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An experimental test of noise-dependent voice amplitude regulation in Cope's grey treefrog (*Hyla chrysoscelis*)

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Abstract

One strategy for coping with the constraints on acoustic signal reception posed by ambient noise is to signal louder as noise levels increase. Termed the 'Lombard effect', this reflexive behaviour is widespread among birds and mammals and occurs with a diversity of signal types, leading to the hypothesis that voice amplitude regulation represents a general vertebrate mechanism for coping with environmental noise. Support for this evolutionary hypothesis, however, remains limited due to a lack of studies in taxa other than birds and mammals. Here, we report the results of an experimental test of the hypothesis that male grey treefrogs increase the amplitude of their advertisement calls in response to increasing levels of chorus-shaped noise. We recorded spontaneously produced calls in quiet and in the presence of noise broadcast at sound pressure levels ranging between 40 dB and 70 dB. While increasing noise levels induced predictable changes in call duration and rate, males did not regulate call amplitude. These results do not support the hypothesis that voice amplitude regulation is a generic vertebrate mechanism for coping with noise. We discuss the possibility that intense sexual selection and high levels of competition for mates in choruses place some frogs under strong selection to call consistently as loudly as possible.

Keywords

acoustic communication; frog; gray treefrog; Lombard effect; noise

Biotic and abiotic sources of environmental noise can constrain acoustic communication, for example, by making signals more difficult to detect and recognize (Narins & Zelick 1988; Klump 1996; Brumm & Slabbekoorn 2005; Bee & Micheyl 2008). Determining how animals overcome noise-related constraints has important implications for understanding the mechanisms, function and evolution of acoustic communication systems, as well as the potential for human-induced impacts on these systems. One familiar strategy used by humans in noisy situations is to simply talk louder (i.e. the 'Lombard effect'; Lane & Tranel 1971). The tendency to increase voice amplitude as the level of background noise increases also occurs in many birds, including songbirds (Cynx et al. 1998; Brumm & Todt 2002; Kobayasi & Okanoya 2003; Leonard & Horn 2005), budgerigars (Manabe et al. 1998), hummingbirds (Pytte et al. 2003) and galliforms (Potash 1972; Brumm et al. 2009), as well as in many nonhuman mammals, such as cats (Nonaka et al. 1997), bats (Tressler & Smotherman 2009),

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monkeys (Sinnott et al. 1975; Brumm et al. 2004; Egnor & Hauser 2006) and cetaceans (Wiggins et al. 2001; Scheifele et al. 2005; Holt et al. 2009). Moreover, these studies of nonhuman animals have demonstrated the Lombard effect with a diversity of signals having different functions, including mating signals (e.g. song: Cynx et al. 1998; Brumm & Todt 2002; Kobayasi & Okanoya 2003), territorial calls (Pytte et al. 2003), contact calls (e.g. Manabe et al. 1998; Brumm et al. 2004; Egnor & Hauser 2006), begging calls (Leonard & Horn 2005) and echolocation calls (Tressler & Smotherman 2009). This considerable diversity of taxa and signal types has led to the view that voice amplitude regulation constitutes part of a generic behavioural response to the problem of noise common among all vertebrates (e.g. Tressler & Smotherman 2009). Importantly, however, this evolutionary hypothesis remains unsubstantiated because voice amplitude regulation in response to noise has rarely been tested, and never demonstrated, in a nonavian or nonmammalian vertebrate (Brumm & Slabbekoorn 2005).

