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Spatial hearing in Cope's gray treefrog: I. Open and closed loop experiments on sound localization in the presence and absence of noise

Michael S. Caldwell and Mark A. Bee

Department of Ecology, Evolution and Behavior, University of Minnesota, Ecology 100, 1987 Upper Buford Circle, St. Paul, MN 55108, USA

Abstract

The ability to reliably locate sound sources is critical to anurans, which navigate acoustically complex breeding choruses when choosing mates. Yet, the factors influencing sound localization performance in frogs remain largely unexplored. We applied two complementary methodologies, open and closed loop playback trials, to identify influences on localization abilities in Cope's gray treefrog, *Hyla chrysoscelis*. We examined localization acuity and phonotaxis behavior of females in response to advertisement calls presented from 12 azimuthal angles, at two signal levels, in the presence and absence of noise, and at two noise levels. Orientation responses were consistent with precise localization of sound sources, rather than binary discrimination between sources on either side of the body (*lateralization*). Frogs were unable to discriminate between sounds arriving from forward and rearward directions, and accurate localization was limited to forward sound presentation angles. Within this region, sound presentation angle had little effect on localization acuity. The presence of noise and low signal-to-noise ratios also did not strongly impair localization ability in open loop trials, but females exhibited reduced phonotaxis performance consistent with impaired localization during closed loop trials. We discuss these results in light of previous work on spatial hearing in anurans.

Keywords

anura; sound localization; source segregation; ear; communication

Introduction

Our understanding of sound source localization in anurans is far less comprehensive than it is for humans and other mammals (Middlebrooks and Green 1991; Brown and May 2005; Christensen-Dalsgaard 2005). For most frogs, sound localization forms a key aspect of their sexual behavior (Gerhardt and Huber 2002; Wells 2007), and these acoustically mediated behaviors often take place under adverse listening conditions, such as in the presence of multiple signalers or high levels of background noise (Narins and Zelick 1988; Narins 1992; Feng and Schul 2007; Bee 2012; Vélez et al. 2014). Frogs generally lack the external physical structures (e.g., pinnae in mammals; facial ruffs in barn owls) that aid some animals in localizing sound sources. Frogs are also quite small and thus have small inter-aural distances (e.g., Gerhardt and Rheinlaender 1980), which limits the use of external binaural

cues related to inter-aural time and level differences (ITDs and ILDs) (Gerhardt and Bee 2007). Nevertheless, frogs accurately localize sound sources, and some exceptional species exhibit remarkable localization acuity on par with humans and other animals, such as barn owls and dolphins (Shen et al. 2008). Previous physiological and behavioral studies demonstrate that internal mechanical coupling of the left and right tympana through the air spaces of the Eustachian tubes and mouth cavity allows frogs to exploit cues to source location (Christensen-Dalsgaard 2005, 2011), but the capabilities of such ‘pressure difference’ ears are still poorly understood. There is particularly little information on the innate localization abilities of anurans independent of behavioral strategies, such as body and head scanning movements (Rheinlaender and Klump 1988), that can improve location estimates. Similarly, there has been little experimental attention paid to how localization performance is affected by important challenges that frogs face in nature, such as variation in sound incident angle and the presence of background noise (but see Gerhardt and Rheinlaender 1980; Ursprung et al. 2009).

Most studies of sound localization in vertebrates have employed a *closed loop* experimental methodology (Brown and May 2005; Christensen-Dalsgaard 2005). In closed loop trials, the animal is permitted to adjust its head, ear, or body position during or between presentations of a sound stimulus, and thereby refine its estimation of sound source location by sampling several points of the sound field. Closed loop methodologies are a powerful tool, as they allow an animal to behave very much as it would during localization tasks in nature. They are limited, however, in that they cannot measure localization acuity independently of behavioral strategies that refine this acuity. In *open loop* studies of localization performance, a sound is presented and playback is stopped before the animal is able to resample the sound field and subsequently update its estimate of source location. Any response is, therefore, based upon location estimates derived from a single position of the head, ears, and body. While several previous studies have investigated sound localization performance in anurans using closed loop methods (Feng et al. 1976; Rheinlaender et al. 1979; Gerhardt and Rheinlaender 1980, 1982; Passmore et al. 1984; Jørgensen and Gerhardt 1991; Bosch and Márquez 2000; Shen et al. 2008; Ursprung et al. 2009), only one has done so using open loop methods (Klump and Gerhardt 1989). That study of localization in the barking treefrog, *Hyla gratiosa*, tested responses to sounds originating from forward azimuthal angles only. To our knowledge, localization acuity to sounds originating from behind the animal has never been measured in any frog.

In the present study, we address some of the current gaps in knowledge of anuran spatial hearing by investigating the sound localization abilities of Cope’s gray treefrog (*Hyla chrysoscelis*). Females of *H. chrysoscelis* locate calling males within dense, noisy, mixed-species breeding choruses, and have become a model for the study of acoustically mediated sexual behavior and anuran hearing (Gerhardt 2001; Bee 2012; Vélez et al. 2014). While many treefrogs communicate in complex three dimensional environments (Gerhardt and Rheinlaender, 1982; Passmore et al., 1984), females in Minnesota populations of *H. chrysoscelis* usually assess and localize potential mates on what is essentially an azimuthal plane, as they move across the surface of a pond, approaching males calling from the same pond surface (Caldwell and Bee, pers. obs). We used both an open loop and a closed loop behavioral experiment (Klump 1995) to ask four major questions about sound source localization by females of this species. First, we used open loop trials to ask whether *H. chrysoscelis* is capable of azimuthal localization precision surpassing simple lateralization, defined here as the determination of whether a sound originates from the left or right of the body’s midline. Many, but not all, frogs approach a sound source with a characteristic ‘zig zag’ path (Feng et al. 1976; Rheinlaender et al. 1979; Gerhardt and Rheinlaender 1980; Passmore and Telford 1981; Hödl et al. 2004; but see Ursprung et al. 2009). While jump and

body orientation angles relative to the speaker along this path are typically under 30° (Christensen-Dalsgaard 2005), an ear with much poorer angular acuity, used in combination with lateralization, could nevertheless result in relatively small orientation errors.

Second, we asked if *H. chrysoscelis* is capable of determining whether a sound originates from in front of (rostral to) the inter-aural axis or from behind (caudal to) the inter-aural axis in open loop conditions. In anurans, the use of inter-aural comparisons for sound localization is potentially susceptible to ambiguity between sound incident angles equidistant from the inter-aural axis (e.g., sounds arriving at 45° versus 135° relative to the animal's snout). This is because, in the absence physical structures shaping the level or spectrum of sounds arriving at each ear, binaural cues will be identical at these angles (Wallach 1939). Indeed, laser vibrometer studies of the directionality of the frog ear generally show that tympanum responses are approximately symmetrical around the inter-aural axis (Michelsen et al. 1986; Jørgensen 1991; Jørgensen and Gerhardt 1991; Ho and Narins 2006). Our companion study using laser vibrometry confirms that this pattern holds for *H. chrysoscelis* as well (Caldwell et al. submitted). These results suggest that frogs may be unable to discriminate between sounds originating from directions in front of or behind the inter-aural axis without sampling the sound field from more than one position, but this possibility has not been previously tested.

Third, we used open loop trials to examine how localization acuity varies with sound incident angle. In addition to ambiguity between sounds arriving from forward and rearward directions, the precision of azimuthal location estimates may vary on finer spatial scales. ITDs and IIDs become smaller at increasingly acute azimuthal sound incident angles relative to the midline (Christensen-Dalsgaard 2005; Ho and Narins 2006), and we might expect this to affect localization acuity. Indeed, in *H. gratiiosa*, orientation errors are larger for sound sources directly in front of the animal than they are for sources offset by 30° or 45° (Klump and Gerhardt 1989). The dependence of localization acuity on sound presentation angle has not been tested in any other frog.

Finally, we used both open loop and closed loop trials to investigate the extent to which localization performance is impaired by background noise. Two previous studies measured a high level of accuracy in closed loop phonotaxis trials conducted in natural (and presumably noisy) settings (Gerhardt and Rheinlaender 1980; Ursprung et al. 2009), but neither study reported the levels or spectral characteristics of the background noise. Yet, it is clear that the presence of ambient background noise can interfere with the ability of frogs to respond appropriately to acoustic signals. Females of *H. chrysoscelis*, for example, take longer to arrive at a speaker playing male advertisement calls in the presence of chorus noise or noise from abiotic sources (Bee and Swanson 2007; Bee and Schwartz 2009). Likewise, the presence of noise, especially at relatively low signal-to-noise ratios (SNRs), can interfere with the ability of frogs to discriminate between sexual signals (Schwartz et al. 2001; Wollerman and Wiley 2002; Bee 2008b, a; Ward et al. 2013a, b). In each of these cases, it is possible that reduced performance in phonotaxis trials was due, at least in part, to impaired localization acuity in noisy conditions. It is well documented that humans suffer reduced localization acuity at low SNRs (Good and Gilkey 1996; Lorenzi et al. 1999; Lingner et al. 2012), but it remains unknown whether anurans suffer similar noise-induced reductions in acuity.

Materials and methods

Subjects

The subjects in this study were females of the western mtDNA lineage (Ptacek et al. 1994) of Cope's gray treefrog, *H. chrysoscelis*. During May and June, 2011 and 2012, we collected amplexed pairs from ponds at three sites in eastern Minnesota, USA (Carver Park Reserve, Carver county; Crow-Hassan Park Reserve, Hennepin county; and Lake Maria State Park, Wright county). We placed pairs individually into plastic containers and transported them to our laboratory in St. Paul, MN. There, each container was partially filled with aged tap water and kept in the dark at ~2° C until shortly before testing. A minimum of 30 min prior to testing, pairs were placed in a dark incubator at 20° C. Females were temporarily separated from their mates during each trial. All pairs were returned to their original site of collection the day after testing. Pairs were housed in the laboratory for a maximum of 3 days.

A total of 225 females were collected and tested for this study. Of these, 190 females completed all designated trials successfully; 127 of these females were tested in the open loop experiment and 63 were tested in the closed loop experiment.

