Spring peepers *Pseudacris crucifer* modify their call structure in response to noise

Dalal E. L. HANNA[1*] , David R. WILSON[2], Gabriel BLOUIN-DEMERS[3], Daniel J. MENNILL[4]

1 Department of Biological Sciences, McGill University, Montréal, Québec. H3A 1B1, Canada

2 Department of Biological Sciences, University of Windsor, Windsor, Ontario, N9B 3P4, Canada; email: drwilson76@gmail.com

3 Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada; email: gblouin@uottawa.ca

4 Department of Biological Sciences, University of Windsor, Windsor, Ontario, N9B 3P4, Canada; email: dmennill@uwindsor.ca

Abstract Acoustic interference can impede effective communication that is important for survival and reproduction of animals. In response to acoustic interference, some animals can improve signalling efficacy by altering the structure of their signals. In this study, we played artificial noise to 46 male spring peepers *Pseudacris crucifer*, on their breeding grounds, and tested whether the noise affected the duration, call rate, and peak frequency of their advertisement calls. We used two experimental noise treatments that masked either the high- or low-frequency components of an average advertisement call; this allowed us to evaluate whether frogs adaptively shift the peak frequency of their calls away from both types of interference. Our playback treatments caused spring peepers to produce shorter calls, and the high-frequency noise treatment caused them to lower the frequency of their calls immediately after the noise ceased. Call rate did not change in response to playback. Consistent with previous studies, ambient temperature was inversely related to call duration and positively related to call rate. We conclude that noise affects the structure of spring peeper advertisement calls, and that spring peepers therefore have a mechanism for altering signal structure in response to noise. Future studies should test if other types of noise, such as biotic or anthropogenic noise, have similar effects on call structure, and if the observed changes to call structure enhance or impair communication in noisy environments [*Current Zoology* 60 (4) : – , 2014].

Many animals depend on acoustic communication for survival and reproduction (Bradbury and Vehrencamp, 2011), yet acoustic interference from other animals, or from abiotic sources such as streams and wind, can impede communication and impose significant costs (Greenfield, 1994; Penna et al., 2005; Feng et al., 2006; Lengagne, 2008; McNett et al., 2010). In areas populated by humans, animals must also contend with anthropogenic noise from sources such as traffic, construction sites, and airports (Slabberkoorn and Peet, 2003; Lengagne, 2008). The interference caused by these various forms of noise may reduce an individual's fitness and jeopardize the health of populations, species, and ecosystems (Slabberkoorn and Peet, 2003; Tyack 2008; Barber et al., 2010; Halfwerk et al., 2011). For example, natural noise created by wind has the potential to completely inhibit communication in plant feeding insects (McNett et al.,

2010), and anthropogenic noise lowers reproductive success in great tits (Halfwerk et al., 2011). Determining how individuals respond to noise is therefore important for understanding how animals optimize communication in noisy environments. It is also important for understanding how noise might affect populations and species distributions, and, ultimately, for establishing conservation policies (Lengagne, 2008; Laiolo, 2010).

In response to ambient noise, some animals exhibit adaptations that minimize interference and improve signal transmission. For example, many animals improve signal transmission by altering the structure of their signals (Sun and Narins, 2005; Kaiser and Hammers, 2009; Parris et al., 2009; Dunlop et al., 2010; Goodwin and Podos, 2013). Common marmosets *Callithrix jacchus,* olive baboons *Papio anubis*, and green hylias *Hylia prasina*, for example, alter temporal characteristics of calls, such as call duration, call rate, and call timing (Brumm et al., 2004; Ey et al., 2009; Kirschel et al., 2009). Some animals, such as great tits *Parus major*, concave-eared torrent frogs *Amolops tormotus*, song sparrows *Melospiza melodia*, and house finches Hae*morhous mexicanus* increase the frequency of their calls (Slabbekoorn and Peet, 2003; Feng et al., 2006; Wood and Yezerinac, 2006; Bermúdez-Cuamatzin et al., 2009), and others, such as, blue-throated hummingbirds *Lampornis clemenciae*, cotton top tamarins *Saguinus oedipus*, and southern right whales *Eubalaena australis* increase the amplitude of their calls (Pytte et al., 2003; Egnor and Hauser, 2006; Parks et al., 2011; i.e., Lombard Effect; Brumm and Todt, 2002; Brumm et al., 2004; Bee and Micheyl, 2008; Brumm et al., 2009). Some animals even alter multiple properties of their acoustic signals in response to noise (Brumm et al., 2004; Cunnington and Fahrig, 2010). Others, like the Bornean rock frog *Staurois parvus*, use alternative modes of communication, such as visual signalling, to achieve effective communication in the presence of loud acoustic background noise (Grafe et al., 2012).

Strategies for minimizing acoustic interference can depend on the duration and variability of the competing noise (Brumm and Slabbekoorn, 2005; Patricelli and Blickley, 2006). In response to continuous chronic noise, cultural or genetic evolution can produce long-term acoustic adaptations that persist throughout an individual's lifetime (Luther and Baptista, 2010; Ripmeester et al., 2010; Luther and Derryberry, 2012). For example, certain frog populations living beside noisy streams permanently produce ultrasonic calls, which prevent frequency overlap with surrounding stream noise (Feng et al., 2006). In addition to long-term acoustic adaptation, individuals may also be capable of rapidly adjusting their calls in response to transient noise (Bermúdez-Cuamatzin et al., 2009; Halfwerk and Slabbekoorn, 2009). Green frogs, for example, increase the peak frequency of their calls when suddenly exposed to loud anthropogenic noise (Cunnington and Fahrig, 2010). Of course, it is also possible for animals to exhibit both short- and long-term adaptations to noise (Brumm and Slabbekoorn, 2005; Cunnington and Fahrig, 2010; Hanna et al., 2011).

Acoustic communication is critical to the reproductive success of anurans (Gerhardt and Huber, 2002; Wells, 2007), so understanding how anurans respond to noise is important, particularly in an era of increasing conservation concern for anurans (Stuart et al., 2004). As with other species, a number of factors, both natural and anthropogenic, can interfere with their communication systems. Of particular note is the fact that several species call in a chorus setting, which can impose tremendous acoustic interference on individual callers, and which may ultimately drive and constrain the evolution of communication systems in these species (Grafe, 1996; Greenfield and Rand, 2000; Parris, 2002; Velez and Bee, 2010; Bee et al., 2012). The acoustic environment of an anuran chorus varies over time in terms of amplitude, frequency structure, and overall masking potential (Bridges and Dorcas, 2000). Plasticity in an individual's calling behavior may help compensate for this variable ambient noise (Bee and Swanson, 2007; Love and Bee, 2010).