Our broad aim was to test the hypothesis that voice amplitude regulation is a generic vertebrate response for coping with environmental noise. To this end, we tested the specific hypothesis that males of Cope's grey treefrog, *Hyla chrysoscelis*, regulate the amplitude of their vocalizations in response to variation in the level of background noise simulating social aggregations. Cope's grey treefrog is the diploid member of a cryptic, diploid-tetraploid species complex; the closely related eastern grey treefrog, *H. versicolor*, is the tetraploid (Holloway et al. 2006). During their breeding season, males of both species form dense and noisy choruses in which they produce loud, pulsed advertisement calls that are necessary and sufficient to attract gravid females (Fig. 1a; Gerhardt 2001). Similar to some other anurans (Gerhardt & Klump 1988; Wollerman 1999; Schwartz et al. 2001; Wollerman & Wiley 2002), recent studies of the diploid species have shown that the noise generated in a chorus (Fig. 1b) represents a potent source of auditory masking. In addition to reducing a signal's active space (Bee 2007; Bee & Swanson 2007), auditory masking constrains the abilities of females to discriminate between conspecific and heterospecific calls (Bee 2008a) and to exercise potentially adaptive mate choice preferences (Bee 2008b).

Importantly, however, chorus environments are also quite variable. In grey treefrog choruses, for example, both the density of callers and calling activity (and hence background noise levels) exhibit predictable patterns of seasonal and diel variation, as well as unpredictable variation due to local, short-term weather patterns (Bridges & Dorcas 2000; M. A. Bee, unpublished data). In addition, as in other frogs (Gerhardt & Huber 2002), calling by male grey treefrogs is quite energetically expensive (Wells & Taigen 1986). Therefore, we should generally expect natural selection to favour signallers that behave in ways that are plastic and that maximize the transmission of their signals in variable and cluttered acoustic environments. Previous studies have found that grey treefrog males show some behavioural plasticity with potential relevance to signalling in noisy situations. In response to increases in the local density of nearby callers, males of the tetraploid species (*H. versicolor*) increase the duration of their calls and reduce their rate of calling, thus maintaining a fairly constant calling effort over time (Wells & Taigen 1986; Schwartz et al. 2002, 2008). While the function of this plasticity is currently unknown (Schwartz et al. 2008), one viable hypothesis is that longer signals are more easily detected in high levels of ambient background noise (Brumm & Slabbekoorn 2005; Schwartz et al. 2008). Our specific aim in this study was to assess the extent to which calling males regulate the amplitude of their vocalizations in ways that are tailored to the ambient noise background.

METHODS

Subjects

Twenty-eight males of *H. chrysoscelis* served as subjects in this study. They were collected in amplexus at night (2200–0100 hours) during the breeding season (May–June, 2008–2009) from

several ponds located in the Carver Park Reserve (44°52'49"N, 93°43'3"W; Carver County, MN, U.S.A.) and the Crow-Hassan Park Reserve (45°11'20"N, 93°38'21"W; Hennepin County, MN, U.S.A.). The care and handling of animals followed approved protocols that are described in more detail elsewhere (Bee 2008a, b; Bee & Schwartz 2009). Briefly, the males were kept in small plastic containers with the female they paired with and maintained at 2 °C until 1 h prior to testing to prevent egg deposition by the females, which served as subjects in other experiments not reported here. Tests were conducted within 12–18 h of collection. One hour prior to testing, subjects were transferred in their containers with their mates to a 20 °C incubator where their body temperatures were allowed to reach 20 ± 2 °C, which is a typical temperature at which grey treefrogs breed. Pairs that became separated during maintenance at 2 °C invariably re-entered amplexus after warming up in the incubator. During an experimental treatment, the pair was manually separated, the male was tested, and then the pair was reunited and placed back in the incubator until the next test. Subjects were given a 5–10 min time-out between tests of different treatments. During these time-out periods, the pairs typically re-entered amplexus while in the incubator. All subjects were returned with their mates to their location of capture after testing, usually within 24 h of collection. This work was approved by the University of Minnesota's Institutional Animal Care and Use Committee (protocol no. 0809A46721; last approved 22 September 2009).