Arena and stimuli

The behavioral arena, playback equipment, and all acoustic stimuli were identical for the open and closed loop experiments. Animals were tested in a 2-m diameter circular arena, with a carpeted floor and acoustically transparent, but visually opaque, walls made of black cloth and metal wire. The arena was housed in a custom-built, semi-anechoic sound chamber (Industrial Acoustics Company, Bronx, NY; see Bee and Schwartz, 2009, for a complete description). The inside of the chamber was painted dark gray and lined with sound absorbent panels (IAC Planarchoic™). We maintained temperature inside the chamber at 20 ± 2 °C, which is a typical breeding temperature for *H. chrysoscelis*. Digital stimulus files (.wav format, 16-bit, 44.1 kHz) were played from Adobe Audition software (v.1.5, Adobe Systems Inc., San Jose, CA) on a desktop PC, through an external sound card (Firewire 410, M-Audio, Irwindale, CA). Analog output from the sound card was amplified by an HTD DMA-1275 amplifier (Home Theater Direct Inc., Plano, TX) and output through speakers in the sound chamber (Mod1, Orb Audio, New York, NY). The sound chamber was dimly illuminated by a red incandescent 40 W light bulb, shaded so that all light was reflected off of the dark gray ceiling. An additional infrared LED light source was mounted to the ceiling above the center of the test arena. All trials were recorded from overhead with an IR-sensitive video camera (WV-BP334, Panasonic, Syracuse, NJ), and video was digitized in real time using a PicoLo PCIe video capture card (Euresys, San Jaun Capistrano, CA).

The stimulus was a synthetic *H. chrysoscelis* call generated in MATLAB (7.6.0, MathWorks, Natick, MA). This call had spectral, temporal, and amplitude features approximating the population mean values at our field sites in Minnesota (Ward et al. 2013b). The call had 32 pulses, and two harmonically related spectral peaks at frequencies (and relative amplitudes) of 1250 (-6 dB) and 2500 Hz (0 dB). The call was played from one of four speakers equally spaced, with 90° separation, at floor level outside the wall of the circular test arena. For trials with noise, we generated band-limited white noise (500–4500 Hz) in Audition and played it from an overhead speaker suspended from the sound chamber ceiling at a height of 1.9 m above the center of the test arena. The bandwidth of noise was chosen to encompass the sound frequencies typically present in mix-species frog choruses in eastern Minnesota (see Fig. 1e in Nityananda and Bee 2011).

Noise was equalized to ensure a flat spectrum (± 2 dB) before each new frog entered the arena using a Brüel and Kjær Type 2250 sound level meter (Norcross, GA) in conjunction with a Stanford Research Systems 785 dynamic signal analyzer (Stanford Research Systems, Inc., Sunnyvale, CA) and the parametric equalizer module in Audition. During equalization, the microphone from the sound level meter was placed in the center of the arena, 3 cm off of the ground, pointing in the direction of the speaker 1 m away that would play the calls for the subsequent set of playback trials. The spectrum of the call stimulus was equalized using the same procedure to ensure a 6 dB amplitude difference between the 1250 and 2500 Hz peaks. Calibrations of playback levels were made using the sound level meter with its microphone in the same position used for spectrum equalization. Calls were calibrated to either 79 dB or 85 dB sound pressure level (SPL re 20 μ Pa, flat RMS, C-weighted) at a distance of 1 m. The 85 dB SPL approximates the lower end of the natural range of SPLs measured for *H. chrysoscelis* calls (Gerhardt 1975). Noise from the overhead speaker was calibrated to either 73 dB or 76 dB SPL (LC_{eq}) at the position of the sound level meter microphone. These levels were within the range typical of noise levels we and others have recorded in gray treefrog breeding choruses (Caldwell, M. S. and Bee, M. A., unpublished data; Schwartz et al. 2001). Noise SPL varied within ± 2 dB across the floor of the test arena.

Open loop experiment

Testing procedure—For open loop trials, females were positioned in the center of the test arena, with their snouts oriented at a predetermined angle on the azimuthal plane away from the call speaker located immediately behind the arena wall, 1 m away. Except where specified otherwise, we defined 0° as directly in front of the frog, 90° as directly to the side of the frog, and 180° as directly behind the frog. The side of the frog's body from which calls were played, left or right, was randomized between subjects. After positioning the frog, the experimenter exited the test arena and sat motionless in the corner of the sound chamber. If the frog moved within 10 s of being positioned, or before playback began, the experimenter returned to the arena and repositioned the frog. Playback was controlled by a second experimenter monitoring a video feed of the trial from outside of the chamber. The second experimenter also recorded response latencies for each trial in real time using a stopwatch.

For inclusion in the dataset, each frog had to complete a series of 15 successful trials. These included three closed loop *reference trials*, nine open loop *test trials*, and three open loop *sham trials*. As the first and last trial in the series of 15 trials, we conducted a closed loop reference trial in the absence of noise to confirm that the subject was receptive to playback of calls at the start and end of the trial series. In these closed loop reference trials, subjects were placed with the call speaker at 0° or 180° relative to its snout. The order of 0° and 180° presentation alternated between subjects. Calls were repeated at a rate of one call every 5 s and playback was not stopped until the subject contacted the arena wall within $\pm 7.5^\circ$ (approximately 10 cm) of the call speaker. The second trial in the series of 15 trials was an additional closed loop reference trial conducted in the presence of the broadband noise broadcast from the overhead speaker. This additional reference trial was used to ensure that all frogs, including those that would not be tested in noise, were similarly motivated to localize a call in noise. In this trial, the subjects were always positioned with their snouts 180° relative to the speaker (the most challenging angle; see below), and were given 2 min to make an initial movement and 5 min to reach the speaker. Subjects that did not reach the speaker within 5 min during any reference trial were put in the incubator for 10 min before being re-tested in the same reference trial on a second, and final, attempt. If the subject responded during the second attempt at the reference trial, testing with that subject

continued; otherwise, further testing with that subject was halted. In total, 27 frogs were excluded from further testing because we could not elicit a response during two attempts at a reference trial.

The primary data of interest were collected in the nine open loop test trials using a factorial experimental design. Across these nine trials, we tested each subject once at each of nine different angles (i.e., angle was a within-subjects factor in our design). Different groups of subjects were tested at the two different signal levels (79 dB or 85 dB) and in two different noise conditions (present or absent). In the noise present condition, different groups of subjects were tested with noise broadcast at either 73 dB or 76 dB. Females were randomly assigned to the six combinations of signal level and noise condition/level to provide approximately equal sample sizes for each combination ($N = 20$ to 22 females per group). During each of the nine open loop test trials, the subject was positioned in the arena such that the designated call speaker was at one of nine different angles relative to its snout (0° , 15° , 30° , 45° , 90° , 135° , 150° , 165° , or 180°), with order randomized for each subject. After call playback began, calls were repeated at a rate of one call every 8 s until the frog made any orientation movement. As soon as the frog moved, playback was stopped. We considered any perceptible rotational movement of the frog's body or head (movements greater than $\sim 1^\circ$ were visible in recordings) or any translational movement due to walking or hopping as orientation movements. Rotational movements without translational movements were common (36% of trials), whereas translational movements without some measurable rotational movement were extremely rare. Hence, 64% of test trials involved rotational movement in conjunction with translational movements. During these trials, females generally exhibited translational movement in the same direction to which their rotational movement was directed. Limb movements from a fixed body position that did not result in head or body rotation or translation were not considered orientation movements. The majority of orientation movements scored in test trials were made after the first, second, or third call was broadcast (i.e., within the first 24 s of the trial).

We conducted sham trials to distinguish between genuine responses to call playback and the possibility that the orientations we scored in test trials were simply random movements. Each subject was tested in three sham trials in which it was positioned in the center of the arena as usual (with its snout at 0° , 90° , and 180° relative to the speaker, tested in random order), but was not presented with call playback. For each subject, sham trials were conducted using the same combination of noise condition (present or absent) and noise level (73 dB or 76 dB, if noise present) in which it was tested in the open loop test trials. We monitored subject behavior for up to 120 s during a sham trial. If the frog did not move during this time, its response latency was scored as 120 s.

To control for the possibility of directional biases in the test arena, the position of the call speaker (one of four locations) was randomly selected for each subject. The side of the test subject from which stimuli were presented (left or right) was also randomly selected for each individual. To allow test subjects to acclimate to the noise stimuli, overhead noise began at least 1 min prior to the start of all trials with noise. Likewise, subjects were given at least one minute to acclimate to the quiet conditions when a noise free trial followed a trial with noise.

An additional note about our signal and noise levels is necessary. Across the four factorial combinations of signal level and noise level tested in this study, the nominal SNRs were +3 dB and +6 dB (for the 79-dB signal in 76-dB noise and 73-dB noise, respectively) or +9 dB and +12 dB (for the 85-dB signal in 76-dB noise and 73-dB noise, respectively). In earlier studies of *H. chrysoscelis*, we have reported behavioral response thresholds in noise on the

order of -3 dB to +3 dB SNR, depending on the spectrotemporal characteristics of the noise (e.g., Bee 2007b; Bee and Swanson 2007; Bee and Schwartz 2009; Vélez and Bee 2013). Therefore, our general expectation was that any effects of noise, if present, would be most pronounced at the lowest SNR of +3 dB (79-dB signal in 76-dB noise).

Analysis—We exported still frames, captured immediately after the first movement, from videos of each test and sham trial. From these images we measured the subject's angular orientation relative to its initial orientation and the position of the call speaker using ImageJ software (U. S. National Institutes of Health, Bethesda, MD). We also measured the distance moved away from the starting location at the center of the arena, if any, and the direction of this movement. All measurements were made blind to experimental treatment. In approximately 37% of open loop test trials, frogs made a second, additional movement within 10 s of their first movement and cessation of the stimulus. We explored whether the frog's position after this second movement might be a better indicator of sound source localization acuity by exporting and scoring still frames of the video taken 10 s after the frog's initial movement. Subsequent comparisons of data taken from images of initial movements and of the frog 10 s post movement revealed that the two measures produced quantitatively and qualitatively similar results. We, therefore, focus the analyses presented here on initial movements.

For each open loop test and sham trial, we scored three response variables: turn angle, orientation error, and latency to first movement. We defined *turn angle* as the angle that a subject turned relative to its orientation at the start of each trial (Fig. 1). Hence, turn angles reflect only rotational movement. No turn angles approached a full 360° rotation, and the vast majority were below 90° in magnitude. We defined *orientation error* as the absolute value of a subject's angle away from pointing directly at the speaker following its first orientation movement. Orientation error was our primary measure of open loop localization acuity. We calculated orientation error in two ways (Fig. 1b, 1c). The first assumed that a frog's estimate of sound source location was based solely on speaker position relative to the frog's snout during playback, and did not account for any translational movement of the frog away from its initial location during its first movement (Fig. 1b, 1c). The second method of determining orientation error assumed that females used the direction and distance they traveled as a result of any translational movement during their response to calculate an adjusted angle to the call speaker (Fig. 1b, 1c). Hence, this second measure of orientation error accounts for both rotational and translational movements. Orientation errors calculated without regard for translational movement were consistently lower than orientation errors that took translational movements into account. Moreover, results from preliminary statistical analyses produced similar output for both measures.