Spring peepers *Pseudacris crucifer* are small frogs that breed in shallow ponds throughout much of North America.

During the breeding season, males compete in choruses with other males by producing advertisement calls that are critical for attracting females (Fig. 1A). Calls are loud (range: 81–104 dB), tonal, and have an average peak frequency of 2.9 kHz (range: 2.5–3.5 kHz; Wilczynski et al., 1984; Forester and Czarnowsky, 1985). In this study, we tested whether spring peepers adjust the duration, call rate, and peak frequency of their calls in response to artificial experimental noise. We examined the flexibility of potential frequency shifting behavior by broadcasting noise that was designed to mask either the higher- or lower-frequency components of the frogs' calls (Fig. 1; as in Potvin and Mulder, 2013). We predicted that frogs would shift the peak frequency of their calls away from the interfering noise, as predicted by the Acoustic Adaptation Hypothesis (Morton, 1975), and that they would increase the call rate and duration of their calls equally in response to both noise treatments.

Due to their widespread distribution and tolerance of disturbed habitat, spring peepers often reside in locations contaminated by loud anthropogenic noise, such as noise from urban centers or busy highways. Although the frequencies contained in spring peeper advertisement calls generally exceed the loudest frequencies contained in anthropogenic noise (e.g., energy in traffic noise is concentrated below 2 kHz; Cornillon and Keane, 1977), anthropogenic noise is known to have immediate and chronic effects on the communication systems of a wide range of species (Bermúdez-Cuamatzin et al., 2009; Halfwerk and Slabbekoorn, 2009; Brumm and Slabbekoorn, 2005; Hanna et al., 2011). We therefore conducted our playback experiment on individuals from both roadside and non-roadside locations to ensure that we sampled individuals living in both types of environment, and that our results were thus broadly applicable.

1 Materials and Methods

We recorded spring peepers in marshes along a noisy highway, as well as in nearby marshes located away from noisy highways. For each individual frog (n = 46), we recorded its calls during a silent baseline treatment and then during two experimental treatments in which we broadcast either low- or high-frequency noise. We also recorded calls during silent periods immediately following each experimental noise treatment (i.e., silent post-noise assessment periods) so that we could assess potential residual effects of noise on calling behavior.

1.1 General field methods

We collected data between 20:00 h and 24:00 h on rainless evenings in April 2012 in the vicinity of the Queen's University Biological Station (44°34 N, 76°19 W), approximately 100 km southwest of Ottawa, Ontario, Canada. We visited a different marsh on each evening. We visited three sites located along a noisy roadside and six sites located away from noisy roads. Roadside marshes were located along Ontario Provincial Highway 15 (average of 1 vehicle per minute between 19:00 h and 24:00 h, according to data collected by the Ministry of Transportation of Ontario in 2010). These marshes have louder ambient noise levels throughout the day, as compared to their surrounding non-roadside marshes (mean ± SE amplitude at non-roadside sites and roadside sites, respectively: 51.7±0.1 dBC SPL, 65.8±1.8 dBC SPL; see Hanna et al. 2011 for further details). All recorded frogs were located within 75 m of the highway. Non-roadside sites were located at least 10 km from any major roads (i.e., national and provincial highways), and at least 50 m from any minor rural roads that are used by the public. All sites were located at least 2 km from each other to minimize the probability that subjects moved between recording sites during the study. Movement among sites is unlikely, however, as previous studies have shown that spring peepers occupy the same positions on successive nights over the course of a breeding season (Rosen and Lemon, 1974).

Air temperature is known to affect call structure in many anurans (Gayou, 1984; Brenowitz et al., 1985; Sullivan and Hinshaw, 1990). We therefore measured air temperature during our recording sessions with a Hobo U23 ProV2

External Temperature data logger (model 23-02, Onset HOBO Data Loggers, Cape Cod, MA, USA) that was placed 50 cm above the surface of the ground at one of our non-roadside sites. The logger measured air temperature every hour during the recording sessions with an accuracy of 0.21°C. We considered air temperature measurements as approximations, since they were taken as much as 30 min from the actual time of recording, up to 15 km away from the recording location, and in a microhabitat that might have differed slightly in temperature from that of the microhabitat occupied by the subject (e.g., surface of the water vs. on a reed 0.5 m above the water). We consider temperature variance owing to location, to time differences between the temperature measure and the time of recording, and to microhabitat effects as unmeasured random error.

1.2 Playback experiment

We located male spring peepers by listening to their advertisement calls. After locating an individual, we placed a wire pin flag immediately beside him (within 2 cm) to ensure that we did not test the same individuals more than once. In all cases, males could be found in the same small area near their wire pin flags for the remainder of the evening's recording session. Once an individual had been found, we exposed it to a sequence of three treatments. In the first treatment (i.e., silent baseline treatment), we played a silent stimulus track through our playback apparatus (see detailed description of stimuli below). This treatment served as a control period so that we could obtain a baseline measure of the subject's behavior before we broadcast any noise. In the second treatment, we broadcast either low- or high-frequency noise (order determined randomly). The second treatment was followed by a silent post-noise assessment period during which we evaluated potential residual effects of the first noise treatment. In the third treatment, we broadcast the remaining noise stimulus. The third treatment was similarly followed by a silent post-noise assessment period. Because the treatments for a particular individual were completed within a short period of time (2.6 ± 0.8 min, average ± SE), variation in ambient conditions (e.g., temperature) and in calling behaviors related to time of day should have been minimal throughout the trial.

There is a concern that experimental noise stimuli could affect measurements of call structure (Verzijden et al., 2010), though previous work using similar experimental noise treatments showed that such noise did not influence structural measurements obtained with the same technique used in the current study (see 'Measuring Call Structure' below; Hanna et al., 2011). Nevertheless, we cannot exclude the possibility that experimental noise would obscure or confound our measurements, particularly in the case of call duration, where background noise could mask the precise onset and offset of calls (Bee, 2004). The two post-noise assessment periods allowed us to address this concern, since they allowed us to continue assessing the subject's response to the preceding noise treatment, but without the potentially obscuring effects of the playback noise on our measurements of call structure.

