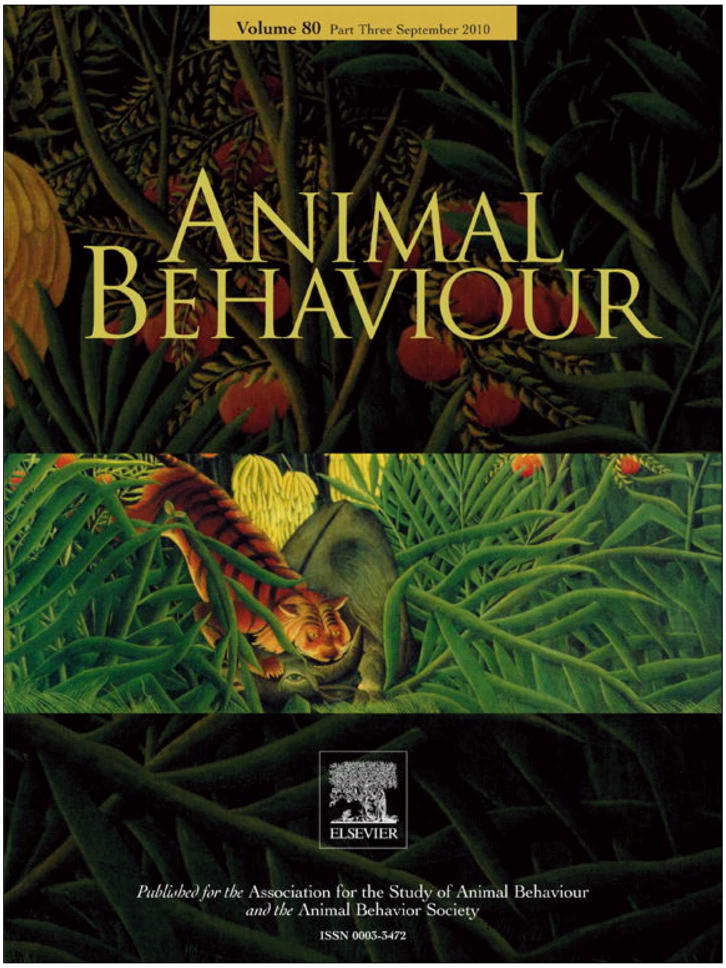
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Auditory sensitivity and the frequency selectivity of auditory filters in the Carolina chickadee, *Poecile carolinensis*

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Chickadees produce elaborate vocalizations, but their basic auditory capabilities remain unexplored. We used auditory brainstem responses to tone bursts to assess the auditory sensitivity of Carolina chickadees at frequencies from 0.5 to 8 kHz and the percentage of frequency selectivity (quality) of auditory filters at frequencies from 2 to 4 kHz. The high-frequency limit of sensitive hearing was relatively high in chickadees compared to other songbirds tested using the same method, while the low-frequency limit was similar among species. These results support a previously noted correlation across songbirds between the high-frequency limit of sensitive hearing and the maximum frequency of vocalizations. The frequency selectivity of auditory filters in chickadees increased with increasing frequency. Frequency selectivity was intermediate compared to other species at 2 and 3 kHz, and relatively high at 4 kHz. This pattern suggests that frequency selectivity is generally greatest within the frequency range of long-range communication signals, and maximum frequency selectivity may be greater in woodland species than in open habitat species. Greater frequency selectivity may have evolved for greater frequency resolution of vocal signals, which are relatively tonal in woodland species, or for better signal detection in noise. Finally, males had greater auditory sensitivity than females, whereas females had greater frequency selectivity than males. Greater frequency selectivity may enhance perception of the frequency difference between song notes, which indicates male quality in another chickadee species.