Therefore, measurements of orientation error for all further analyses were made using our first method (Fig. 1). This decision also allowed us to make direct comparisons to data on barking treefrogs reported by Klump and Gerhardt (1989). We defined *latency* to first movement as the time from the start of call playback until the frog made its first movement.

We analyzed our response variables (turn angle, ln-transformed orientation error, and ln-transformed latency) using a combination of factorial multivariate and univariate analysis of variance (MANOVA and ANOVA, respectively). In preliminary analyses, we also included mean-centered body size (snout-to-vent length in mm) as a covariate, but it was not significant and inclusion of this covariate did not influence the significance or effect sizes of other variables of interest in the model. Hence, body size was not included in the models reported here. We compared all three response variables simultaneously in a 9 *sound presentation angle* (0°, 15°, 30°, 45°, 90°, 135°, 150°, 165°, and 180°; within subjects) $\times$ 2

signal level (79 dB versus 85 dB; between subjects) $\times$ 2 *noise condition* (present versus absent; between subjects) $\times$ 2 *noise level* (76 dB versus 73 dB, nested within the noise present treatment; between subjects) MANOVA. This analysis revealed several significant effects; therefore, we analyzed response variables separately in subsequent univariate ANOVAs designed to address specific questions of interest.

To test whether *H. chrysoscelis* uses a simple strategy of lateralization during phonotaxis, or it more accurately localizes sound sources, we compared the response variable turn angle in a 9 *sound presentation angle* $\times$ 2 *signal level* $\times$ 2 *playback side* (left versus right side of the animal; between subjects) ANOVA. For this analysis only, turns to the left were scored as negative turn angles, and turns to the right were scored as positive turn angles. (For all other analyses turns towards the speaker were scored as positive turn angles, and turns away from the speaker were scored as negative turn angles.) We limited this analysis to the noise absent condition to examine the animals' capabilities in optimal listening conditions unconstrained by background noise. If *H. chrysoscelis* only lateralizes sound sources, we expected turn angle to be independent of sound presentation angle (Klump and Gerhardt 1989). We, therefore, predicted a significant main effect of playback side (left versus right), but no significant main effect of sound presentation angle and no interaction between playback side and sound presentation angle. In contrast, if frogs more accurately localize sound sources beyond left/right judgments, we predicted a significant main effect of playback side and a significant interaction between sound presentation angle and playback side, but no significant main effect of sound presentation angle. This result would be expected if the magnitudes of turn angles varied with the magnitudes of sound presentation angles on opposite sides of the frog, and if the pattern of this variation was identical but of opposite sign for sounds presented on the two sides of the animal. In this scenario, the main effect of sound presentation angle would still not be significant. This follows because the opposite signs of turn angles directed to the left (negative) and right (positive) would result in an expected mean turn angle of 0° for pairs of sound presentation angles of identical magnitude but on opposite sides of the animal (e.g., -30° and 30°). We had no specific prediction related to the factor of signal level.

To determine if *H. chrysoscelis* is able to identify whether a sound source is located in front of or behind the animal, we compared the response variable turn angle using a 4 *sound presentation angle* (0°, 15°, 30°, and 45°; within subjects) $\times$ 2 *signal level* $\times$ 2 *playback direction* (forward versus rearward; within subjects) ANOVA. The two playback directions corresponded to forward (0°–45°) and rearward (135°–180°) angles relative to the inter-aural axis. For this analysis, rearward angles were re-coded to indicate angular distance from the midline relative to the cloaca of the animal, such that angles of 180°, 165°, 150°, and 135° became 0°, 15°, 30°, and 45°, respectively. Forward angles already indicated their distance from midline relative to the snout of the animal, and were not re-coded. As in the previous analysis, this analysis was restricted to the quiet noise condition to observe behavior under optimal listening conditions. If *H. chrysoscelis* is able to distinguish between sound sources located in front of (rostral to) the inter-aural axis versus those located behind (caudal to) the inter-aural axis, we predicted a significant main effect of playback direction (forward versus rearward), with frogs making greater turns to rearward speaker angles. We also predicted a significant interaction between playback direction and sound presentation angle, because turn angles would have decreased as the call speaker angle approached the front of the frog, but would have increased as speaker angle approached the rear of the frog. By contrast, if frogs are unable to discriminate between sounds originating from the front, and those originating from the back, we expected subjects to behave similarly in response to forward and rearward playback directions. Under this scenario, we predicted a significant main effect of sound presentation angle, but no significant effect of playback direction and

no interaction between playback direction and sound presentation angle. We again had no specific prediction related to the factor of signal level.

To assess how sound presentation angle and noise might impact localization acuity and behavior, we compared the response variables orientation error and latency in separate 4 *sound presentation angle* (0°, 15°, 30°, and 45°; within subjects) $\times$ 2 *signal level* $\times$ 2 *noise condition* $\times$ 2 *noise level* (nested within noise present) ANOVAs. We focused this analysis on the forward angles relative to the inter-aural axis based on the outcome of the previous analysis (see Results). If localization acuity or behavior varied with sound presentation angle, we predicted a significant main effect of sound presentation angle in the models for orientation error and latency, respectively. If the presence of noise impaired localization, we expected the main effect of noise condition (i.e., present versus absent) would be significant in models for both response variables, as would the effect of noise level (i.e., 73 dB versus 76 dB). If localization ability were most impaired at the lowest SNR, as expected, then we also predicted significant interactions between signal level and noise level in models for orientation error and latency that would directly reflect an effect of the nominal SNR. Such an interaction would be expected if orientation errors and latencies were inflated at the lowest SNR compared to similar values across all other SNRs. As an alternative and direct test of the effects of SNR, we conducted additional 4 *sound presentation angle* (0°, 15°, 30°, and 45°; within subjects) $\times$ 4 *SNR* (+3, +6, +9, or +12 dB; between subjects) ANOVAs for orientation errors and latencies.

For this experiment, and the closed loop experiment described below, all statistical analyses were conducted using Statistica (v. 10, StatSoft Inc, Tulsa, OK). For each univariate model we report Greenhouse and Geisser (1959) corrected *P* values for within-subjects effects with more than a single numerator degree of freedom. We also report partial η^2 , which ranges in value from 0 to 1, as a measure of effect size for all main effects and interactions. Some response variables were non-normally distributed. Transformations were used, as indicated in the description of our MANOVA and ANOVA models, to meet the assumptions of parametric analyses. We used a significance criterion of $\alpha = 0.05$.

Closed loop experiment

Testing procedure—Methods for the closed loop experiment closely followed those for the open loop experiment, with the following important exceptions. In closed loop trials, calls repeated every 8 s for all stimuli, and playback did not stop until the female had contacted the arena wall within $\pm 7.5^\circ$ of the speaker. Each subject underwent four closed loop trials, varying in signal level and the presence of overhead noise (79 dB signal without noise, 85 dB signal without noise, 79 dB signal with noise, and 85 dB signal with noise). Treatment order was randomized for each subject. For all trials, subjects were placed with their snouts directed 90° from the speaker broadcasting calls. The speaker was on the right side for a randomly selected two trials, and on the left side for the other two trials. For approximately half of subjects, the overhead noise was played at a level of 73 dB ($N = 32$), and for the other half it was played at 76 dB ($N = 31$), yielding the same four nominal SNRs as in the open loop experiment. There were no reference or sham trials for the closed loop experiment. If a subject failed to reach the playback speaker for any trial, it was given a 2-min timeout, after which it was re-tested with the same stimulus a second and final time. If the subject failed its second attempt, it was excluded from the dataset ($N = 8$).

Analysis—We used automated behavior tracking software (Ethovision XT8, Noldus, Leesburg, VA, USA) to analyze the phonotactic approach path of subjects from the videos of each trial. Occasionally, the software was unable to detect the frog for a portion of its

path. In these instances, we corrected tracking errors by hand. There was often a marked change in behavior once frogs arrived within the immediate vicinity of the playback speaker (e.g., they stopped moving and pointed away from the speaker, or made repeated approaches to and contacts with the arena wall). Path measurements taken from within 20 cm of the speaker ("speaker zone") were, therefore, not included in our statistical analyses.

We focused on four measures of localization performance. We defined *error angle at wall* as the angular distance away from the approximate center of the speaker broadcasting calls at which subjects first contacted the arena wall. We defined *path turn angle* as the mean absolute value of the change in a subject's movement angle between two successive movements across the arena floor. We restricted estimates of path turn angle to movements greater than 1.5 cm to eliminate error due to jitter in the automated tracking software's successive estimates of a stationary subject's exact position between consecutive samples (8 Hz sampling rate). We defined *path length* as the total length of the frog's phonotactic path between the starting point at the center of the arena and the 20 cm zone surrounding the speaker. The shortest possible path length was 80 cm. Finally, *travel time* was defined as the latency from the frog's initial movement until reaching the speaker zone. We examined travel time, instead of total latency from the start of playback until arrival at the speaker zone, to determine whether variation in arrival time to the playback speaker under certain signal and noise conditions was due solely to increased initial response latencies (determined in our open loop experiment), or whether changes in frog behavior as it moved across the arena floor contributed to this variation.

We analyzed measures of localization performance (ln-transformed error angle at wall, ln-transformed path turn angle, reciprocal-transformed path length, and ln-transformed travel time) using MANOVA and ANOVA. Again, initial analyses included body size as a covariate, but the covariate itself was not significant and removing it from the models did not appreciably change the results, so it was not included in our final analyses, which included two different model constructions. In the first model, we compared the four response variables simultaneously in a 2 *signal level* (79 dB versus 85 dB; within subjects) $\times$ 2 *noise condition* (present versus absent; within subjects) MANOVA. This analyses revealed several significant effects related to signal level and noise condition; therefore, we analyzed each response variable separately in a comparable univariate ANOVA. In the second model construction, we excluded tests conducted in the absence of noise and compared our four response variables in a 2 *signal level* (79 dB versus 85 dB; within subjects) $\times$ 2 *noise level* (73 dB versus 76 dB; between subjects) MANOVA.

There were significant effects related to both of these factors, so we subsequently conducted comparable univariate ANOVAs on each response variable separately. We chose this approach of two different model constructions because our design precluded nesting noise level (a between-subjects factor) within one level of the within-subjects factor of noise condition (i.e., noise present).

Univariate analyses of closed loop trials were used to assess the extent to which the presence of overhead noise and the SNR affected localization performance. If the noise impaired localization abilities during phonotaxis, we predicted larger error angles at wall, larger path turn angles, longer path lengths, and longer latencies. To the extent that the different SNRs we tested had differential influences on localization during phonotaxis, we also expected to find significant effects of noise level, as well as possible interactions between noise level and signal level for each of our four response variables. We generally expected these interactions to reflect the influence of different nominal SNRs, with higher values for each variable occurring at the lowest SNR of +3 dB. Unfortunately, we were unable to compare

the effect of SNR directly using ANOVA (as in our open loop analyses) due to the particular mix of within-subject and between-subject effects in our factorial experimental design. Therefore, we used t-tests to compare responses at each SNR to responses at the next highest and lowest SNR. For these analyses, we reduced α by a factor of three to account for multiple comparisons.