We recorded subjects throughout the trial using a digital recorder (Marantz PMD 660; WAVE format, 44.1 kHz sampling rate, 16 bit amplitude encoding; Kawasaki, Kanagawa, Japan) and a shotgun microphone (Audio-Technica, model 8,015b; 40–20,000 Hz frequency response; Stow, OH, USA) that was fitted with a foam windscreen. The microphone was attached to a tripod, which we placed in the marsh directly above the subject. We pointed the microphone downwards and adjusted its position so that its tip was 50 cm above the subject. By fixing the microphone to a tripod, we ensured that its distance to the subject remained constant within and among trials, as advocated by Brumm and Zollinger (2011). Once the recording apparatus was set up and the subject was producing advertisement calls, we played the three playback treatments from a digital playback device (Apple iPod; Apple, Cupertino, CA, USA) through a loudspeaker (Mini-Vox, PB 25; Anchor Audio Inc., Torrance, CA, USA). We positioned the loudspeaker 50 cm away from the subject at the same height as the subject, and pointed the cone of the speaker directly at the subject. The playback apparatus was calibrated so that it broadcast the two experimental noise treatments at 89–90 dB SPL, as

measured at the subject's position (50 cm) with a sound level meter (C-weighting, fast response; RadioShack Corporation, Fort Worth, TX, USA). This was the maximum playback amplitude at which we could distinguish the spring peeper calls from the experimental noise on the audio recordings, as determined during pilot trials at the beginning of the experiment. It also ensured that the experimental noise was the loudest source of ambient noise at the subject's location (typical spring peeper chorus noise ranges between 75 and 86 dB SPL; Schwartz and Gerhardt, 1998); this was confirmed by inspecting our trial recordings, which showed that the experimental noise caused an immediate and substantial increase in the amplitude of the background noise. This is important because anuran signals are known to be heavily influenced by loud neighbors in their immediate vicinity (Greenfield and Rand, 2000). Treatments were considered complete when we had recorded a minimum of 10 calls from each individual.

1.3 Playback Stimuli

We created the silent playback stimuli by generating 5 min of silence in Audition software (version 2.0; WAVE format, 44.1 kHz sampling rate, 16 bit amplitude encoding; Adobe Systems Inc., San Jose, CA, USA). To create the two experimental noise treatments (see Fig. 1), we began by generating two 5 min samples of white noise (WAVE format, 44.1 kHz sampling rate, 16 bit amplitude encoding). For the low-frequency noise treatment, we filtered one of the white noise samples with an 1,850–2,850 Hz bandpass filter. We created the high-frequency noise treatment by filtering the other white noise sample with a 2,850–3,850 Hz bandpass filter. We selected these frequency ranges based on preliminary recordings of 10 spring peepers that were found near our study sites. We selected a threshold frequency between our two noise treatments (i.e., 2,850 Hz) that corresponded to the mean peak frequency of these 10 individuals (see below for details of sound analysis), and which was similar to the mean peak frequency observed in other studies of this species (2.9 kHz; Wilczynski et al., 1984; Forester and Czarnowsky, 1985; Sullivan and Hinshaw, 1990). The 1000-Hz bandwidth of our stimuli ensured that no component of the subject's call could be less than the lowest frequencies of our low-frequency treatment or more than the highest frequencies of our high-frequency treatment, given the known frequency range of spring peeper calls (Fig. 1). Our playback stimuli were therefore designed with respect to the known characteristics of spring peeper advertisement calls, and not with respect to the auditory sensitivity of the receivers.

The two noise treatments were normalized to -15 dB root-mean-square amplitude using Raven software (version 1.4 Pro; Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, USA). We verified that our playback system broadcast the two stimuli at precisely the same amplitude by measuring their amplitude with a sound level meter (C-weighting, fast response) held 50 cm in front of the speaker, where the subject would normally reside. Our measurements showed that the realized amplitude of the high- and low-frequency stimuli were identical at the position of the subject (89-90 dB SPL).

1.4 Measuring call structure

We recorded a total of 4,689 calls from 46 individuals, including 19 individuals found in roadside marshes and 27 individuals found in non-roadside marshes. We used the 'automatic parameter measurement' feature (threshold setting: −10 dB; hold time: 50 ms) in AviSoft SASLab Pro (version 4.38; Avisoft Bioacoustics, R. Specht, Berlin, Germany) to analyze calls with minimal subjectivity. We created a separate spectrogram (1,024 point fast Fourier transform; 87.5% overlap; Blackman window; 43 Hz frequency resolution; 2.9 ms temporal resolution) for each call and inspected it to ensure that the automatic selection procedure had properly selected its onset and offset. We normalized the peak amplitude to 0 dB, and then measured call duration and peak frequency. Peak frequency was measured at the center of each call, as determined by the automatic parameter measurements feature in AviSoft. Finally, we calculated the rate at which subjects produced calls during each phase of the playback sequence, since previous studies have suggested that anurans adjust call rate in response to noise (Kaiser and Hammers, 2009; Cunnington and Fahrig, 2010), and that call

duration and call rate might be coupled (Love and Bee, 2010).

We derived our measurements of duration, call rate, and peak frequency from all calls produced by the subject during the silent baseline treatment and the two experimental noise treatments, but from only the first 10 calls of the two silent post-noise assessment periods, since the purpose of the post-noise assessment periods was to test for residual and potentially transient effects of experimental noise on call structure.

For some calls produced during the two experimental noise treatments, the automatic parameter measurement feature could not properly define the calls' onset and offset because they were too faint in relation to the broadcasted experimental noise. We excluded these calls from the analysis of duration and peak frequency, but included them in the analysis of call rate. As a result, our sample size was slightly larger for analyses involving call rate (see below). In addition, some subjects failed to produce any calls during one or more treatments and were thus excluded from analyses involving those treatments; sample sizes therefore varied slightly among analyses (see below). The removal of some signals from the analysis of the experimental noise treatments might have biased our results, particularly if the probability of being excluded (which is based on low signal-to-noise ratios) was correlated with the traits of interest (such as call duration). Our second set of analyses, in which we compared calls produced during the silent baseline treatment with the first 10 calls produced during the two silent post-noise assessment periods, allowed us to exclude this possibility, as no signals were excluded from any of these periods.

1.5 Statistical analyses

The number of calls varied considerably among individuals and playback treatments, so we calculated average values of duration, call rate, and peak frequency within each playback period (i.e., silent baseline treatment, high-frequency noise treatment, silent post-high-frequency noise assessment period, low-frequency noise treatment, silent post-low-frequency noise assessment period) for each individual. Only the averaged values of each response variable were used in subsequent statistical analyses.