Apparatus

We recorded spontaneous calling inside a custom-built, hemi-anechoic sound chamber (Industrial Acoustics Corporation (IAC), Bronx, NY, U.S.A.; inside dimensions: 220 × 280 × 216 cm, L × W × H). The floor and walls of the ceiling were treated with IAC's Planarchoic™ panelling to reduce reverberations. The sound chamber was temperature controlled with an external HVAC unit and was maintained at a temperature of 20 ± 1 °C throughout testing. With the ventilation unit running, the sound pressure level (SPL re. 20 µPa, fast RMS, flat weighting) of the chamber's ambient noise floor ranged between 2 and 12 dB SPL in the 1/3-octave bands centred between 500 and 5000 Hz, which span the most sensitive part of the hearing range of grey treefrogs (Hillery 1984).

During recordings, subjects were restrained in a small plastic cup (5.7 cm diameter, 5.0 cm deep) with an acoustically transparent lid and placed in the centre of the sound chamber directly below a ceiling-mounted speaker (Kenwood KFC-1680ie; Kenwood USA Corporation, Long Beach, CA, U.S.A.) that was used to broadcast chorus-shaped noise (Fig. 1c). Noise was broadcast using Adobe Audition 1.5 (Adobe Systems Inc., San Jose, CA, U.S.A.) running on a PC interfaced with an M-Audio FireWire 410 soundcard (M-Audio, Irwindale, CA, U.S.A.) and HTD 1235 amplifier (Home Theater Direct, Inc., Plano, TX, U.S.A.). We recorded calls using a Sennheiser ME66 microphone and K6 power supply (Sennheiser USA, Old Lyme, CT, U.S.A.) suspended from the chamber ceiling so that the recording tip was positioned 50 cm directly above the subject. The low-frequency roll-off filter (≈ -11 dB/octave @ 100 Hz) of the K6 microphone power supply was turned on. Recordings were made onto a Marantz PMD670 (D&M Professional, Itasca, IL, U.S.A.) located outside the chamber (44.1 kHz, 16 bits).

Experimental Design

We used a within-subjects design that included five treatments tested in a different random order for each subject. We recorded 20 calls per subject during each treatment. In four experimental noise treatments, we broadcast the chorus-shaped noise at sound pressure levels of 40 dB, 50 dB, 60 dB, or 70 dB SPL (fast RMS, C-weighted). The highest experimental noise level corresponds to natural sound levels we have recorded in active grey treefrog choruses (Swanson et al. 2007). The level of the 70 dB noise was calibrated at the approximate position of a subject's head using a Brüel & Kjær Type 2250 sound level meter (Brüel & Kjær, Norcross,

GA, U.S.A.). All other noise levels were adjusted in 10 dB steps relative to the 70 dB noise using software attenuation settings, the accuracy of which were verified at the start of the experiment prior to collecting data.

The chorus-shaped noise (Fig. 1c) was broadcast continuously during the noise treatments and was created as follows. First, we filtered a 6 min long segment of white noise two different times to create one low-frequency band and one high-frequency band. The low-frequency band was created using a band-pass finite impulse response (FIR) filter of order 300, pass-band frequencies of 1200 and 1300 Hz, and stop-band frequencies of 1000 and 1500 Hz. The high-frequency band was created using a band-pass FIR filter of order 150, with pass-band frequencies of 2400 and 2600 Hz and stop-band frequencies of 2000 and 3000 Hz. Both FIR filters had pass-band ripples of 0.1 Hz and stop-band attenuations of 60 dB. Next, the amplitude of the low-frequency band was attenuated by 6 dB in relation to that of the high-frequency band. Finally, the two bands were digitally added together to create a noise with a long-term spectrum resembling that of a natural chorus (cf. Fig. 1b, c). The noise had a continuous, steady-state envelope. Similar chorus-shaped noises have been used in several recent studies of auditory masking in grey treefrogs (Bee 2007, 2008a, b; Bee & Schwartz 2009; Vélez & Bee, in press).