Results

Open loop experiment

In approximately 96% of test trials, subjects made rotational or translational movements within 120 s. In contrast, this was true for only 67% of sham trials. That a high proportion of frogs would exhibit movement in sham trials is not surprising given previous findings showing that female gray treefrogs tend to wander around the test arena in the absence of a stimulus call (Vélez and Bee 2013). Importantly, across all trials in which movements were scored, mean ($\pm$ s.e.m. here and elsewhere) latencies to movement were significantly shorter during call playback than during sham trials (14.8 ± 1.2 s vs. 50.7 ± 2.1 s), even in the lowest SNR condition (+3 dB; 79-dB signal in 76-dB noise; Wilcoxon signed-rank test: $Z = -3.48$, $P = 0.001$, $N = 16$ with complete sham data for this SNR). These results indicate that subjects detected and were attracted to calls at all SNRs. Moreover, across signal and noise conditions, orientation responses were much more accurate during call playback than during sham trials. For example, mean orientation error was significantly ($Z = -7.18$, $P < 0.001$, $N = 113$) lower in response to the 90° sound presentation angle in test trials ($51.7 \pm 2.1^\circ$) compared with the equivalent angle in sham trials ($88.8 \pm 2.3^\circ$). These results demonstrate that orientation responses were directed towards the call speaker, and did not result from some inherent tendency of the frogs to turn in a particular direction in the absence of a stimulus.

A MANOVA of responses in test trials that included turn angle, orientation error, and latency as response variables identified significant effects of sound presentation angle, noise condition, and noise level and a significant signal level $\times$ noise condition interaction (Table ESM1). The main effect of signal level did not significantly influence responses.

Lateralization versus more accurate localization—In Figure 2a, we summarize turn angles in open loop test trials conducted in quiet as a function of sound presentation angle. Positive values along the abscissa and ordinate correspond, respectively, to sound presentation angles to the right of the animal's midline and turn angles toward the right. As illustrated here, frogs generally turned to the right when the speaker was on the right and to the left when the speaker was on the left. Moreover, as illustrated in Figure 3a, the accuracy of the subjects' source location estimates in quiet was quite high between -45° (left) and $+45^\circ$ (right) in the forward direction. Beyond this range, and especially beyond $\pm 90^\circ$, turn angles did not continue to increase in absolute magnitude, but instead became smaller with further increases in sound presentation angle. These general patterns were broadly consistent across both signal levels (Fig. 2b) and across noise conditions and noise levels (Fig. 2c).

In our analyses of turn angles in quiet conditions (Fig. 2a), there was a significant main effect of playback side (left or right relative to the frog) and a significant interaction between sound presentation angle and playback side (Table 1). The main effects of sound presentation angle, signal level, and all other interactions in the model, were not significant (Table 1). This general pattern of results, and in particular, the direct relationship observed between turn angles and sound presentation angles between -45° and $+45^\circ$ (Fig. 3a), is consistent with the hypothesis that subjects were able to do more than simply lateralize the

sound as coming from the left or the right side, but instead were able to localize the sound to specific angles of presentation from the left and right sides.

Discrimination between forward and rearward sound source directions—In an ANOVA that compared turn angle as a function of sound presentation angle, ranging from 0° to 45° from the midline relative to either the snout (forward) or the cloaca (rearward), the main effect of sound presentation angle was significant ($F_{(3,123)} = 35.2$, $P < 0.001$, partial $\eta^2 = 0.462$), but the effect of playback direction ($F_{(1,41)} = 0.0$, $P = 0.997$, partial $\eta^2 < 0.001$) and the interaction between sound presentation angle and playback direction ($F_{(3,123)} = 0.8$, $P = 0.502$, partial $\eta^2 < 0.019$) were not significant. In fact, playback direction was associated with one of the smallest effect sizes observed in this study. This pattern of statistical results can be explained as follows (see Fig. 4). As sound presentation angle increased from 0° to 45° from the midline relative to either the snout or the cloaca of the frogs, so did the angle to which the frogs turned. However, the distances they turned in response to sounds originating from forward and rearward angles were statistically indistinguishable. Together with data from Figure 3a, the results shown in Figure 4 indicate that while female gray treefrogs possess a high degree of angular acuity in the frontal direction, they do not discriminate behaviorally between sounds coming from forward versus rearward directions under open loop test conditions.

Effects of sound presentation angle and noise on open loop localization—For forward angles (0°–45°), mean turn angle varied in near direct proportion with sound presentation angle (Figs. 2 and 3). For more rearward sound presentation angles, orientation errors appeared to be driven by the effects of confusion over playback direction (forward versus rearward), rather than by localization acuity (Fig. 4). Our analyses of the effects of sound presentation angle and noise on localization acuity, therefore, focused on angles at which females were able to localize the sound sources effectively in quiet conditions, namely between 0° and 45° in the forward direction.

An ANOVA model including sound presentation angle, signal level, noise condition (present versus absent), and noise level did not identify any factors that strongly affected orientation errors (Table 2; Fig. 5a). While there was a significant effect of sound presentation angle, and a significant interaction between this factor and noise condition, all of the main effects and interactions in this model were associated with uniformly small effect sizes (0.001 partial η^2 0.044; Table 2). Orientation error varied with sound presentation angle, such that frogs were slightly less accurate when orienting toward stimuli presented from angles approaching 0° (Fig. 5a), but again, the size of this effect was quite small (partial $\eta^2 = 0.044$). Most notably, while orientation error was slightly lower in the presence of noise (Fig. 5a), the effect of noise condition (present versus absent) was also quite small (partial $\eta^2 = 0.028$; Table 2). In contrast to the broader pattern, at some signal and noise level combinations orientation errors were larger in the presence of noise. Averaged across sound presentation angles between 0° and 45°, the mean orientation error to advertisement calls broadcast at 85 dB in the absence of noise was $23.5 \pm 2.2^\circ$. Performance was slightly worse ($25.6 \pm 3.2^\circ$) in response to the 79-dB signal in 76-dB noise, the lowest SNR tested. The interaction between signal level and noise level was nonsignificant, however, and was associated with a small effect size (partial $\eta^2 = 0.026$; Table 2), suggesting differences in SNR had small effects on orientation error. This result was confirmed in a separate ANOVA comparing the four SNRs directly, in which there was a significant effect of sound presentation angle on orientation error ($F_{(3,243)} = 4.1$, $P = 0.009$, partial $\eta^2 = 0.048$), but no effect of SNR ($F_{(3,81)} = 1.4$, $P = 0.241$, partial $\eta^2 = 0.050$) and no interaction between these two independent variables ($F_{(9,243)} = 1.5$, $P = 0.167$, partial $\eta^2 = 0.051$).

We also examined the effects of sound presentation angle, signal level, noise condition, and noise level, on latency to first orientation response using ANOVA (Table 2; Fig. 5c). By far the most important influence on latency to respond was the presence or absence of noise, as indicated by the significant effect of noise condition (Table 2). For example, in the absence of noise, mean latencies were on the order of 5 to 10 s for forward angles, corresponding to responses to the first or second presented stimulus (call period = 8 s). Latencies were typically 15 s to 25 s, corresponding to the second or third stimulus, across these same forward angles in the presence of noise (Fig. 5c). We also found a weaker but significant main effect of noise level on latency. On average, females took slightly longer to respond to playback in the presence of noise at the higher noise level (Fig. 5c). There was also a weak but significant interaction between noise level and signal level, suggesting differences in nominal SNRs had some, albeit small, effect on latency. Neither the main effect of sound presentation angle, nor any interaction that included this factor, were significant (Table 2). In a separate analysis, ANOVA revealed a significant effect of SNR ($F_{(3,81)} = 4.7$, $P = 0.005$, partial $\eta^2 = 0.148$), but no effect of sound presentation angle ($F_{(3,243)} = 1.1$, $P = 0.348$, partial $\eta^2 = 0.013$) or interaction between SNR and sound presentation angle ($F_{(9,243)} = 0.5$, $P = 0.902$, partial $\eta^2 = 0.017$). Mean latencies for forward angles (0° to 45°) ranged between 20.4 ± 2.0 s and 22.6 ± 2.3 s across SNRs of +3 dB to +9 dB, with that at the highest SNR of +12 dB being significantly lower (12.8 ± 2.0 s) in post-hoc comparisons (Tukey's HSD: all $P < 0.023$).

Closed loop experiment

During closed loop trials conducted in the absence of noise, the phonotaxis paths taken by some female gray treefrogs closely resembled the zig-zag paths reported previously in studies of this (e.g., Bee and Riemersma 2008) and other (e.g., Feng et al. 1976; Rheinlaender et al. 1979; Gerhardt and Rheinlaender 1980) anuran species (Fig. 6a). Other females moved directly towards the speaker (Fig. 6b), or approached the speaker with long, arcing paths (Fig. 6c). Occasionally, females actively responded to playback, but took indirect, looping paths to the speaker (Fig. 6d), giving the impression that they were having difficulty localizing the sound. In noise free trials, females arrived at the arena wall, on average, $6.9^\circ \pm 0.8^\circ$ away from the speaker ($N = 62$ females). They turned an average of $13.0 \pm 1.5^\circ$ between walks and hops, had a mean phonotactic path length of 120.4 ± 6.6 cm, and took an average of 69.9 ± 3.3 s to reach the speaker zone after their initial orientation movement.

Effects of noise on closed loop localization performance—In a MANOVA for all closed loop trials, which included error angle at wall, path turn angle, path length, and travel time as the response variables (Table ESM2), there were significant main effects of signal level and noise condition (i.e., present versus absent, collapsed over both noise levels), as well as a significant interaction between these two variables. In subsequent univariate ANOVAs, the main effect of signal level was significant in analyses of error angle at wall and path length, and approached significance ($P = 0.053$) in the analysis of travel time (Table 3). The main effect of noise condition was not significant for any response variable, though it approached statistical significance for path turn angle ($P = 0.074$) and travel time ($P = 0.079$). The signal level $\times$ noise condition interaction was significant in the analysis of path length only (Table 3).