We conducted two complementary sets of analyses. In the first, we used univariate repeated measures ANCOVA to compare the three averaged response variables between the silent baseline treatment and the two noise treatments. We included treatment (silent baseline, high-frequency noise, low-frequency noise) as a within-subject factor, site type (roadside, non-roadside) as a between-subject factor, and temperature as a covariate due to its influence on call structure (Gayou, 1984; Brenowitz et al., 1985; Sullivan and Hinshaw, 1990). Where an overall repeated measures ANCOVA was significant, we conducted three post-hoc pairwise comparisons that compared the two experimental noise treatments to each other and to the silent baseline period. We corrected for multiple comparisons by applying a Bonferroni correction to the *P*-values derived from the post-hoc analyses (i.e., each p-value was multiplied by three and then assessed for statistical significance relative to α = 0.05). Our sample size in this first set of analyses was 29 individuals (13 roadside, 16 non-roadside) for analyses of duration and peak frequency, and 40 individuals (17 roadside, 23 non-roadside) for the analysis of call rate. The second set of analyses was identical to the first, except that it compared the response variables between the silent baseline treatment and the two silent post-noise assessment periods. Our sample size in this second set of analyses was 26 individuals (14 roadside, 12 non-roadside) for analyses of duration and peak frequency, and 37 individuals (16 roadside, 21 non-roadside) for the analysis of call rate.

For all analyses, we reported effect sizes as partial eta-squared ($\eta^2_{partial}$; Cohen 1973). Following each analysis, we inspected histograms of the residuals and found that they always complied with the assumptions of normality and homogeneity of variance. Analyses also complied with the assumption of sphericity, as determined by Mauchly's test of

sphericity (all $P > 0.05$). All analyses were two-tailed, and results were considered statistically significant when $P \leq 0.05$.

2 Results

Experimental playback of noise significantly affected the duration of spring peeper advertisement calls (Fig. 2A; univariate repeated measures ANCOVA: $F_{2,52} = 7.94$, $P = 0.001$, $\eta^2_{partial} = 0.23$). Bonferroni-corrected post-hoc analyses showed that calls were shorter during the low-frequency noise treatment (mean $\pm$ SE $= 134 \pm 4$ ms) than during the high-frequency noise treatment (146 ± 5 ms; $P_{corrected} = 0.005$) or the silent baseline treatment (155 ± 5 ms; $P_{corrected} < 0.001$). Call duration was also significantly and inversely related to air temperature (Fig. 3A; $F_{1,26} = 13.95$, $P = 0.001$, $\eta^2_{partial} = 0.35$), which ranged between -1.7 °C and 15.7 °C during the recording sessions (mean $\pm$ SD $= 6.7 \pm 4.9$ °C), but was unaffected by site type (i.e., roadside versus non-roadside; $F_{1,26} = 2.00$, $P = 0.284$, $\eta^2_{partial} = 0.04$). We detected a similar pattern when we compared the silent baseline treatment and post-noise assessment periods. The preceding experimental noise treatment significantly affected the duration of spring peeper calls during the post-noise assessment periods (Fig. 2B; univariate repeated measures ANCOVA: $F_{2,46} = 9.23$, $P < 0.001$, $\eta^2_{partial} = 0.29$). Specifically, calls were significantly shorter during the post-low-frequency noise assessment period (133 ± 3 ms; $P_{corrected} < 0.001$) and the post-high-frequency noise assessment period (140 ± 6 ms; $P_{corrected} = 0.034$) than they were during the initial silent baseline treatment (157 ± 5 ms). Call duration was again significantly and inversely related to air temperature ($F_{1,23} = 20.57$, $P < 0.001$, $\eta^2_{partial} = 0.47$) and unrelated to site type ($F_{1,23} = 0.11$, $P = 0.743$, $\eta^2_{partial} = 0.01$).

The rate at which spring peepers produced calls was not affected by the experimental noise treatment (Fig. 2C; univariate repeated measures ANCOVA: $F_{2,74} = 2.77$, $P = 0.069$, $\eta^2_{partial} = 0.07$) or by site type ($F_{1,37} = 2.12$, $P = 0.154$, $\eta^2_{partial} = 0.05$), but it was significantly and positively related to ambient temperature (Fig. 3B; $F_{1,37} = 78.61$, $P < 0.001$, $\eta^2_{partial} = 0.68$). We detected a similar pattern when we compared the baseline and post-noise assessment periods. Call rate was not affected by the preceding experimental noise treatment (Fig. 2D; $F_{2,68} = 0.18$, $P = 0.833$, $\eta^2_{partial} = 0.01$) or by site type ($F_{1,34} = 3.09$, $P = 0.088$, $\eta^2_{partial} = 0.08$), but was again significantly and positively related to ambient temperature ($F_{1,34} = 73.80$, $P < 0.001$, $\eta^2_{partial} = 0.69$).

The peak frequency of spring peeper calls was unaffected by the experimental playback of noise (Fig. 2E; univariate repeated measures ANCOVA: $F_{2,52} = 0.64$, $P = 0.530$, $\eta^2_{partial} = 0.02$), by temperature (Fig. 3C; $F_{1,26} = 2.52$, $P = 0.125$, $\eta^2_{partial} = 0.09$), or by site type ($F_{1,26} = 2.67$, $P = 0.115$, $\eta^2_{partial} = 0.09$). However, the experimental playback of noise did affect the peak frequency of calls produced during the post-noise assessment periods (Fig. 2F; $F_{2,46} = 9.76$, $P < 0.001$, $\eta^2_{partial} = 0.30$). Specifically, peak frequency was significantly lower during post-high-frequency noise assessment period (2823 ± 22 Hz) than during the initial silent baseline treatment (2864 ± 20 Hz; $P_{corrected} = 0.018$). The comparison of post-noise assessment periods and baseline treatments again showed that peak frequency was unaffected by temperature ($F_{1,23} = 4.24$, $P = 0.051$, $\eta^2_{partial} = 0.16$) and site type ($F_{1,23} = 2.15$, $P = 0.156$, $\eta^2_{partial} = 0.09$).

3 Discussion

Spring peepers varied their advertisement calls in response to experimental noise. In response to noise, spring peepers produced advertisement calls that were, on average, 9 to 21 ms shorter than calls produced during the silent baseline treatment. This result was not an artifact caused by experimental noise masking the onset and offset of calls, since calls were also shorter during the silent post-noise assessment periods. The call rate and peak frequency of spring peeper calls did not change during the noise treatments, although peak frequency did decrease in the silent post-high-frequency noise assessment period. We conclude that noise significantly affects the structure of spring peeper calls.