Natural selection for efficient communication generally favours a close match between signal design and sensory capabilities (Endler 1992). Indeed, studies of communication systems reveal correlations between signal form and receiver physiology in a broad variety of taxa (e.g. insects: Lall et al. 1980; frogs: Feng et al. 2006; lizards: Nava et al. 2009). In other cases, however, the correlation may be disrupted by constraints acting on the sender or receiver (Ryan et al. 1990), or additional selective pressures acting on the sensory system (e.g. detection of prey; Konishi 1973). In songbirds, species differences in vocal communication signals are well documented (Nelson & Marler 1990), while differences in auditory capabilities are less explored. As a result, the extent to which auditory capabilities have diversified in parallel with vocal signals is not well understood.

In general, natural selection is expected to favour sensitive hearing across the frequency range of vocal signals. Estimates of auditory sensitivity are available for approximately 25 of 5000 songbird species (Dooling et al. 2000). Audiograms plotting auditory thresholds (minimum detectable sound pressure levels) as a function of frequency are broadly similar in these species, with best sensitivity from 2 to 3 kHz and moderate sensitivity (i.e. auditory thresholds within 30 dB of best sensitivity) between 0.5 and 6–8 kHz. A few species with relatively high-frequency vocal signals, however, are more sensitive to frequencies above 3–4 kHz (Konishi 1969, 1970; Okanoya & Dooling 1988; Langemann et al. 1998; Henry & Lucas 2008). The emerging pattern suggests that the high-frequency limit of sensitive hearing may coevolve with the maximum frequency of vocal signals in songbirds.

The peripheral auditory system of vertebrates performs a spectral decomposition of sound that determines the frequency resolution, or minimum detectable frequency difference, of the system. The system acts as an array of band-pass auditory filters, each representing a place on the sensory epithelium of the cochlea, that vary in centre frequency across the frequency range of hearing (Fletcher 1940; Moore 1993). Narrower filter bandwidth increases frequency resolution because signals of closely adjacent frequency are more likely to stimulate different filters. However, narrower bandwidth also decreases temporal resolution, or sensitivity to rapid changes in sound intensity. This is because bandwidth, in the frequency domain, and damping, in the time domain, are inversely

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related properties of a band-pass filter. For example, a narrower filter has lower damping, and therefore resonates for a prolonged period in response to a brief period of stimulation (like a tuning fork; reviewed in Viemeister & Plack 1993). In theory, tonal signals requiring precise frequency resolution are processed more effectively with narrow filters whereas modulated signals requiring greater temporal resolution are processed more effectively with broader filters (Viemeister & Plack 1993; Thyer & Mahar 2006). However, the extent to which auditory filters have coevolved with vocal modulation is not clear.

Studies of peripheral frequency selectivity in songbirds are largely limited to a few model species including the European starling, *Sturnus vulgaris* (Manley et al. 1985; Gleich 1994; Marean et al. 1998) and domestic canary, *Serinus canaria* (Lauer et al. 2009), or based on indirect methods such as critical ratios (Dooling et al. 2000). Frequency selectivity is commonly described in terms of the equivalent rectangular bandwidth (ERB; see Table 1 for abbreviations) of the auditory filter (i.e. the bandwidth of a rectangular filter with the same total area as the auditory filter), or %ERB, which is ERB expressed as a percentage of centre frequency. Taken together, these studies find that ERB increases with increasing frequency, while %ERB may decrease slightly. More recently, a comparative study of five species found that frequency selectivity may vary with habitat-based differences in the modulation rate of long-range signals (Henry & Lucas 2010). Woodland species, which tend to communicate with tonal signals, appear to have greater frequency selectivity than open habitat species, which tend to communicate using faster modulations.

Several studies have used auditory brainstem responses (ABRs) to tone bursts to evaluate the frequency range of auditory sensitivity in songbirds (Woolley & Rubel 1999; Woolley et al. 2001; Lucas et al. 2007; Henry & Lucas 2008, 2009) and other small birds (Brittan-Powell et al. 2002; Brittan-Powell & Dooling 2004). ABRs are voltage waveforms recorded from the scalp that reflect action potentials generated within the cochlea and auditory brainstem nuclei (reviewed in Hall 2007). ABR waveforms consist of three to five voltage peaks occurring within 10 ms of stimulus onset. ABR amplitude is positively related to the number of neural responses and their synchrony, and hence, generally reflects the audibility of the stimulus. ABR latency, or the reaction time to the stimulus, is inversely related to the sensation level of the stimulus (i.e. dB above threshold), and hence generally decreases with increasing auditory sensitivity (Brittan-Powell et al. 2002). The ABR threshold is the lowest stimulus intensity that evokes a detectable response. ABR thresholds in birds are generally 25–30 dB higher than behavioural auditory thresholds (Brittan-Powell et al. 2002, 2005).