In a separate MANOVA that excluded trials conducted in quiet and assessed the effects of signal level and noise level, only the main effect of signal level was significant (Table ESM3). The signal level $\times$ noise level interaction approached significance ($P = 0.066$, Table ESM3). In subsequent univariate ANOVAs, there were significant effects of signal level and

the signal level $\times$ noise level interaction on the response variables of error angle at wall and path length (Table 4). Path turn angle and travel time were not significantly affected by any factors in the model. In subsequent t-tests comparing each variable across adjacent SNRs (Table 5), we found a significant difference between path lengths at +3 and +6 dB. After correcting for multiple comparisons, there were no other significant effects of SNR, but there were trends towards larger error angles at the wall ($P = 0.048$) and longer travel times ($P = 0.065$) between these same two SNR conditions.

We interpret the gross patterns of statistical significance in the main effects and interactions reported here and the data illustrated in Fig. 7 as evidence that sound localization performance deteriorated in the most challenging nominal SNR condition tested (+3 dB; i.e., 79-dB signal in 76-dB noise) compared with other conditions. Evidence of this deterioration was most pronounced in analyses of error angle at wall, path length, and, to a lesser extent, also travel time (Tables 3–5; Fig. 7). Compared with quiet conditions, subjects were less accurate in their approach to the speaker in the presence of noise, as measured by larger error angles at the wall (Fig. 7a), but only when the signal level was 79 dB, and this difference was most pronounced at the higher noise level of 76 dB (Fig. 7a). Path lengths showed a somewhat similar trend in that they were longest when subjects responded to the 79-dB signal in 76-dB noise (cf. Fig. 7a and 7c). This influence of signal and noise levels on path length was considerable, with paths 62% longer in the lowest SNR (79-dB signal, 76-dB noise) compared with those in response to the 85-dB signal in the absence of noise. As illustrated in Fig 7b, the various signal and noise conditions tested in this study had little overall discernible effect on path turn angle, which varied between about 10° and 14° , on average, across test conditions. The effects of noise condition (Table 3) and noise level (Table 4) on travel time approached statistical significance, and this trend was largely driven by longer travel times in the presence of 76-dB noise (Fig. 7d).

Discussion

The combination of open and closed loop perspectives proved to be a powerful tool for investigating sound localization behavior *H. chrysoscelis*. Open loop playback allowed us to focus on fine scale orientation responses based on the frog's localization estimates while static in the sound field, and closed loop playbacks allowed us to view these static estimates of sound source location *in situ* as elements of a female's phonotactic approach to a calling male. We discuss our results with respect to the four major questions guiding this study of factors affecting azimuthal sound localization acuity in *H. chrysoscelis*.

Lateralization versus localization acuity

Our results strongly support the hypothesis that females of *H. chrysoscelis* do not simply lateralize sounds prior to their first orientation movements. If females were lateralizing sound source location, then turn angle in open loop trials should have been independent of sound presentation angle. This was not the case, as documented by the interaction between sound presentation angle and playback side in our analysis of factors affecting turn angle (Table 1, Figs. 2 and 3a). In open loop trials, in response to calls presented from forward angles, turn angle was almost directly proportional to sound presentation angle (Fig. 3a). This result is strikingly consistent with that reported by Klump and Gerhardt (1989) in their investigation of localization acuity and lateralization in females of the barking treefrog, *H. gratiola* (cf. Figs. 3a and 3b). This earlier study did not examine sound incident angles greater than 45° , however. Our results show that turn angle continues to vary with sound presentation angle for sounds presented across all azimuthal angles (Fig. 2). Even for rearward angles, where there appears to be considerable ambiguity about sound source

location, *H. chrysoscelis* did not exhibit simple lateralization behavior (Fig. 4). This result is consistent with the idea that, despite their small head size, which likely precludes the use of ITDs and IIDs at the *external* surfaces of the tympana for accurate localization (Feng and Capranica 1978; Rheinlaender et al. 1979), frogs can apply fine scale location information extracted from variation in the amplitude or phase (or both) of tympanum vibrations due to mechanical coupling of the two ears (Christensen-Dalsgaard 2005). In a companion study, we use laser Doppler vibrometry to demonstrate how the pressure-difference ear of *H. chrysoscelis* converts small ILDs (e.g., 1 dB for calls) into larger (e.g., 3.5 dB for calls) interaural differences in the vibration amplitude of the two tympana (Caldwell et al. submitted). In the same study, however, we did not find that the pressure-difference system produced similar increases in inter-aural phase differences (Caldwell et al. submitted).

Discrimination between forward and rearward sound source directions

In open loop trials, females of *H. chrysoscelis* appeared unable to discern whether sounds originated from in front of or from behind their inter-aural axes. While we would expect an animal correctly localizing sound sources to turn at ever increasing angles as the sound incident angle increased, during open loop trials, subjects started turning at smaller and smaller angles as the sound presentation angle increased beyond 90° and approached 180° relative to their snouts (Fig. 2). This behavior is not simply indicative of poorer localization acuity at rearward angles, but suggests that females are responding to rearward sound sources as if they were *in front* of the inter-aural axis. In fact, responses to sound sources at angles from 135°–180° were statistically indistinguishable from those to corresponding angles (relative to the midline) ranging from 0°–45° (Fig. 4). As demonstrated in our companion study, information that could resolve ambiguity between forward and rearward sound sources is apparently not encoded by the inherent directionality of the amplitude or phase responses of the tympana (Caldwell et al. submitted).

Some frogs exhibit head scanning movements when listening to acoustic stimuli (Rheinlaender et al. 1979; Gerhardt and Rheinlaender 1982). Such movements could help to overcome this front versus back confusion (Wallach 1939). Indeed head movements largely resolve front versus back sound source ambiguities for human listeners (Wightman and Kistler 1999). We did not observe any head scanning movements during our study. Similarly, head scanning has not been observed in the closely-related eastern gray treefrog, *H. versicolor*, which is the sister species to *H. chrysoscelis* (Jørgensen and Gerhardt 1991), or in some other frogs (Klump and Gerhardt 1989; Ursprung et al. 2009). Nonetheless, females in our study were able to overcome forward versus rearward ambiguity during closed loop reference trials in which the speaker was initially positioned directly behind the frog (180°). It seems likely that the frogs accomplished this by integrating location information across several successive head or body positions during closed loop trials.

Dependence of localization on sound presentation angle

In open loop trials, we measured orientation errors of ~21° in the absence of noise for sounds originating from between 0° and 45° relative to the frog's snout. This is on par with the orientation errors observed in the only other open loop study of localization acuity in frogs (Klump and Gerhardt 1989), as well as those reported during closed loop phonotaxis in other species (Christensen-Dalsgaard 2005; Ursprung et al. 2009). While sound presentation angle significantly affected localization acuity in *H. chrysoscelis*, the effect appeared to be relatively minor (Table 2). Localization errors were slightly larger for forward sound presentation angles closer to 0° (Fig. 5a). As illustrated in Figure 5b, similar results were reported previously for *H. gratiiosa* by Klump and Gerhardt (1989). In that study, however, improvement in orientation (i.e., a decrease in error) with movement of the sound away from

the midline (i.e., from 0° to 45°) was perhaps more pronounced compared with *H. chrysoscelis* (cf. Figs. 5a and 5b). This difference might be related to species differences in body size, as barking treefrogs are one of the largest indigenous treefrogs in North America (50–70 mm snout-vent length; Conant and Collins 1998) and are larger than the Cope's gray treefrogs tested in the present study (34 – 45 mm snout-vent length).

Orientation by *H. chrysoscelis* in response to sounds presented from greater than 45° from the midline was distinct from that to sound presented within $\pm 45^\circ$. In response to calls broadcast from $\pm 90^\circ$, for example, average turn angles were far smaller than the 90° expected (Fig. 2a). This is an interesting finding considering that biomechanical measures of tympanum vibrations indicate that ear directionality is maximal at 90° in this species (Caldwell et al. submitted). Subjects occasionally turned at angles greater than 90° during open loop trials, and so are capable of such turns. It is possible, however, that as a general pattern, individuals do not turn more than 45° based on a single estimate of sound source location, regardless of whether they have accurately assessed the position of a sound source 90° from their snout. At sound source angles to the rear of the inter-aural axis, orientation errors were extremely large, but this trend was entirely driven by the ambiguity between forward and rearward sound source directions, rather than a loss of localization precision *per se* (Fig. 2). In fact, turns in response to sounds at rearward angles were comparably precise but consistently inaccurate (Fig. 4).

It is important to note that orientation errors scored from either closed loop or open loop responses to sound playback may be a product of other factors in addition to the localization acuity of the subject. For instance, animals may intentionally orient their ears at an optimum angle relative to the sound source for assessing source location (Gerhardt and Bee 2007). For *H. chrysoscelis* (Caldwell et al. submitted), and other frogs too (Christensen-Dalsgaard 2005; Ho and Narins 2006), the angle of optimum directionality of the auditory system does not fall at 0°. Such “off-axis” listening presumably provides the frog with more useful information for sound localization.

Effects of noise

Open loop experiment—Our open loop results provide limited support for the hypothesis that ambient (overhead) noise impairs localization acuity, at least under the conditions tested in this study (Fig. 2c). In open loop trials, for example, we saw no significant effects of noise condition (present versus absent) or noise level (73 dB versus 76 dB) on orientation errors, nor was there any indication that signal level and noise level together (i.e., SNR) impacted localization acuity (see Table 2; Fig. 5a). In contrast, there was a moderately sized significant effect of noise condition on response latency, which was longer in the presence of noise. There were also much smaller, though still significant, effects of noise level and a signal level $\times$ noise level interaction (Table 2; Fig. 5c). Latencies were longer at SNRs of +3 dB, +6 dB, and +9 dB compared with +12 dB. To summarize these findings, females required more time (i.e., more call presentations) to respond in the presence of noise, but when they did respond, they did so with nearly the same localization acuity exhibited in the absence of noise.

At least three different hypotheses could explain these results for orientation errors and latency in open loop trials. First, females might simply have been less motivated to respond in the presence of high noise levels. While we cannot rule out this explanation, it seems unlikely to us considering that female frogs have evolved to respond to male advertisement calls in noisy social environments, and the spectrum of our artificial noise encompassed frequencies present in the natural, mixed-species choruses in which reproduction by *H. chrysoscelis* naturally occurs. Second, it is possible that calls were presented near some

animals' signal detection or recognition thresholds at some combinations of signal and noise levels. For these animals, stochastic effects might have resulted in detection or recognition first occurring in response to stimulus presentations subsequent to the first, resulting in longer response latencies in trials with noise. If this explanation were correct, we might have expected latencies at the lowest SNR (+3 dB) to be longer than those at higher SNRs. However, the data are only partially consistent with this expectation, as mean latencies for forward angles ranged between about 20 s and 23 s across SNRs of +3 dB to +9 dB, with a mean of about 13 s at the highest SNR of +12 dB. Given the 8 s period of the repeating stimulus in open loop trials, these latencies are equivalent to 1.5 to 2.8 call periods, which corresponds to two to three presentations of the stimulus call, on average. In quiet conditions, subjects typically responded after the first call presentation, on average. Hence these reported differences in latency are not large when considered in terms of the number of call repetitions required to elicit a response. A third hypothesis to explain noise-related increases in latency, but no noise-related changes in orientation error, is that all sounds were above behavioral response thresholds, but subjects compensated for noise-induced uncertainty in sound location by listening to more than one call in noise to improve their location estimates before their initial movement. Such an explanation would require the use of an integrative mechanism akin to a "multiple looks" strategy (Viemeister and Wakefield 1991; Hofman and Van Opstal 1998). Under this scenario, females would be required to integrate and update their location estimates over relatively long timespans on the order of seconds given the typical rate of calling by males in a chorus.