The fifth treatment condition was a no-noise treatment that served as a control to assess male calling behaviour in quiet conditions in the absence of any broadcast chorus-shaped noise. The ambient noise level in the sound chamber during the no-noise conditions was 59 dB SPL (fast RMS, flat weighting). However, a spectral analysis using 1/3-octave bands revealed that most of the noise energy occurred at frequencies below 40 Hz. Hence, most of the ambient noise in the sound chamber was not only well outside the range of sensitive airborne hearing in grey treefrogs (Hillery 1984), it was also below the low-frequency cutoff of the Sennheiser microphone used to record them. To establish the effective contribution of ambient noise to our recordings of males in quiet conditions, we applied the attenuation settings from the low-frequency roll-off filter of the recording microphone to the output of the 1/3-octave band analysis. This adjustment resulted in an estimated ambient background noise level of 28 dB SPL in the absence of any broadcast noise.

Acoustical and Statistical Analyses

We used Avisoft SASLab Pro v1.2 (Avisoft Bioacoustics, Berlin, Germany) and custom-written scripts in Matlab v7.6 (MathWorks Inc., Natick, MA, U.S.A.) to extract four dependent variables from our recordings. Our determination of signal amplitudes followed procedures similar to those used in recent studies of the Lombard effect in other animals (Brumm & Todt 2002; Brumm et al. 2004, 2009). For each subject, we determined call amplitude by calibrating all measurements relative to that of a 2 s segment of the 70 dB noise containing no calls recorded during the 70 dB treatment for that subject. We used logarithmic subtraction to remove the increment in the measured signal level due to the presence of the ambient noise in the quiet condition or to the combination of ambient and chorus-shaped noise in the four experimental conditions (Hartmann 1997). Measurements of root-mean-square amplitude were averaged over 10 pulses from the middle of each call and then over all 20 calls. Below, we report values of amplitude in dB SPL (re. 20 μ Pa); however, all statistical analyses were conducted after converting amplitude values in dB to a linear pressure scale (Pa). We determined the duration of each of the 20 recorded calls by counting the number of pulses per call, and we determined call rate as the number of calls produced per minute. Measures of call duration and call rate were made using the oscillogram and spectrogram (FFT size = 1024, Hanning window) functions of the SASLab Pro software. From our measures of call rate and call duration, we also determined call effort, which is a measure of total sound output per unit time. Call effort was calculated by multiplying call duration (in units of pulses/call) by call rate (in units of

calls/min) and is expressed in units of pulses/min. Because we expected a priori that males would increase call duration and decrease call rate while maintaining a constant call effort (Wells & Taigen 1986; Schwartz et al. 2002, 2008), our measures of these three variables served as a control for assessing the extent to which our noise manipulations were sufficient to induce species-typical plasticity in calling behaviour.

After a log transformation of sound pressure levels (in Pa), none of the four response variables departed from normality (Kolmogorov–Smirnov tests: all $d < 0.18$, all $P > 0.20$). We used repeated measures multivariate analysis of variance (MANOVA) to test the general hypothesis that calling behaviour depended on noise treatment. Subsequent univariate repeated measures ANOVAs were used to test this hypothesis separately for each dependent variable, and for these omnibus tests, we report Greenhouse & Geisser (1959) corrected P values. Focused linear contrasts were used to test the specific hypotheses that call amplitude, duration, rate and effort varied as increasing or decreasing functions of increasing noise level. For all analyses, we determined significance using a criterion of $\alpha = 0.05$, and we report partial η^2 as a measure of effect size (i.e. an estimate of the extent to which the null hypothesis of ‘no effect’ is false). Partial η^2 , which can vary between 0.0 and 1.0, represents a nonadditive ‘variance-accounted-for’ measure of effect size that corresponds to the proportion of the combined effect and error variance that can be attributed to the effect. The interpretation of partial η^2 is similar to that of the coefficient of determination (r^2). All statistical analyses were computed using Statistica 7.1 (StatSoft 2006).

