog chorus) versus chorus noise (white noise filtered with spectral envelope of a chorus recording). For this experiment, we analyzed the first call produced after sound offset. The amplitude of the whine ($N = 12$; df = 1; RMS: $\chi^2 = 13.9$, $P < 0.001$; peak: $\chi^2 = 11.4$, $P < 0.001$; Figure 4a) and the chuck (RMS: $\chi^2 = 9.93$, $P = 0.002$; peak: $\chi^2 = 8.70$, $P = 0.003$; Figure 4b) increased with exposure levels, but we did not find any significant difference between the 2 treatments (Figure 4; all $P > 0.2$).

DISCUSSION

The Lombard effect is a common strategy among birds and mammals to increase signal-to-noise ratio of acoustic signals in high levels of background noise. Despite the reliance of frogs on acoustic signals for mate recognition, and given that frogs also are often confronted by high noise levels, it is surprising that there have been few investigations of and no conclusive evidence supporting a Lombard effect in these highly vocal animals.

We tested for a direct effect of noise on male calling behavior in our initial experiment. Males increased call rate, call complexity, and peak amplitude in response to masking low-frequency noise, but not to non-masking high-frequency noise. The lack of impact of high-frequency noise was not surprising giving the relatively low sensitivity of our study species for this frequency range (see Wilczynski et al. 2001, for an electrophysiological audiogram recorded in the midbrain). A second experiment, using RMS amplitude as an additional measurement, confirmed the positive direct effect of noise on signal amplitude. In a third experiment, we measured signals directly after noise was terminated and found an indirect effect of noise on amplitude that was not biased by any measurement errors. Taken together, our 3 independent experiments, using 3 independent measurements, provide conclusive evidence for the Lombard effect in túngara frogs.

Amplitude regulation has been reported for several leptodactylid frogs (Lopez et al. 1988; Penna et al. 2005) and even for fish (Holt and Johnston 2014), but recent debate on methodological issues has evoked caution as to the presence of the Lombard effect in taxa other than mammals or birds (Love and Bee 2010; Schwartz and Bee 2013). An important methodological issue regarding signal amplitude measurements in a noisy background concerns possible additive effects when combining sound waves of similar frequencies and amplitudes. Most individuals in our experiments exhibited a Lombard effect in the range of 1–3 dB with every 10 dB increase in noise. Furthermore, during experiments 1 and 2 some individuals called only a few dB above the recorded noise levels. We used a noise subtraction method to ensure that our signal amplitude measurements were not simply an artifact of our recording setup. Using a reference signal of known amplitude, we estimated that this noise-subtracting method is reliable and provides conservative estimates under low signal-to-noise ratios. Another technical issue concerns directionality, as many animals (e.g., Patricelli et al. 2007), and túngara frogs in particular (Bernal, Page, et al. 2009), are not omnidirectional sound sources. Túngara frogs focus most of their acoustic energy between 45 and 90 degrees in an angle to the water surface (Bernal, Page, et al. 2009) and recording frogs from these angles therefore assured that our amplitude measurements were not confounded by the directionality pattern.

Despite technical issues and limitations, amplitude regulation in response to noise might be more widespread in frogs than currently appreciated. Studies on anthropogenic noise, for instance, have shown that frogs increase spectral call characteristics in response to low-frequency urban noise (Parris et al. 2009; Cunnington and