Closed loop experiment—Interestingly, although we saw no clear effect of noise on orientation error in open loop trials, localization performance was reduced at the lowest SNR of +3 dB (79-dB signal, 76-dB noise) under closed loop conditions (Fig. 7). Under this condition, females arrived at the arena wall significantly further away from the speaker and took significantly less direct paths to arrive at the speaker zone. It is not immediately apparent why these effects emerged in the closed loop experiment but were less evident in our measures of open loop orientation error. As already noted, one possibility is that females in open loop trials did not move until they had a good estimate of sound source location. Subsequent translational movements in the direction to which they initially oriented would have brought them closer to the speaker, on average, and thus increased the SNR ratio of subsequent calls in a closed loop trial. With each subsequent move toward the speaker, the SNR would again increase and uncertainty about source location would presumably decrease. Such an explanation might account for why noise condition had a larger and significant effect on latency to first movement in open loop trials (partial $r^2 = 0.234$; Table 2) compared with its smaller and nonsignificant effect on travel time (which began *after* the first movement) in closed loop trials (partial $r^2 = 0.051$; Table 3). Any benefit from an improvement in SNR and reduction in location uncertainty resulting from movement in closed loop trials was apparently less pronounced at the lowest SNR of +3 dB, which as noted earlier, is close to behavioral response thresholds measured for this species. Together, the pattern of results observed across open and closed loop trials suggests the window of SNRs over which noise produces meaningful impairments to localization before rendering a sound inaudible may be quite narrow.

Other considerations—We chose to use overhead broadband noise for our study, because frogs are attracted to some noise sources (Gerhardt and Klump 1988; Bee 2007a; Swanson et al. 2007; Christie et al. 2010), and an overhead source should not introduce directional biases into the experiments. We readily point out that other types of noise, or noise from positions other than overhead, may cause greater impairment to localization acuity, however. Noise sources on the azimuthal plane, for instance, would increase the

relative amplitude of sound arriving at one ear more than at the other. If the noise were to contain spectral or temporal features similar to those in the signal the animal is trying to localize, this could potentially bias localization estimates, as shown in crickets (Wendler 1989).

The extent to which the localization difficulties we have discussed here constitute serious biological problems for females in nature remains an open empirical question. Potential localization deficits caused by noise may become most relevant in especially dense choruses by preventing females from effectively discriminating the positions of attractive mates and those of nearby, low quality conspecifics or even heterospecifics. Some frogs make use of multimodal, acoustic and visual, cues during social interactions (Narins et al. 2003, 2005; Rosenthal et al. 2004; Taylor et al. 2007, 2008, 2011a, b; Gomez et al. 2009). Combining acoustic localization information with that gained from visual cues might be an effective tactic for mitigating uncertainty in sound source location introduced by the presence of noise or ambiguity about whether sounds originate from in front of or behind the animal. Along the same lines, impediments to sound localization acuity could interfere with the perceptual binding of multimodal signals, reducing their active space and effectiveness.

Effects of signal level

We found no evidence that signal level influenced localization acuity in the open loop experiment. This result is perfectly in line with analyses of ear directionality as measured at the tympanum, which indicate that the anuran ear behaves approximately linearly (Pinder and Palmer 1983; Jørgensen et al. 1991; Ho and Narins 2006), with no substantial increase in directionality at higher signal levels (Caldwell et al. submitted). In the closed loop experiment, however, females showed reduced localization performance at the lower signal level, with smaller error angles at the arena wall and shorter paths lengths. It seems unlikely that our manipulation of signal level (79 dB and 85 dB) should, in itself, affect localization performance, as both signal levels were likely at or above the animals' behavioral response thresholds (e.g., Bee 2007b; Bee and Swanson 2007; Bee and Schwartz 2009; Vélez and Bee 2013). Instead, poorer localization performance in tests with lower signal levels reflects the relatively lower SNRs in trials with noise. Indeed, whether measured as error angle at the wall or as path length, mean localization performance for treatments with noise was inversely related to SNR, with the poorest performance in the highest SNR treatment, and the best performance at the lowest two SNRs (Fig. 7a and 7c).

Past studies of gray treefrogs have shown shorter latencies to arrive at a speaker playing calls at 79 dB compared with 85 dB (Beckers and Schul 2004; Bee and Swanson 2007). We, however, observed similar initial response latencies and travel times at these two signal levels. This apparent discrepancy, at least in part, might be due to differences in playback methodology between our study and this earlier work. Those studies exposed females to call playback for a period of time before they were released from a cage at the center of the arena, whereas frogs in our study were not enclosed at the start of trials, and were free to respond at any time.

Conclusion

While source localization is critical to finding calling mates in acoustically and structurally complex habitats, recent studies of auditory grouping (Farris et al. 2002, 2005; Bee and Riemersma 2008; Bee 2010; Farris and Ryan 2011), spatial release from masking (Schwartz and Gerhardt 1989; Bee 2007b, 2008a; Nityananda and Bee 2012; Ward et al. 2013a), and cross-modal binding (Narins et al. 2005; Taylor et al. 2011b) are highlighting additional functions of spatial hearing in frogs beyond simple source localization. While the frog's

tympanic ear shares many convergent features with those of other vertebrates, it nevertheless appears to be the result of a largely independent evolutionary derivation (Christensen-Dalsgaard and Carr 2008) and is characterized by several distinct features, including its usually smaller size, unique anatomy, and mechanical coupling with other structures in the body. The implications of this unique physiology for the range of perceptual tasks that rely on spatial hearing remain largely unexplored. Hence, there is a critical need for additional and more in-depth studies into the functional roles of directionality in hearing and sound communication across a greater diversity of anuran species. In a companion paper, we extend our investigation of spatial hearing in Cope's gray treefrog using laser Doppler vibrometry to investigate frequency-dependent directionality of the amplitude and phase responses of the tympanum (Caldwell et al. submitted).

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Definitions

IAD	Interaural amplitude difference
ITD	Interaural time difference
SNR	Signal-to-noise ratio
SPL	Sound pressure level

References

- Beckers OM, Schul J. Phonotaxis in *Hyla versicolor* (Anura, Hylidae): the effect of absolute call amplitude. *J Comp Physiol A*. 2004; 190:869–876.
- Bee MA. Selective phonotaxis by male wood frogs (*Rana sylvatica*) to the sound of a chorus. *Behav Ecol Sociobiol*. 2007a; 61:955–966.
- Bee MA. Sound source segregation in grey treefrogs: Spatial release from masking by the sound of a chorus. *Anim Behav*. 2007b; 74:549–558.
- Bee MA. Finding a mate at a cocktail party: spatial release from masking improves acoustic mate recognition in grey treefrogs. *Anim Behav*. 2008a; 75:1781–1791. [PubMed: 19412318]
- Bee MA. Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog. *Anim Behav*. 2008b; 76:845–853. [PubMed: 19727325]
- Bee MA. Spectral preferences and the role of spatial coherence in simultaneous integration in gray treefrogs (*Hyla chrysoscelis*). *J Comp Psychol*. 2010; 124:412–424. [PubMed: 20853948]
- Bee MA. Sound source perception in anuran amphibians. *Curr Opin Neurobiol*. 2012; 22:301–310. [PubMed: 22265243]
- Bee MA, Riemersma KK. Does common spatial origin promote the auditory grouping of temporally separated signal elements in grey treefrogs? *Anim Behav*. 2008; 76:831–843. [PubMed: 19727419]
- Bee MA, Schwartz JJ. Behavioral measures of signal recognition thresholds in frogs in the presence and absence of chorus-shaped noise. *J Acoust Soc Am*. 2009; 126(5):2788–2801. [PubMed: 19894854]
- Bee MA, Swanson EM. Auditory masking of anuran advertisement calls by road traffic noise. *Anim Behav*. 2007; 74:1765–1776.