The frequency selectivity of auditory filters is commonly determined by measuring auditory thresholds in notched noise (reviewed in Moore 1993). Notched noise is white masking noise with a band of spectral energy, or notch, filtered out around a test frequency. Auditory thresholds are measured at the test frequency as the bandwidth of the notch is increased from zero. Auditory thresholds decrease with increasing notch bandwidth, and in general, thresholds should decrease more rapidly when the auditory filter is narrower. The specific shape of the auditory filter can be derived from the relationship between the auditory threshold and notch bandwidth based on the power spectrum model of auditory masking (Fletcher 1940). Auditory filter shapes can be determined from behavioural auditory thresholds (e.g. Moore & Glasberg 1983; Marean et al. 1998) or from ABR thresholds (e.g. Henry & Lucas 2010).

North American chickadees are known to produce elaborate vocal communication signals, but their basic auditory capabilities are largely unexplored. Previous studies of the Carolina chickadee, *Poecile carolinensis* (Paridae) assessed sensitivity to clicks (Lucas et al. 2002) and tone bursts ranging in frequency from 1 to 4 kHz using ABR amplitude and latency (Lucas et al. 2007). Here, we examine auditory sensitivity over a broader range of stimulus frequencies (0.5–8 kHz) using not only ABR amplitude and latency, but also ABR thresholds. Furthermore, we examine the frequency selectivity of auditory filters from 2 to 4 kHz (i.e. within the frequency range of best sensitivity) using ABR thresholds in notched masking noise.

The Carolina chickadee is a small (10 g), nonmigratory, woodland songbird species (Monstrom et al. 2002). Individuals defend territories as breeding pairs during spring and early summer, but live in small flocks of two to eight birds outside of the breeding season. Vocalizations generally fall into three categories based on acoustic structure and function (Fig. 1; Smith 1972; Hailman 1989). Fee-bee-fee-bay songs contain four to five slowly delivered, tonal notes with little or no frequency modulation and average

Table 1
Commonly used abbreviations and definitions

ABR	Auditory brainstem response
AM	Amplitude modulation
ERB	Equivalent rectangular bandwidth; the bandwidth of a rectangular filter with the same total area as the auditory filter; 4 times centre frequency divided by p
FM	Frequency modulation
K'	Efficiency; the signal-to-noise ratio of the auditory filter in dB
%ERB	ERB of the auditory filter expressed as a percentage of centre frequency