RESULTS

There were significant differences in male calling behaviour across the five treatment conditions (MANOVA: Wilks’ $\lambda = 0.08$, $F_{16,12} = 9.2$, $P < 0.01$, partial $\eta^2 = 0.92$). These overall differences could be attributed to significant, noise-dependent differences in call duration (ANOVA: $F_{4,108} = 41.9$, $P < 0.01$; $\eta^2 = 0.61$) and call rate (ANOVA: $F_{4,108} = 4.6$, $P < 0.01$; $\eta^2 = 0.15$), and did not result from differences in call amplitude (ANOVA: $F_{4,108} = 1.4$, $P = 0.24$; $\eta^2 = 0.05$) or call effort (ANOVA: $F_{4,108} = 1.2$, $P = 0.32$; $\eta^2 = 0.04$). The mean sound pressure levels of recorded calls varied less than 1 dB across all five treatment conditions and did not exhibit any significant trends associated with increasing noise level (linear contrast: $F_{1,27} = 3.6$, $P = 0.07$; $\eta^2 = 0.12$). Average values ranged between 92.0 and 92.9 dB SPL (Fig. 2a), and were quite similar to the average sound pressure level reported by Gerhardt (1975) based on field measurements in natural choruses (93.5 dB SPL RMS). In contrast, increases in noise level resulted in significant increases in call duration (Fig. 2b; linear contrast: $F_{1,27} = 105.9$, $P < 0.01$; $\eta^2 = 0.80$) and significant decreases in call rate (Fig. 2c; linear contrast: $F_{1,27} = 3.6$, $P < 0.01$; $\eta^2 = 0.33$). Between the no-noise and 70 dB noise conditions, call duration increased by about 20%, and call rate decreased by about 21%. Simultaneous increases in duration and decreases in rate resulted in measures of call effort that did not vary with increasing noise levels (Fig. 2d; linear contrast: $F_{1,27} < 0.1$, $P = 0.99$; $\eta^2 < 0.01$).

DISCUSSION

We found no support for the hypothesis that male grey treefrogs regulate the amplitude of their vocalizations as a function of background noise levels. Instead, males produced calls of equivalent amplitudes (92–93 dB SPL) in quiet and noisy conditions. In contrast, the noise treatments induced other, species-typical forms of plasticity in calling behaviour. As expected based on previous studies (Wells & Taigen 1986; Schwartz et al. 2002, 2008), males increased call duration and decreased call rate as functions of increasing noise level, while maintaining a fairly constant call effort. These results for call duration and rate are particularly revealing because they serve as an important check on the efficacy of our experimental manipulations. Moreover, they establish that the study had adequate statistical power to detect species-typical

changes in calling behaviour. The effect sizes associated with differences in call duration ($0.60 \leq \eta^2 \leq 0.80$) and call rate ($0.15 \leq \eta^2 \leq 0.33$) were moderate to large, and they were uniformly larger than those associated with differences in call amplitude ($0.05 \leq \eta^2 \leq 0.12$). We would also point out that the magnitude of between-treatment differences observed in call amplitude were quite small, falling within the typical range of reported measurement error for type I sound level meters (e.g. ± 0.7 to 1.5 dB). A conservative conclusion based on our findings is that male grey treefrogs readily increased call duration and decreased call rate as a function of increasing ambient noise levels; the effects of noise levels on the regulation of call amplitude were relatively smaller and were negligible under the conditions tested in this study.

Our results stand in stark contrast to those reported in previous experimental studies of birds and mammals, in which individuals commonly increased the amplitude of their vocalizations by as much as 10–15 dB (i.e. by factors of about three to six) or more over ranges of noise levels similar to those used in the present study. For example, over a 37 dB range of noise levels, ranging between ambient (~43 dB) and broadcasts of broadband noise at 80 dB, domestic fowl, *Gallus gallus domesticus*, increased the amplitude of their separation calls by 15 dB, with mean call amplitudes increasing from 61 dB in quiet to 76 dB at the highest noise level (Brumm et al. 2009). Across a 35 dB range in noise levels between ambient (~30 dB) and broadcasts of white noise at 65 dB, individual common marmosets, *Callithrix jacchus*, increased the amplitude of their twitter calls by magnitudes ranging between 10 dB and 30 dB (Brumm et al. 2004). Average call amplitudes ranged between about 40 dB and 80 dB across conditions. Cottontop tamarins, *Saguinus oedipus*, increased the amplitude of their combination long calls by magnitudes ranging between 7 dB and 13 dB in response to a 20 dB increase in background noise level from 50 dB to 70 dB (Egnor & Hauser 2006). In a survey of both experimental and observational studies of the Lombard effect in humans and other animals, Scheifele et al. (2005) reported that typical magnitudes of voice amplitude modulation were in the range of 0.3–3.3 dB per decibel increase in noise level. These results bring into sharp focus those reported here for grey treefrogs, in which call amplitude varied less than 1.0 dB over a range of noise levels greater than 40 dB.