- Bosch J, Márquez R. Tympanum fluctuating asymmetry, body size and mate choice in female midwife toads (*Alytes obstetricans*). *Behaviour*. 2000; 137:1211–1222.
- Brown, CH.; May, BJ. Comparative mammalian sound localization. In: Popper, AN.; Fay, RR., editors. Sound source localization, vol 25. Springer handbook of auditory research. New York: Springer; 2005. p. 124–178.
- Caldwell MS, Lee N, Johns AR, Christensen-Dalsgaard J, Bee MA. Spatial hearing in Cope's gray treefrog: II. Frequency-dependent directionality of the amplitude and phase of tympanum vibrations. *J Comp Physiol A*. (submitted).
- Christensen-Dalsgaard, J. Directional hearing in nonmammalian tetrapods. In: Popper, AN.; Fay, RR., editors. Sound source localization, vol 25. Springer handbook of auditory research. New York: Springer; 2005. p. 67–123.
- Christensen-Dalsgaard J. Vertebrate pressure-gradient receivers. *Hearing Res*. 2011; 273(1–2):37–45.
- Christensen-Dalsgaard J, Carr CE. Evolution of a sensory novelty: Tympanic ears and the associated neural processing. *Brain Res Bull*. 2008; 75(2–4):365–370. [PubMed: 18331899]
- Christie K, Schul J, Feng AS. Phonotaxis to male's calls embedded within a chorus by female gray treefrogs, *Hyla versicolor*. *J Comp Physiol A*. 2010; 196(8):569–579.
- Conant, R.; Collins, JT. A field guide to reptiles & amphibians of eastern & central North America. New York: Houghton Mifflin; 1998.
- Farris HE, Rand AS, Ryan MJ. The effects of spatially separated call components on phonotaxis in túngara frogs: Evidence for auditory grouping. *Brain Behav Evol*. 2002; 60(3):181–188. [PubMed: 12417822]
- Farris HE, Rand AS, Ryan MJ. The effects of time, space and spectrum on auditory grouping in túngara frogs. *J Comp Physiol A*. 2005; 191(12):1173–1183.
- Farris HE, Ryan MJ. Relative comparisons of call parameters enable auditory grouping in frogs. *Nat Commun*. 2011; 2:410. [PubMed: 21811239]
- Feng AS, Capranica RR. Sound localization in anurans II. Binaural interaction in superior olivary nucleus of the green tree frog (*Hylacinerea*). *J Neurophysiol*. 1978; 41(1):43–54. [PubMed: 621545]
- Feng AS, Gerhardt HC, Capranica RR. Sound localization behavior of the green treefrog (*Hyla cinerea*) and the barking treefrog (*Hylagratiosa*). *J Comp Physiol A*. 1976; 107(3):241–252.
- Feng, AS.; Schul, J. Sound processing in real-world environments. In: Narins, PA.; Feng, AS.; Fay, RR.; Popper, AN., editors. Hearing and sound communication in amphibians. New York: Springer; 2007. p. 323–350.
- Gerhardt HC. Sound pressure levels and radiation patterns of vocalizations of some North American frogs and toads. *J Comp Physiol A*. 1975; 102(1):1–12.
- Gerhardt HC. Acoustic communication in two groups of closely related treefrogs. *Adv Stud Behav*. 2001; 30:99–167.
- Gerhardt, HC.; Bee, MA. Recognition and localization of acoustic signals. In: Narins, PM.; Feng, AS.; Fay, RR.; Popper, AN., editors. Hearing and sound communication in amphibians, vol 28. Springer handbook of auditory research. New York: Springer; 2007. p. 113–146.
- Gerhardt, HC.; Huber, F., editors. Acoustic communication in insects and anurans: common problems and diverse solutions. Chicago: Chicago University Press; 2002.
- Gerhardt HC, Klump GM. Phonotactic responses and selectivity of barking treefrogs (*Hyla gratiosa*) to chorus sounds. *J Comp Physiol A*. 1988; 163(6):795–802.
- Gerhardt HC, Rheinlaender J. Accuracy of sound localization in a miniature dendrobatid frog. *Naturwissenschaften*. 1980; 67(7):362–363.
- Gerhardt HC, Rheinlaender J. Localization of an elevated sound source by the green tree frog. *Science*. 1982; 217(4560):663–664. [PubMed: 17817539]
- Gomez D, Richardson C, Lengagne T, Plenet S, Joly P, Lena JP, Thery M. The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proc R Soc Lond, Ser B: Biol Sci*. 2009; 276(1666):2351–2358.

- Good MD, Gilkey RH. Sound localization in noise: the effect of signal-to-noise ratio. *J Acoust Soc Am*. 1996; 99(2):1108–1117. [PubMed: 8609294]
- Greenhouse SW, Geisser S. On methods in the analysis of profile data. *Psychometrika*. 1959; 24(2): 95–112.
- Ho CCK, Narins PM. Directionality of the pressure-difference receiver ears in the northern leopard frog, *Rana pipiens pipiens*. *J Comp Physiol A*. 2006; 192(4):417–429.
- Hödl W, Amézquita A, Narins PM. The role of call frequency and the auditory papillae in phonotactic behavior in male Dart-poison frogs *Epipedobates femoralis* (Dendrobatidae). *J Comp Physiol A*. 2004; 190(10):823–829.
- Hofman PM, Van Opstal AJ. Spectro-temporal factors in two-dimensional human sound localization. *J Acoust Soc Am*. 1998; 103(5):2634–2648. [PubMed: 9604358]
- Jørgensen MB. Comparative studies of the biophysics of directional hearing in anurans. *J Comp Physiol A*. 1991; 169(5):591–598.
- Jørgensen MB, Gerhardt HC. Directional hearing in the gray tree frog *Hyla versicolor*: Eardrum vibrations and phonotaxis. *J Comp Physiol A*. 1991; 169(2):177–183. [PubMed: 1748974]
- Jørgensen MB, Schmitz B, Christensen-Dalsgaard J. Biophysics of directional hearing in the frog *Eleutherodactylus coqui*. *J Comp Physiol A*. 1991; 168(2):223–232.
- Klump, GM. Studying sound localization in frogs with behavioral methods. In: Klump, GM.; Dooling, RJ.; Fay, RR.; Stebbins, WC., editors. *Methods in comparative psychoacoustics*. Basel: Birkhäuser Verlag; 1995. p. 221-233.
- Klump GM, Gerhardt HC. Sound localization in the barking treefrog. *Naturwissenschaften*. 1989; 76(1):35–37. [PubMed: 2704420]
- Lingner A, Wiegrebe L, Grothe B. Sound localization in noise by gerbils and humans. *JARO*. 2012; 13(2):237–248. [PubMed: 22245918]
- Lorenzi C, Gatehouse S, Lever C. Sound localization in noise in normal-hearing listeners. *J Acoust Soc Am*. 1999; 105(3):1810–1820. [PubMed: 10089604]
- Michelsen A, Jørgensen MB, Christensen-Dalsgaard J, Capranica RR. Directional hearing of awake, unrestrained treefrogs. *Naturwissenschaften*. 73(11):682–683. [PubMed: 3808081]
- Middlebrooks JC, Green DM. Sound localization by human listeners. *Annu Rev Psychol*. 1991; 42:135–159. [PubMed: 2018391]
- Narins, PM. Biological constraints on anuran acoustic communication: auditory capabilities of naturally behaving animals. In: Webster, DB.; Popper, AN.; Fay, RR., editors. *The evolutionary biology of hearing*. New York: Springer; 1992. p. 439-454.
- Narins PM, Grabul DS, Soma KK, Gaucher P, Hödl W. Cross-modal integration in a dart-poison frog. *Proc Natl Acad Sci USA*. 2005; 102(7):2425–2429. [PubMed: 15677318]
- Narins PM, Hödl W, Grabul DS. Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. *Proc Natl Acad Sci USA*. 2003; 100(2):577–580. [PubMed: 12515862]
- Narins, PM.; Zelick, R. The effects of noise on auditory processing and behavior in amphibians. In: Fritzsch, B.; Ryan, MJ.; Wilczynski, W.; Hetherington, TE.; Walkowiak, W., editors. *The evolution of the amphibian auditory system*. New York: Wiley & Sons; 1988. p. 511-536.
- Nityananda V, Bee MA. Finding your mate at a cocktail party: Frequency separation promotes auditory stream segregation of concurrent voices in multi-species frog choruses. *PLoS ONE*. 2011; 6(6):e21191. [PubMed: 21698268]
- Nityananda V, Bee MA. Spatial release from masking in a free-field source identification task by gray treefrogs. *Hearing Res*. 2012; 285:86–97.
- Passmore NI, Capranica RR, Telford SR, Bishop PJ. Phonotaxis in the painted reed frog (*Hyperolius marmoratus*): The localization of elevated sound sources. *J Comp Physiol A*. 1984; 154(2):189–197.
- Passmore NI, Telford SR. The effect of chorus organization on mate localization in the painted reed frog (*Hyperolius marmoratus*). *Behav Ecol Sociobiol*. 1981; 9(4):291–293.

- Pinder AC, Palmer AR. Mechanical properties of the frog ear: Vibration measurements under free- and closed-field acoustic conditions. *Proc R Soc Lond, Ser B: Biol Sci.* 1983; 219(1217):371–396. [PubMed: 6139817]
- Ptacek MB, Gerhardt HC, Sage RD. Speciation by polyploidy in treefrogs: Multiple origins of the tetraploid, *Hyla versicolor*. *Evolution.* 1994; 48(3):898–908.
- Rheinlaender J, Gerhardt HC, Yager DD, Capranica RR. Accuracy of phonotaxis by the green treefrog (*Hyla cinerea*). *J Comp Physiol A.* 1979; 133(4):247–255.
- Rheinlaender, J.; Klump, GM. Behavioral aspects of sound localization. In: Fritzsche, B.; Ryan, MJ.; Wilczynski, W.; Hetherington, T., editors. *The evolution of the amphibian auditory system*. New York: Wiley & Sons; 1988. p. 297–305.
- Rosenthal GG, Rand AS, Ryan MJ. The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. *Anim Behav.* 2004; 68:55–58.
- Schwartz JJ, Buchanan BW, Gerhardt HC. Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behav Ecol Sociobiol.* 2001; 49(6):443–455.
- Schwartz JJ, Gerhardt HC. Spatially mediated release from auditory masking in an anuran amphibian. *J Comp Physiol A.* 1989; 166(1):37–41.
- Shen JX, Feng AS, Xu ZM, Yu ZL, Arch VS, Yu XJ, Narins PM. Ultrasonic frogs show hyperacute phonotaxis to female courtship calls. *Nature.* 2008; 453(7197):914–916. [PubMed: 18469804]
- Swanson EM, Tekmen SM, Bee MA. Do female anurans exploit inadvertent social information to locate breeding aggregations? *Can J Zool.* 2007; 85:921–932.
- Taylor RC, Buchanan BW, Doherty JL. Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. *Anim Behav.* 2007; 74:1753–1763.
- Taylor RC, Klein BA, Ryan MJ. Inter-signal interaction and uncertain information in anuran multimodal signals. *Curr Zool.* 2011a; 57(2):153–161.
- Taylor RC, Klein BA, Stein J, Ryan MJ. Faux frogs: Multimodal signalling and the value of robotics in animal behaviour. *Anim Behav.* 2008; 76:1089–1097.
- Taylor RC, Klein BA, Stein J, Ryan MJ. Multimodal signal variation in space and time: how important is matching a signal with its signaler? *J Exp Biol.* 2011b; 214(5):815–820. [PubMed: 21307068]
- Ursprung E, Ringler M, Hödl W. Phonotactic approach pattern in the neotropical frog *Allobates femoralis*: a spatial and temporal analysis. *Behaviour.* 2009; 146:153–170.
- Vélez A, Bee MA. Signal recognition by green treefrogs (*Hyla cinerea*) and Cope's gray treefrogs (*Hyla chrysoscelis*) in naturally fluctuating noise. *J Comp Psychol.* 2013; 127:166–178. [PubMed: 23106802]
- Vélez, A.; Schwartz, JJ.; Bee, MA. Anuran acoustic signal perception in noisy environments. In: Brumm, H., editor. *Animal communication and noise*. New York: Springer; 2014. in press
- Viemeister NF, Wakefield GH. Temporal integration and multiple looks. *J Acoust Soc Am.* 1991; 90(2):858–865. [PubMed: 1939890]
- Wallach H. On sound localization. *J Acoust Soc Am.* 1939; 10:270–274.
- Ward JL, Buerkle NP, Bee MA. Spatial release from masking improves sound pattern discrimination along a biologically relevant pulse rate continuum in gray treefrogs. *Hearing Res.* 2013a; 306:63–75.
- Ward JL, Love EK, Vélez A, Buerkle NP, O'Bryan LR, Bee MA. Multitasking males and multiplicative females: Dynamic signalling and receiver preferences in Cope's grey treefrog (*Hyla chrysoscelis*). *Anim Behav.* 2013b; 86:231–243.
- Wells, KD. *The ecology and behavior of amphibians*. Chicago: University of Chicago Press; 2007.
- Wendler G. Acoustic orientation of crickets (*Gryllus campestris*) in the presence of two sound sources. *Naturwissenschaften.* 1989; 76(3):128–129.
- Wightman FL, Kistler DJ. Resolution of front-back ambiguity in spatial hearing by listener and source movement. *J Acoust Soc Am.* 1999; 105(5):2841–2853. [PubMed: 10335634]
- Wollerman L, Wiley RH. Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Anim Behav.* 2002; 63:15–22.