Producing longer calls is a common strategy used by many animals for reducing interference in noisy environments (reviewed in Brumm et al., 2004). Longer calls are also known to be more attractive to female anurans when choosing their mates (Gerhardt, 1994; Gerhardt et al., 2000; Sullivan and Kwiatkowski, 2007; but see Richardson et al., 2010). It is therefore surprising that calls in our study became shorter in response to playback noise, as shorter calls should be less detectable and less attractive to females. One possible explanation for this result is that female anurans may lose their preference for longer calls as ambient noise increases (Schwartz et al., 2001; Bee, 2008). Another possible explanation is that subjects shortened their calls so that they were less likely to be overlapped by the loud and transient experimental noise. Indeed, some anurans are known to shorten their calls as a strategy for minimizing the probability of being overlapped by the loud calls of nearby competitors (Grafe, 1996). Whatever the reason, frogs continued to produce shorter calls even after the noise treatments ceased, suggesting that noise has potentially lasting residual effects on call structure. Our study also showed that calls became shorter as temperature increased. This relationship is expected, as it has been described in several anuran species, including the gray treefrog and the spring peeper (Gayou, 1984; Brenowitz et al., 1985; Sullivan and Hinshaw, 1990). Finally, call duration was even shorter in response to low-frequency noise than in response to high-frequency noise. We did not expect the duration of signals to differ between noise treatments, as they were broadcast at equal amplitudes. A possible explanation is that receivers had greater auditory sensitivity to the frequency characteristics of the low-frequency stimulus; however, a study on the peripheral auditory system suggests that male spring peepers should be more sensitive to the high-frequency noise treatment than to the low-frequency noise treatment (Wilczynski et al., 1984). Therefore, additional research will be needed to explain why call duration was affected more by low-frequency noise than by high-frequency noise.

Some previous studies suggest that amphibians increase their call rate in response to noise as a mechanism for increasing call detectability (Penna et al., 2005; Kaiser and Hammers, 2009). Furthermore, in several frog species, females have been found to prefer calls with higher call rates (Sullivan and Hinshaw, 1990), although this might only be the case when rate differences are extreme (Arak, 1988). Other studies suggest that call rate is inversely related to duration, and that this tradeoff maintains a constant duty cycle throughout the calling bout (i.e., the proportion of time that a call can be detected; Wells and Taigen, 1986; Schwartz et al., 2002; Cunnington and Fahrig, 2010; Love and Bee, 2010). In our study, call rate was not significantly influenced by noise, despite significant effects of noise on call duration. This shows that call duration and call rate are not completely dependent on one another (Wells and Taigen, 1986), and that they have the potential to vary independently, either through phenotypic plasticity or through adaptations driven by female mate choice or other forms of selection. As with call duration, however, call rate demonstrated a clear positive relationship with temperature.

Increasing the frequency of calls is a common strategy used by many animals for minimizing the masking effects of low-frequency noise (Slabbekoorn and Peet, 2003; Wood and Yezerinac, 2006; Bermúdez-Cuamatzin et al., 2009), but previous studies show that this strategy is used only rarely by anurans (Lengagne, 2008; Cunnington and Fahrig, 2010). In our study, spring peepers decreased the peak frequency of their advertisement calls during the silent post-high-frequency noise assessment period. This finding is consistent with spring peepers shifting the frequency of their calls away from interfering noise. Given that we did not see comparable effects during the post-low-frequency noise assessment period, or during either of the two noise treatments, the effect is weak at best. In addition, the magnitude of the documented change is probably insufficient for conspecifics to detect (Gerhardt and Huber, 2002), further emphasizing the minor importance of this variation. An alternative explanation for this result is that subjects may have perceived the broadcasted noise as a competing signal from a rival male, as opposed to signal interference *per se*. In other anuran species, males have been found to decrease the frequency of their signals in response to other males'

signals during aggressive interactions (Bee and Perrill, 1996). Furthermore, some anuran males are known to continue calling at lower frequencies after winning a vocal interaction with a rival male (Reichert and Gerhardt, 2013), which may explain our observation of lowered frequencies during the post-high-frequency assessment period.

One possible explanation for the weak effect of noise on peak frequency is that female anurans may prefer to mate with males that produce low-frequency calls (Richardson et al., 2010). Therefore, the benefits of producing low-frequency calls that attract females may outweigh the benefits of producing frequency-shifted calls that optimize call detectability, such as we would have expected during the low-frequency noise treatment. This explanation seems unlikely, however. A previous study on spring peepers showed that there was no preference for calls of any frequency in the absence of background noise, but a preference for higher frequency calls in the presence of background noise (Gerhardt and Schwartz, 2001). Another study showed that females seem to lose the capability to discriminate between frequencies when background noise is present (Wollerman and Wiley, 2002). A second possible explanation is that changing the frequency structure of calls may entail significant energetic costs that outweigh the associated benefits of increased call detectability (Bradbury and Vehrencamp, 2011). A final possible explanation is that changing the peak frequency of calls may have little or no effect on call detectability in noisy environments, despite theoretical arguments to the contrary (Slabbekoorn and Peet, 2003; Brumm and Slabbekoorn 2005; Bradbury and Vehrencamp 2011). A recent empirical study showed that the transmission distance of low- and high-frequency calls did not differ, regardless of whether they were broadcast in a noisy or quiet environment (Nemeth and Brumm, 2010). Future studies should further quantify the relationships between call frequency, call detectability, ambient noise, and female preferences.

Noise can disrupt an animal's communication system, which, in turn, can have adverse effects on the animal's reproductive success (Bee and Swanson, 2007; Lengagne et al., 2008). It is therefore important for future studies to document the effects of noise on animal communication, and for conservation biologists to consider these effects. We showed that noise affects the structure of spring peeper advertisement calls, and that spring peepers therefore have the capacity to alter signal structure in response to noise. Future studies on this species should test if other types of noise, such as biotic or anthropogenic noise, have similar effects on call structure, and if the observed changes to call structure enhance or impair communication in noisy environments.

References

Arak A, 1988. Female mate selection in the natterjack toad: Active choice or passive attraction? Behav. Ecol. Siociobiol. 22: 317–327.

Barber JR, Crooks KR, Fristrup KM, 2010. The costs of chronic noise exposure for terrestrial organisms. Trends Ecol. Evol. 25: 180–189.

Bee MA, Perrill, SA, 1996. Responses to conspecific advertisement calls in the green frog *Rana clamitans* and their role in male-male communication. Behaviour 133: 283–301.

Bee MA, 2004. Sound ruler acoustical analysis: A free, open code, multi-platform sound analysis and graphing package. Bioacoustics 14: 171–178.

Bee MA, Swanson EM, 2007. Auditory masking of anuran advertisement calls by road traffic noise. Anim. Behav. 74: 1765–1776.

Bee MA, 2008, Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog. Anim. Behav. 76: 845–853.