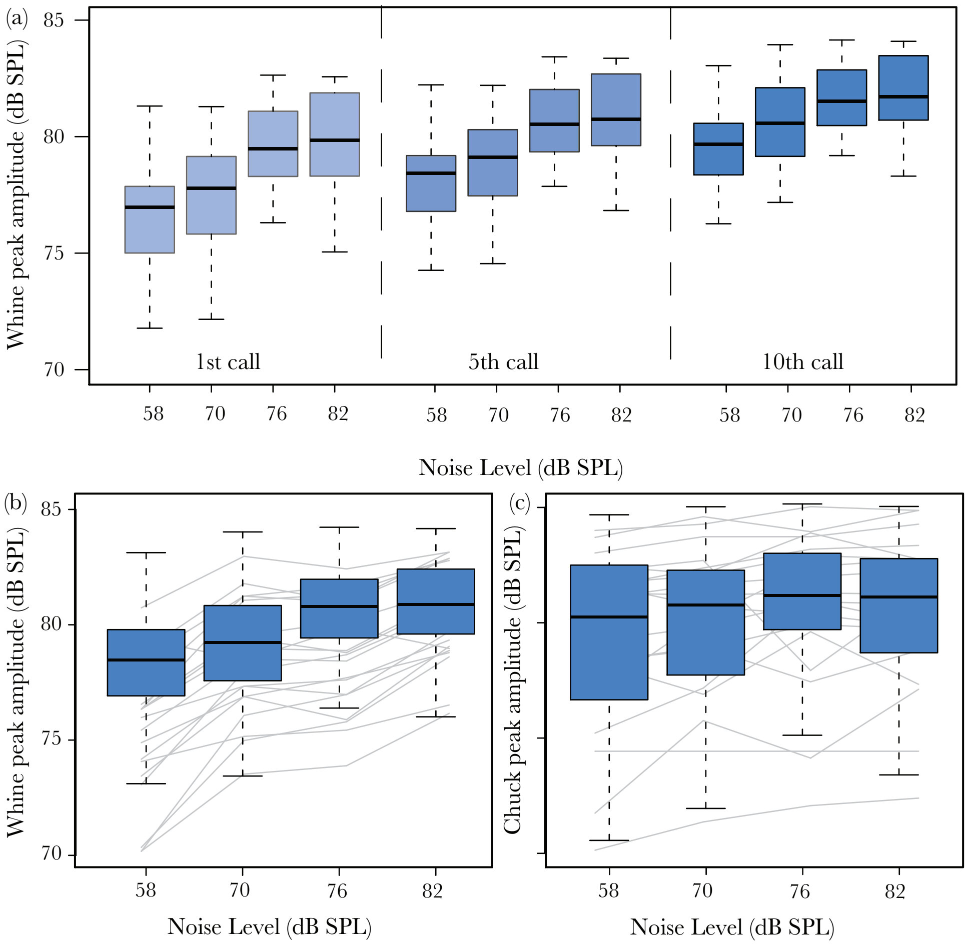

Figure 3

Increased amplitude with increasing background levels demonstrates the existence of the Lombard effect for túngara frogs. (a) Amplitude increases across a bout of calling, but the Lombard effect is independent of call order. Male frogs increase the peak amplitude of the whine (b) and the chuck component (c) of their call. Boxplots depict model estimates, and lines depict individual responses to noise averaged over call order. Eighteen out of 19 males increased peak amplitude of their whine with rising noise levels. Males also significantly increased chuck amplitude in relation to increasing noise levels, but individual patterns are less clear.

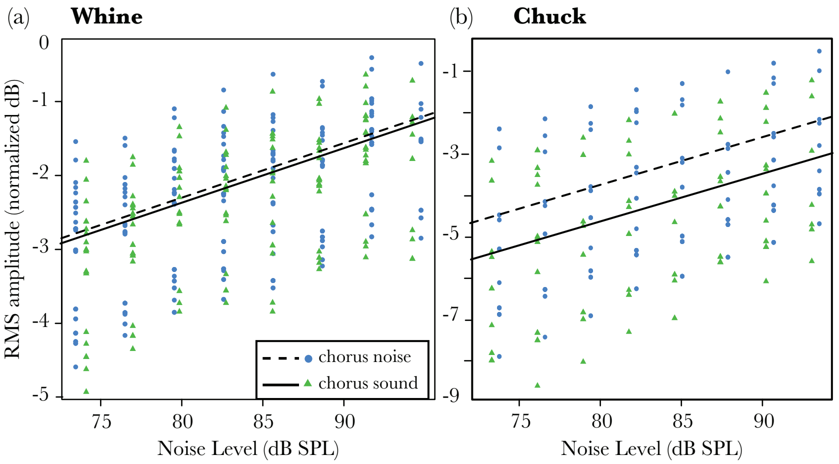

Figure 4

Chorus sounds and chorus-shaped noise induce the same signal amplitude increase. (a) Effect of different levels of chorus sound (green triangles) and chorus-shaped noise (blue dots) on the amplitude of the whine component of a male frog's call. (b) Effect on the chuck component of the call. *X* axes show experimental noise levels at the position of the focal male. *Y* axes show log-transformed RMS amplitudes of the different call components, normalized by the loudest call for each individual. Lines indicate fixed effect of sound level for both treatments.