While we found no evidence of voice amplitude regulation in grey treefrogs, it was not unreasonable to expect that they might show a Lombard effect similar to that seen in birds and mammals. We noted earlier the constraints that chorus noise imposes on the reception of calls by female frogs. Not surprisingly, calling male frogs use a suite of evolutionarily adaptive signalling strategies to maximize the transmission of their advertisement calls to intended receivers (reviewed in: Narins & Zelick 1988; Gerhardt & Huber 2002; Wells & Schwartz 2007). For example, some species call at different times and places than acoustically signalling heterospecifics, and thereby call under conditions of reduced ambient noise levels (Duellman & Pyles 1983; Wells & Schwartz 2007). Males of some species precisely adjust the timing of their calls to avoid overlap from the calls of other nearby males (Brush & Narins 1989; Wells & Schwartz 2007). In habitats with high levels of (usually) low-frequency abiotic noise, such as those near fast flowing streams and waterfalls, male frogs may call using ultrasonic frequencies (Feng et al. 2006; Arch et al. 2008) or augment their vocal signals with visual displays (Hödl & Amézquita 2001). Male frogs also use vocalizations to maintain intermale separation in choruses (Brenowitz 1989), which can improve the ability of females to recognize and locate potential mates and to exercise preferences for particular call properties (Telford 1985; Dyson & Passmore 1992; Schwartz & Gerhardt 1995). As noted earlier, the longer calls produced by male grey treefrogs in the presence of noise may also be an adaptation for improving the signal's detectability in noise.

It is important to consider why grey treefrogs failed to increase their call amplitude in response to increasing noise levels. Based on our results, we believe the more relevant and informative question is, why did our subjects call so loudly when it was quiet? Recall that the amplitudes

recorded when males called in isolated, quiet conditions in our study were similar to those recorded in the presence of both broadcast chorus noise (this study) and the real chorus noise present in the frogs' natural habitat (Gerhardt 1975). We suggest the hypothesis that chorus-breeding species, like grey treefrogs, are under strong selection (or have been so in the past) to vocalize consistently at sound levels constrained only by the upper morphological or physiological limits on vocal production. In the context of sexual selection, call amplitude is an important determinant of signal active space that potentially influences male mating success in frogs because females typically choose the loudest signal among otherwise similar alternatives (Ryan & Keddy-Hector 1992; Gerhardt & Huber 2002). Female grey treefrogs, for example, show significant preferences for the more intense of two otherwise identical calls differing by only 2 dB (Fellers 1979). Because there is a tight linkage in frogs between vocal behaviour and reproduction, and hence evolutionary fitness, and because breeding episodes are often short and seasonally restricted, selection may well favour males that always call as loudly as possible. This ultimate-level hypothesis might be tested by comparing different species that typically breed in dense versus sparse assemblages, as well as those that differ widely in the amplitudes of their calls (e.g. see Gerhardt 1975; Penna & Solis 1998). Species with relatively lower-amplitude calls might have a greater available dynamic range for regulating voice amplitude. At a proximate level, various features of call production (e.g. impedance matching) might constrain calls to be loud, or frogs may lack the neural or neuromuscular mechanisms necessary to monitor and regulate simultaneously their own call amplitude in response to changes in ambient noise levels.