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

Bee MA, Velez A, Forester JD, 2012. Sound level discrimination by gray treefrogs in the presence and absence of chorus-shaped noise. J. Acoust. Soc. Am. 131: 4188–4195.

Bermúdez-Cuamatzin E, Rios-Chelen AA, Gil D, Garcia CM, 2009. Strategies of song adaptation to urban noise in the house finch: Syllable pitch plasticity or differential syllable use? Behaviour 146: 1269–1286.

Bradbury JW, Vehrencamp SL, 2011. Principles of Animal Communication, 2nd edn. Sunderland, MA: Sinauer Associates.

Brenowitz EA, Rose G, Capranica RR, 1985. Neural correlates of temperature coupling in the vocal communication system of the Gray treefrog *Hyla versicolor*. Brain Res. 359: 36–367.

Bridges AS, Dorcas, ME, 2000. Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. Copeia 2: 587–592.

Brumm H, Schmidt R, Schrader L, 2009. Noise-dependent vocal plasticity in domestic fowl. Anim. Behav. 78: 741–746.

Brumm H, Slabbekoorn H, 2005. Acoustic communication in noise. Adv. Stud. Behav. 35: 151–209.

Brumm H, Todt D, 2002. Noise-dependent song amplitude regulation in a territorial songbird. Anim. Behav. 63: 891–897.

Brumm H, Voss K, Kollmer I, Todt D, 2004. Acoustic communication in noise: regulation of call characteristics in a New World monkey. J. Exp. Biol. 207: 443–448.

Brumm H, Zollinger SA, 2011. The evolution of the Lombard effect: 100 years of psychoacoustic research. Behavior 148: 1173–1198.

Cohen J, 1973. Eta-squared and partial eta-squared in fixed factor ANOVA designs. Educ. Psychol. Meas. 33: 107–112.

Cornillon PC, Keane MA, 1976. Simple model for simulating traffic noise spectra. J. Acoust. Soc. Am. 61: 739–743.

Cunnington GM, Fahrig L, 2010. Plasticity in the vocalizations of anurans in response to traffic noise. Acta Oecol. 36: 463–470.

Dunlop RA, Cato DH, Noad MJ, 2010. Your attention please: increasing ambient noise levels elicits a change in communication behavior in humpback whales *Megaptera novaeangliae*. Proc. R. Soc. B. 277: 2521–2529.

Egnor SER, Hauser MD, 2006. Noise-induced vocal modulation in cotton-top tamarins *Saguinus oedipus*. Am. J. Primatol. 68: 1183–1190.

Ey E, Rahn C, Hammerschmidt K, Fischer J, 2009. Wild female olive baboons adapt their grunt vocalizations to environmental conditions. Ethology 115: 493–503.

Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL et al., 2006. Ultrasonic communication in frogs. Nature 440: 333–336.

Forester DC, Czarnowsky R, 1985. Sexual selection in the spring peeper *Hyla crucifer* (Amphibia, Anura): Role of the advertisement call. Behavior 92: 112–128.

Gayou DC, 1984. Effects of temperature on the mating call of *Hyla versicolor*. Copeia 1984: 733–738.

Gerhardt HC, 1994. Selective responsiveness to long range acoustic signals in insects and anurans. Amer. Zool. 34: 706–714.

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

Gerhardt HC, Bee MA, 2007. Recognition and localization of acoustic signals. In: Narins PM, Feng AS, Fay RR, Popper AN ed. Hearing and sound Communication in Amphibians. New York: Springer, 113–146.

Gerhardt HC, Tanner SD, Corrigan CM, Walton HC, 2000. Female preference functions based on call duration in the gray tree frog *Hyla versicolor*. Behav. Ecol. 11: 663–669.

Gerhardt HC, Schwartz JJ, 2001. Auditory tuning and frequency preferences. In: Ryan MJ ed. Anuran Communication. Washington and London: Smithsonian Institution Press, 73–85.

Grafe TU, 1996. The function of call alternation in the African reed frog *Hyperolius marmoratus*: Precise call timing prevents auditory masking. Behav. Ecol. Sociobiol. 38: 149–158.

Grafe TU, Preininger D, Sztatecsny M, Kasah R, Dehling JM et al., 2012. Multimodal communication in a noisy environment: A case study of the Bornean rock frog *Staurois parvus*. Plos One 7: e37965.

Greenfield MD, 1994. Cooperation and conflict in the evolution of signal interactions. Annu. Rev. Ecol. Evol. Syst. 25: 97–126.

Greenfield MD, Rand AS, 2000. Frogs have rules: Selective attention algorithms regulate chorusing in *Physalaemus pustulosus* (Leptodactylidae). Ethology 106: 331–347.

Halfwerk W, Bot S, Buikx J, Van der Velde M, Komdeur J et al., 2011. Low-frequency songs lose their potency in noisy urban conditions. Proc. Natl. Acad. Sci. U. S. A. 108: 14549–14554.

Halfwerk W, Slabbekoorn H, 2009. A behavioral mechanism explaining noise-dependent frequency use in urban birdsong. Anim. Behav. 78: 1301–1307.

Hanna D, Blouin-Demers G, Wilson DR, Mennill DJ, 2011. Anthropogenic noise affects song structure in red–winged blackbirds *Agelaius phoeniceus*. J. Exp. Biol. 214: 3549–3556.

Kaiser K, Hammers JL, 2009. The effect of anthropogenic noise on male advertisement call rate in the neotropical treefrog *Dendropsophus triangulum*. Behaviour 146: 1053–1069.

Kirschel ANG, Blumstein DT, Cohen RE, Buermann W, Smith TB et al., 2009. Birdsong tuned to the environment: Green hylia song varies with elevation, tree cover, and noise. Behav. Ecol. 20: 1089–1095.

Laiolo P, 2010. The emerging significance of bioacoustics in animal species conservation. Biol. Cons. 143: 1635–1645.

Lengagne T, 2008. Traffic noise affects communication behavior in a breeding anuran *Hyla arborea*. Biol. Cons. 141: 2023–2031.

Love EK, Bee MA, 2010. An experimental test of noise-dependent voice amplitude regulation in Cope's grey treefrog *Hyla chrysoscelis*. Anim. Behav. 80: 509–515.

Luther D, Baptista L, 2010. Urban noise and the cultural evolution of bird songs. Proc. R. Soc. B. 277: 469–473.

Luther DA, Derryberry EP, 2012. Birdsongs keep pace with city life: Changes in song over time in an urban songbird affects communication. Anim. Behav. 83: 1059–1066.