Fahrig 2013). In some species, changes in frequency and amplitude can covary due to biomechanical linkages (Hage et al. 2013; Nemeth et al. 2013). Despite covariance between call frequency and amplitude, we did not find call frequency to increase with noise level, which suggests that frequency production is uncoupled from amplitude regulation under certain conditions (Nemeth et al. 2012; Slabbekoorn et al. 2012). We did find, however, call complexity and rate to covary with amplitude level as well as noise exposure. Previous studies on anthropogenic noise have shown that frogs change their call rate, call duration, and number of call elements in much the same way as we found and may thus also reflect covarying amplitude changes (Sun and Narins 2005; Lengagne 2008; Kaiser and Hammers 2009).

Technical issues make it difficult to compare results across taxa, but it appears that amplitude regulation in response to noise is not as widespread in anurans as it is in mammals and birds. Two previous studies have reported no change, or even a decrease in amplitude with increasing noise levels (Cunnington and Fahrig 2010; Love and Bee 2010). Why would frogs evolve different strategies to deal with the same cocktail-party-problem? One explanation for the absence of the Lombard effect has focused on the general pattern of reproductive biology of frogs. The males of many frog species congregate in large groups to compete acoustically for females (Grafe 1997; Gerhardt and Huber 2002; Wells 2010). Higher amplitude calls are usually more attractive to females, thus males might be under strong selection to always maximize signal amplitude (Love and Bee 2010). On other hand, calling at maximum signal amplitude may also come at a cost. Many frogs can vary their general call behavior in response to increased threats by rivals (Grafe 1995; Gerhardt and Huber 2002; Bernal, Akre, et al. 2009), or perceived costs of calling (Phelps et al. 2007). There is no reason to assume that varying call amplitude cannot be a similar strategy to balance costs and benefits of calling as we know that predators are typically also more attracted to higher amplitude signals (Page et al. 2013; Steinberg et al. 2014). We do know that increased acoustic complexity of a frog chorus increases attack latencies of frog-eating bats (Halfwerk, Dixon, et al. 2014; Rhebergen et al. 2015); however, whether eavesdropping bats also benefit from increased signal amplitudes remains to be tested.

The Lombard effect occurs in mammals and birds both in the presence and absence of social cues (Cynx et al. 1998). In the absence of social cues, the Lombard effect is thought to require monitoring of the signal-to-noise ratio of an animal's own voice through auditory feedback (Brumm and Zollinger 2011). In this study, túngara frogs also increase the amplitude of their calls in the presence and absence of social cues. But we do not think it is necessary that males monitor the signal-to-noise ratios of their own voices. Male túngara frogs respond to a variety of acoustic stimuli that are quite different from the conspecific calls and which do not elicit phonotaxis from females (Bernal et al. 2007). So it seems that males respond to any sound that matches frequency-content and sound amplitude of rival males, although, interestingly, males do not seem to respond to their own voice when calling in isolation. In the field, males can call alone, or as part of a chorus, ranging in size from 2 till hundreds of individuals. We predict that the increase in male call effort will quickly reach a maximum with increasing chorus size.

The Lombard effect observed in our study hardly seemed to improve signal-to-noise ratios. Call amplitude did not increase proportionally with masking noise levels. The increase in background noise of 24 dB in experiment 2, for instance, only resulted in an average increase of less than 3 dB in túngara call amplitude. For comparison, most studies on humans and birds report an increase of approximately 10 dB over a similar range of noise levels, with some individuals even showing an increase of 20 dB (Cynx et al. 1998; Brumm 2004; Schuster et al. 2012). Male túngara frogs respond stronger to a mimicked rival that calls at high compared with low amplitudes (Halfwerk, Page Rachel, et al. 2014). The small increase in amplitude may therefore primarily function as a way to communicate a male's readiness to fight when challenged by rivals and only secondarily function to restore signal-to-noise ratios.

To summarize, we have shown that túngara frogs increase their call amplitudes in response to increased background noise and in the presence and absence of other acoustic social cues. Thus, the Lombard effect is also present in a vertebrate group other than mammals and birds, and it would be interesting to know how widespread the Lombard effect occurs among other anurans. We call for more, carefully calibrated, measurements of signal amplitudes during noise and across a wide range of taxa to get insight into the evolutionary history of this important communicative trait.

FUNDING

This work was supported by a Smithsonian Institute fellowship to W.H., a graduate research fellowship of the National Science Foundation to A.M.L., and an National Science Foundation grant (grant number: IBN 0517328) to M.J.R. and R.A.P.

I. Geipel and 3 anonymous referees provided useful feedback on earlier versions of the manuscript.

Handling editor: Bob Wong

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