Fig. 1.

Schematic illustrations of open loop orientation measures. **a** A frog positioned at the start of a trial with a sound presentation angle (θ_{sp}) of 90° to the left side of the frog. **b** The frog has made a rotational movement by turning, but no translational movement by walking or hopping away from its starting position. This animal has a turn angle (θ_{ta}) of $\sim 40^\circ$. A measure of orientation error that does not take into account translational movement of the frog across the arena floor ($\theta_{oe I}$) and one that does take such movements into account ($\theta_{oe II}$) are equivalent in this case, and equal to $\sim 50^\circ$. **c** The frog has made the same rotational movement as in panel **b**, but has also made a translational movement by jumping some distance (d) away from its starting position. θ_{ta} and $\theta_{oe I}$ are the same as in panel **b**, but $\theta_{oe II}$ is now larger, because moving across the arena has altered the position of the speaker relative to the frog's snout. The arena wall is not shown, and the frog and speaker are not drawn to scale.

Fig. 2.

Mean $\pm$ s.e.m. turn angle as a function of sound presentation angle in open loop trials. **a** Turn angles in quiet conditions, averaged over both signal levels (79 dB and 85 dB). **b** Turn angles shown separately for each signal level, averaged over all noise conditions (quiet, noise present, noise absent) and both noise levels (73 dB and 76 dB). **c** Turn angles shown separately for each noise condition and level, averaged over both signal levels. Note that results depicted in **a** and the “no-noise” line from **c** are the same data.

Fig. 3. Mean turn angle as a function of sound presentation angles within $\pm 45^\circ$ in open loop trials conducted in quiet conditions. Positive values indicate turns to the right and speakers positioned to the right, whereas negative values indicate turns to the left and speakers positioned to the left. The dotted line shows the expected response for perfect localization acuity. Data are shown separately for **a** Cope's gray treefrogs (*Hyla chrysoscelis*), including $\pm$ s.e.m. values, and **b** barking treefrogs (*Hyla gratiosa*; s.e.m. not available; redrawn from Fig. 1a in Klump and Gerhardt, 1989).

Fig. 4.

Mean $\pm$ s.e.m. turn angle in open loop trials conducted in quiet conditions as a function of sound presentation angles within $\pm 45^\circ$ relative to either the snout (forward angles) or cloaca (rearward angles) of the frog.

Fig. 5. Mean orientation error and latency as a function of sound presentation angle within $\pm 45^\circ$ in open loop trials. Shown here are **a** mean $\pm$ s.e.m. orientation errors for Cope's gray treefrogs (*Hyla chrysoscelis*) in the absence of noise and presence of noise (averaged over both signal levels and noise levels), **b** mean orientation errors for barking treefrogs (*Hyla gratiosa*) in the absence of noise (redrawn from Fig. 2a in Klump and Gerhardt, 1989), and **c** mean $\pm$ s.e.m. latency for Cope's gray treefrogs in the absence of noise and in the presence of noise broadcast at two different levels (73 dB and 76 dB) and averaged over both signal levels.

Fig. 6. Representative phonotaxis paths in the closed loop experiment. The signal speaker (not shown) was positioned at the top of each circle in this figure. Shown here are paths automatically tracked by Ethovision software showing an example of **a** a zig-zag approach path toward the speaker, **b** a direct path toward the speaker, with no significant turns after an initial rotational movement, **c** a less directed path in which the frog took a longer, arcing approach toward the speaker, and **d** a convoluted, looping path to the speaker. Paths like the

one depicted in panel d were typically associated with the highest error angles at wall, longest path lengths, and longest travel times.

Fig. 7.

Measures of phonotaxis behavior during closed loop trials in response to calls presented at two signal levels (79 dB or 85 dB) in the absence of noise and in the presence of noise broadcast at two levels (73 dB or 76 dB). Shown here are the mean $\pm$ s.e.m. values for **a** error angle at wall, **b** path turn angle **c** path length, and **d** travel time.

Table 1

Results of an ANOVA model comparing turn angles in a test of lateralization versus more accurate sound source localization in open loop trials

Factor	df	<i>F</i>	<i>P</i>	Partial η^2
Sound presentation angle	8, 304	0.7	0.640	0.018
Signal level	1, 38	0.4	0.521	0.011
Playback side	1, 38	90.1	< 0.001	0.703
Sound presentation angle $\times$ Signal level	8, 304	1.3	0.235	0.033
Sound presentation angle $\times$ Playback side	8, 304	18.9	< 0.001	0.333
Signal level $\times$ Playback side	1, 38	3.9	0.057	0.092
Sound presentation angle $\times$ Signal level $\times$ Playback side	8, 304	1.3	0.250	0.034

Table 2

Results of ANOVA models comparing orientation error and latency in a test of noise effects on localization acuity and behavior in open loop trials

Response variable	Factor	df	F	P	Partial η^2
Orientation error	Sound presentation angle	3, 363	5.6	0.001	0.044
	Signal level	1, 121	0.6	0.446	0.005
	Noise condition	1, 121	3.4	0.066	0.028
	Noise level (Noise present)	1, 121	0.1	0.744	0.001
	Sound presentation angle $\times$ Signal level	3, 363	0.5	0.670	0.004
	Sound presentation angle $\times$ Noise condition	3, 363	2.8	0.041	0.023
	Sound presentation angle $\times$ Noise level (Noise present)	3, 363	1.6	0.181	0.013
	Signal level $\times$ Noise condition	1, 121	0.4	0.524	0.003
	Signal level $\times$ Noise level (Noise present)	1, 121	3.3	0.073	0.026
	Sound presentation angle $\times$ Signal level $\times$ Noise condition	3, 363	2.0	0.118	0.016
Latency	Sound presentation angle $\times$ Signal level $\times$ Noise level (Noise present)	3, 363	1.0	0.388	0.008
	Sound presentation angle	3, 363	0.7	0.575	0.005
	Signal level	1, 121	1.3	0.266	0.010
	Noise condition	1, 121	36.9	<0.001	0.234
	Noise level (Noise present)	1, 121	5.3	0.023	0.042
	Sound presentation angle $\times$ Signal level	3, 363	0.5	0.697	0.004
	Sound presentation angle $\times$ Noise condition	3, 363	0.4	0.715	0.004
	Sound presentation angle $\times$ Noise level (Noise present)	3, 363	0.7	0.571	0.005
	Signal level $\times$ Noise condition	1, 121	1.7	0.198	0.014
	Signal level $\times$ Noise level (Noise present)	1, 121	4.5	0.037	0.036
	Sound presentation angle $\times$ Signal level $\times$ Noise condition	3, 363	1.0	0.379	0.008
	Sound presentation angle $\times$ Signal level $\times$ Noise level (Noise present)	3, 363	0.3	0.844	0.002

Table 3

ANOVAs for the four response variables examined in closed loop trials testing the effects of signal level (79 dB versus 85 dB) and noise condition (presence versus absence).

Response variable	Factor	df	F	P	Partial η^2
Error angle at wall	Signal level	1, 59	13.8	< 0.001	0.189
	Noise condition	1, 59	1.8	0.181	0.030
	Signal level $\times$ Noise condition	1, 59	0.9	0.343	0.015
Path turn angle	Signal level	1, 59	0.2	0.622	0.004
	Noise condition	1, 59	3.3	0.074	0.053
	Signal level $\times$ Noise condition	1, 59	0.0	0.967	< 0.001
Path length	Signal level	1, 59	16.0	< 0.001	0.213
	Noise condition	1, 59	0.1	0.731	0.002
	Signal level $\times$ Noise condition	1, 59	13.0	0.001	0.181
Travel time	Signal level	1, 60	3.9	0.053	0.061
	Noise condition	1, 60	3.2	0.079	0.051
	Signal level $\times$ Noise condition	1, 60	0.0	0.939	< 0.001

The effect of noise condition collapses over both noise levels.

Table 4

ANOVAs for the four response variables examined in closed loop trials testing the effects of signal level (79 dB versus 85 dB) and noise level (73 dB versus 76 dB)

Response variable	Factor	df	F	P	Partial η^2
Error angle at wall	Signal level	1, 59	11.6	0.001	0.164
	Noise level	1, 59	0.8	0.383	0.013
Path turn angle	Signal level $\times$ Noise level	1, 59	4.1	0.048	0.065
	Signal level	1, 59	0.2	0.653	0.003
	Noise level	1, 59	0.1	0.801	0.001
	Signal level $\times$ Noise level	1, 59	0.7	0.421	0.011
Path length	Signal level	1, 59	30.0	< 0.001	0.337
	Noise level	1, 59	3.0	0.088	0.048
Travel time	Signal level $\times$ Noise level	1, 59	7.6	0.008	0.114
	Signal level	1, 59	2.0	0.161	0.033
	Noise level	1, 59	3.1	0.083	0.050
	Signal level $\times$ Noise level	1, 59	0.2	0.667	0.003

These analyses exclude all closed loop trials conducted in the absence of noise.

Table 5

Results of t-tests comparing response variables in closed loop trials between adjacent signal to noise ratios

	+3 dB versus +6 dB		+6 dB versus +9 dB		+9 dB versus +12 dB	
	<i>t</i> _{df}	<i>P</i>	<i>t</i> _{df}	<i>P</i>	<i>t</i> _{df}	<i>P</i>
Error angle at wall	2.02 ₍₅₈₎	0.048	1.23 ₍₆₀₎	0.224	-0.48 ₍₆₁₎	0.630
Path turn angle	1.16 ₍₅₉₎	0.253	-0.40 ₍₆₀₎	0.689	-0.41 ₍₆₁₎	0.968
Path length	3.00 ₍₅₉₎	0.004	1.77 ₍₆₀₎	0.082	-0.52 ₍₆₁₎	0.958
Travel time	1.88 ₍₅₉₎	0.065	-0.68 ₍₆₀₎	0.499	1.19 ₍₆₁₎	0.239