McNett GD, Luan LH, Cocroft RB, 2010. Wind-induced noise alters signaler and receiver behavior in vibrational communication. Behav. Ecol. Sociobiol. 64: 2043–2051.

Nemeth E, Brumm H, 2010. Birds and anthropogenic noise: Are urban songs adaptive? Amer. Nat. 176: 465–475.

Parks SE, Johnson M, Nowacek D, Tyack PL, 2011. Individual right whales call louder in increased environmental noise. Biol. Lett. 7: 33–35.

Parris KM, 2002. More bang for your buck: the effect of caller position, habitat and chorus noise on the efficiency of calling in the spring peeper. Ecol. Model. 156: 213–224.

Parris KM, Velik-Lord M, North JMA, 2009. Frogs call at a higher pitch in traffic noise. Ecol. Soc. 14: 1–24.

Patricelli GL, Blickley JL, 2006. Avian communication in urban noise: Causes and consequences of vocal adjustment. Auk 123: 639–649.

Penna M, Pottstock H, Velasquez N, 2005. Effect of natural and synthetic noise on evoked vocal responses in a frog of the temperate austral forest. Anim. Behav. 70: 639–651.

Potvin DA, Mulder RA, 2013. Immediate, independent adjustment of call pitch and amplitude in response to varying background noise by silvereyes *Zosterops lateralis*. Behav. Ecol. in press. doi:10.1093/beheco/art075

Pytte CL, Rusch KM, Ficken MS, 2003. Regulation of vocal amplitude by the blue-throated hummingbird *Lampornis clemenciae*. Anim. Behav. 66: 703–710.

Reichert MS, Gerhardt HC, 2013. Gray tree frogs *Hyla versicolor*, give lower-frequency aggressive calls in more escalated contests. Behav. Ecol. Sociobiol. 67: 795–804.

Richardson C, Joly P, Lena JP, Plenet S, Lengagne T, 2010. The challenge of finding a high-quality male: A treefrog solution based on female assessment of male calls. Behaviour 147: 1737–1752.

Ripmeester EAP, Mulder M, Slabbekoorn H, 2010. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. Behav. Ecol. 21: 876—883.

Rosen M, Lemon RE, 1974. The vocal behavior of spring peepers *Hyla crucifer*. Copeia 4: 940–950.

Schwartz JJ, Buchanan BW, Gerhardt HC, 2001. Female mate choice in the gray treefrog *Hyla versicolor* in three experimental environments. Behav. Ecol. Sociobiol. 49: 443–455.

Schwartz JJ, Buchanan BW, Gerhardt HC, 2002. Acoustic interactions among male gray treefrogs *Hyla versicolor* in a chorus setting. Behav. Ecol. Sociobiol. 49: 443–455.

Schwartz JJ, Gerhardt HC, 1998. The neuroethology of frequency preferences in the Spring peeper. Anim. Behav. 56: 55–69.

Slabbekoorn H, Peet M, 2003. Birds sing at a higher pitch in urban noise: Great tits hit the high notes to ensure that their mating calls are heard above the city's din. Nature 424: 267–267.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL et al., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306: 1783–1786.

Sullivan BK, Hinshaw SH, 1990. Variation in advertisement calls and male calling behavior in the spring peeper *Pseudacris crucifer*. Copeia 4: 1146–1150.

Sullivan BK, Kwiatkowski MA, 2007. Courtship displays in anurans and lizards: Theoretical and empirical contributions to our understanding of costs and selection on males due to female choice. Func. Ecol. 21: 666—675.

Sun JWC, Narins PA, 2005. Anthropogenic sounds differentially affect amphibian call rate. Biol. Cons. 121: 419–427.

Tyack PL, 2008. Implications for marine mammals of large-scale changes in the marine acoustic environment. J. Mammol. 89: 549–558.

Velez A, Bee MA, 2010. Signal recognition by frogs in the presence of temporally fluctuating chorus-shaped noise. Behav. Ecol. Sociobiol. 64: 1695–1709.

Verzijden MN, Ripmeester EAP, Ohms VR, Snelderwaard P, Slabbekoorn H, 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. J. Exp. Biol. 213: 2575–2581.

Wollerman L, Wiley H, 2002. Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. Anim. Behav. 63: 15–22.

Wells KD, Taigen TL, 1986. The effect of social interactions on calling energetics in the gray treefrog *Hyla versicolor*. Behav. Ecol. Sociobiol. 19: 9–18.

Wells KD, 2007. The Ecology and Behavior of Amphibians. Chicago: The University of Chicago Press.

Wilczynski W, Zakon HH, Brenowitz EA, 1984. Acoustic communication in spring peepers: Call characteristics and neurophysiological aspects. J. Comp. Physiol. 155: 577–584.

Wilczynski W, Chu J, 2001. Acoustic communication, endrocrine control and the neurochemical systems of the brain. In: Ryan MJ ed. Anuran Communication. Washington and London: Smithsonian Institution Press, 23–35.

Wood WE, Yezerinac SM, 2006. Song sparrow *Melospiza melodia* song varies with urban noise. Auk 123: 650–659.
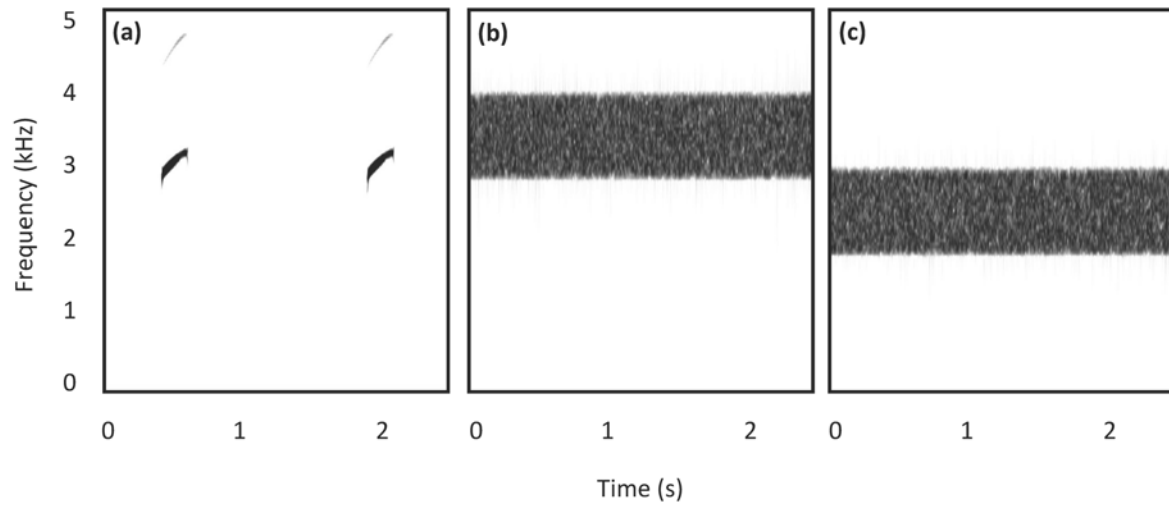
Figure 1