Current results from studies of frogs (including this one) do not lend much support to the view that voice amplitude regulation is a generic vertebrate mechanism for communicating in noise (Tressler & Smotherman 2009). Three previous field studies of frog communication are sometimes incorrectly cited in the literature as providing positive evidence for the Lombard effect in this group of vertebrates. However, careful considerations of the experimental methods used in these playback studies of the 'evoked vocal responses' of male frogs suggest possible alternative interpretations.

In the earliest study, Lopez et al. (1988) investigated call modifications in the Puerto Rican white-lipped frog, *Leptodactylus albilabris*, that were induced by broadcasts of various stimulus calls. They found that males sometimes increased the amplitude of their calls during tests in which the stimulus amplitude sequentially increased and thereby simulated a direct challenge by a single encroaching male. This result is probably best interpreted as aggressive males engaging in the equivalent of a 'shouting match' as a form of escalating agonistic contest, and not as positive evidence for the Lombard effect. In addition, depending on the type of playback stimulus used, between 24% and 38% of males either failed to increase or actually decreased the amplitude of their calls in response to increases in stimulus amplitude. Changes in call amplitude in response to differences in ambient noise levels were not measured, and the authors did not describe their study as a test of the Lombard effect.

In two more recent field playback studies, Penna et al. (2005) and Penna & Hamilton-West (2007) directly investigated whether the males of two leptodactylid frogs in Chile (*Eupsophus calcaratus* and *E. emiliopugini*, respectively) regulated call amplitude as a function of noise level. Both studies used a within-subjects design that involved broadcasting noise at one of seven levels ranging between 48 or 49 dB and 84 or 85 dB (in 6 dB steps). Importantly, neither study tested the different noise levels in randomized orders for different subjects. Instead, all subjects in both studies were tested with noise levels that increased sequentially between the consecutive playback periods during the experiment. In addition, both studies also evoked vocal responses from subjects by repeatedly broadcasting a stimulus call during each test of a different noise level. In terms of their main results, both studies reported that call amplitude increased during the experiment and, hence, with increasing noise levels. On the surface, such

a trend would certainly appear consistent with a Lombard response. This interpretation, however, is not warranted because of the potential confounds resulting from the experimental design, and because it is inconsistent with other results also reported in both studies. Because treatment order was not randomized, the observed trends are confounded both with time and with the potentially cumulative and sensitizing effects of the repeated broadcast of the stimulus call. In addition, both studies reported that call amplitude generally did not differ significantly between periods in which each noise was presented and periods of silence that directly preceded and followed each noise broadcast. Thus, while subjects' call amplitudes tended to increase over the duration of the test sequence, these amplitudes were actually independent of even the presence or absence of a broadcast noise. Although Penna & Hamilton-West (2007) interpreted results from both studies as evidence for a Lombard effect, we concur with the original interpretation of Penna et al. (2005) that their results provide little support for noise-dependent voice amplitude regulation.

Given the results of our study and those of Lopez et al. (1988), Penna et al. (2005) and Penna & Hamilton-West (2007), and given that only a few salamanders and no caecilians are known to vocalize, we believe the currently available evidence from extant amphibians is inconsistent with the hypothesis that noise-dependent voice amplitude regulation is a generic vertebrate strategy for coping with environmental noise. Additional studies of other amphibians, as well as of fish and reptiles that vocalize, are necessary before firm conclusions about general vertebrate mechanisms are warranted.

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Figure 1.

Advertisement calls and chorus noise in grey treefrogs. Waveforms (top) and spectrograms (bottom) depict (a) a single advertisement call, (b) a natural chorus and (c) the artificial chorus noise used as a stimulus. Note that in (b), the calls of American toads (*Bufo americanus*) are also visible between the two dominant, continuous bands of spectral energy comprising the sounds of the grey treefrog chorus.

Figure 2.

Mean + SE change in calling behaviour as a function of noise level: (a) amplitude, (b) duration, (c) rate and (d) effort. Y-axis ranges approximate two standard deviations based on each no-noise condition; for call amplitude, the standard deviation is based on symmetric estimates using dB values.