Fig. 1 Experimental noise was broadcast to 46 free-living male spring peepers to determine its effect on the structure of their advertisement calls

Shown are (**A**) two examples of spring peeper advertisement calls, (**B**) a 2.5-s segment of the 5-min high-frequency noise stimulus, and (**C**) a 2.5-s segment of the 5-min low-frequency noise stimulus. Spectrograms were generated using a 1024-point fast Fourier transform, 87.5% overlap, and Hamming window, which resulted in a frequency resolution of 43 Hz and a temporal resolution of 2.9 ms.
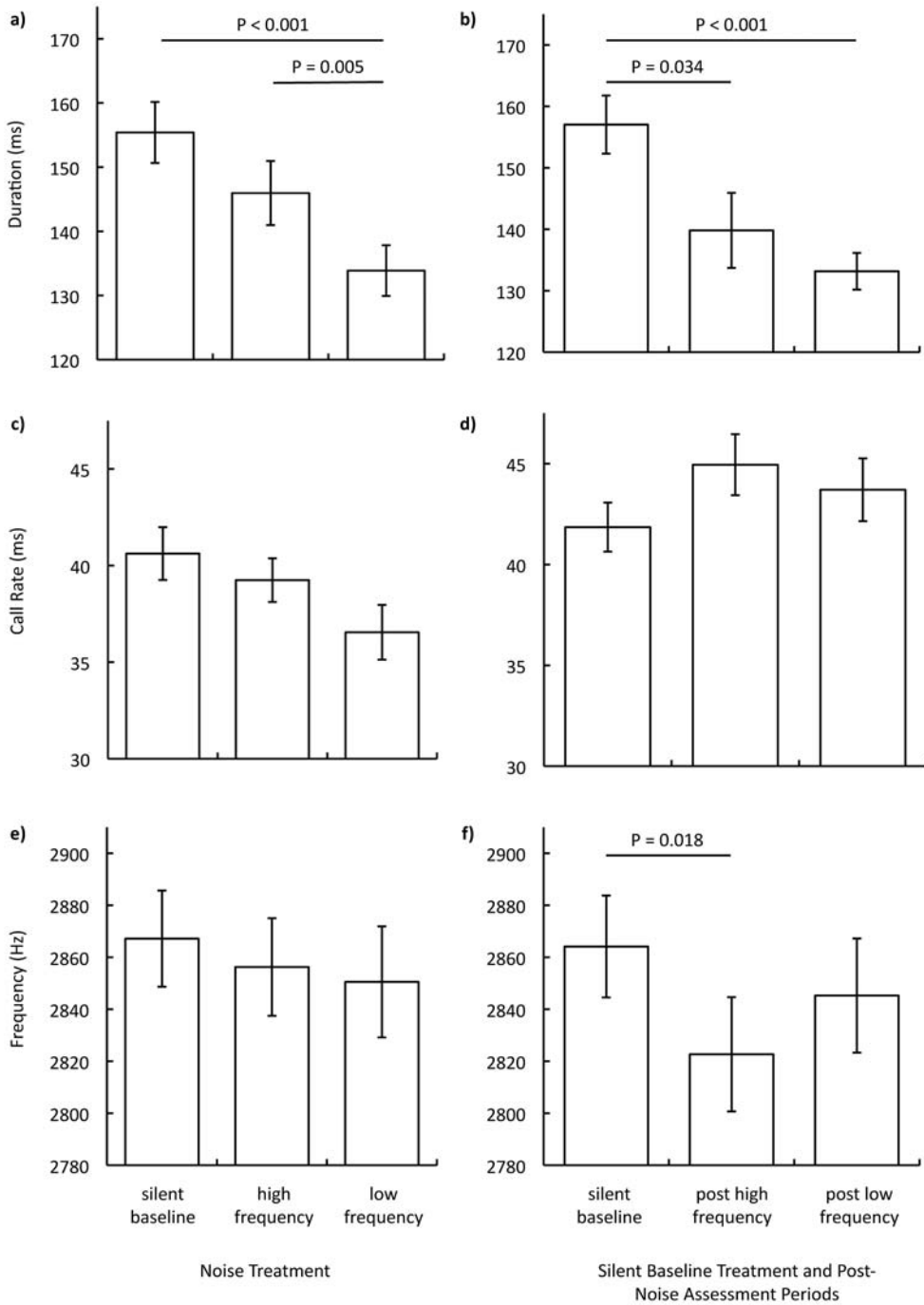
Fig. 2 Effects of noise on the structural characteristics of spring peeper advertisement calls

In the left column of panels (**a**, **c**, **e**), structural characteristics were compared among the silent baseline treatment, the high-frequency noise treatment, and the low-frequency noise treatment. In the right column of panels (**b**, **d**, **f**), structural characteristics were compared among the silent baseline treatment, the silent post-high-frequency noise assessment period, and the silent post-low-frequency noise assessment period. Structural characteristics (mean ± *SE*) include call duration (**a**, **b**), call rate (**c**, **d**), and peak frequency (**e**, **f**). Pairwise comparisons among treatments were conducted only when the overall univariate repeated measures ANCOVA was significant; statistically significant pairwise comparisons are indicated by horizontal lines and Bonferroni-corrected *P*-values.

Fig. 3 Relationship between temperature and structural characteristics of spring peeper advertisement calls during the silent baseline treatment

Structural characteristics include (**A**) call duration, (**B**) call rate, and (**C**) peak frequency. Sample sizes are based on the set of analyses that compared the silent baseline treatment to the high-frequency and low-frequency noise treatments (see text; $n = 29$ males for duration; $n = 40$ males for call rate; $n = 29$ males for peak frequency). Statistical significance and estimates of effect size for temperature, as determined from a univariate repeated measures ANCOVA, are reported at the top of each panel.

Figure 3

(a) $p = 0.001$, $\eta^2_{partial} = 0.35$. Duration (ms) versus Temperature °C.

(b) $p < 0.001$, $\eta^2_{partial} = 0.68$. Call Rate (calls/min) versus Temperature °C.

(c) $p = 0.125$, $\eta^2_{partial} = 0.09$. Frequency (Hz) versus Temperature °C.