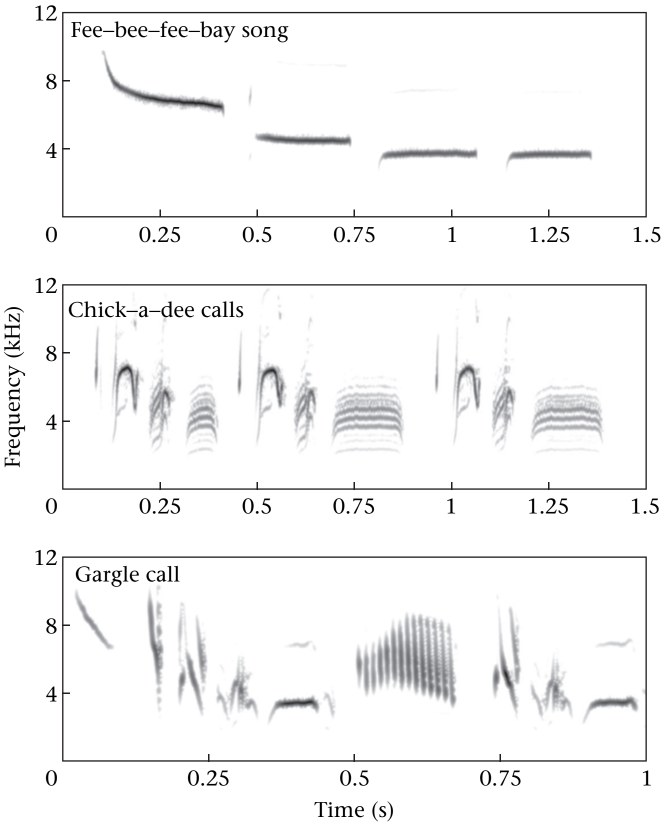

Figure 1. Spectrograms of commonly produced Carolina chickadee vocalizations. Spectrograms were generated in PRAAT based on a Fourier transform of digital recordings (sampling rate = 44.1 kHz) with a 8.7 ms Gaussian analysis window and −3 dB bandwidth of 150 Hz. Fee-bee-fee-bay and chick-a-dee recording are from the Macaulay Library (recording 84817 by Wilbur L. Hershberger; www.macaulaylibrary.org). The gargle recording is from Elliot et al. (1997). Note the difference in timescale for the gargle vocalization.

frequencies between 3 and 7 kHz (Lohr et al. 1991). Songs are produced primarily by males for mate attraction and territory defence over long distances. Chick-a-dee and gargle calls (Smith 1972; Lucas & Freeberg 2007), in contrast, contain a broader variety of acoustically diverse note types including tone complexes (e.g. D notes of chick-a-dee calls), rapid frequency sweeps, and trills (i.e. periodic frequency modulation cycles). Both call types contain high-frequency notes (e.g. 8–10 kHz) and are generally used for communication over short distances. Gargle calls are exchanged primarily between males during dominance interactions (Baker & Gammon 2007), whereas chick-a-dee calls are produced by both sexes in a variety of contexts including foraging and predator mobbing (Lucas & Freeberg 2007; Soard & Ritchison 2009).

To evaluate the extent of auditory coevolution with vocal signals, we compared auditory data from the Carolina chickadees to previously published data from other nondomesticated songbirds collected using the same method (i.e. Henry & Lucas 2008, 2010). Because of differences in stimulus frequencies, we compared the frequency range of sensitive hearing based on +10 dB frequency limits of the audiogram (i.e. the high- and low-frequency limits at which thresholds increase by 10 dB). The high-frequency limit of sensitive hearing should be greater in chickadees than in other species with a lower maximum frequency of vocalizations. Moreover, the low-frequency limit of sensitive hearing may be higher in chickadees than in songbirds with a lower minimum frequency of vocalizations. The predicted frequency selectivity of auditory filters in chickadees, however, is less clear. The tonal acoustic structure of fee-bee-fee-bay songs should favour relatively high-frequency selectivity, while relatively rapid frequency modulation (FM) and amplitude modulation (AM) contained in chick-a-dee and gargle calls should favour less selective auditory filters capable of greater temporal resolution. Finally, recent studies suggest a role of reproductive hormones in modulating auditory anatomy and function (Hultcrantz et al. 2006). Therefore, we tested for auditory differences between male and female chickadees.

METHODS

Subjects

We collected auditory data from 14 wild-caught, adult Carolina chickadees (9 males; 5 females) in November of 2008 and between September and November of 2009. Individuals with a wing chord of 62 mm or greater were classified as males, whereas individuals with a wing chord less than 62 mm were classified as females. Note that this method correctly assigns sex in approximately 96% of individuals (Thirakhupt 1985; Lucas et al. 2006). Mean body mass ± SD was 9.80 ± 0.61 g (males: 9.87 ± 0.71 g; females: 9.67 ± 0.41 g). Subjects were collected near Purdue University in West Lafayette, IN, U.S.A., at Martel Forest and a private residence using treadle traps baited with sunflower seed. Each subject was marked with a numbered aluminium leg band and housed individually in a 1 m^3 wire mesh cage located in an indoor aviary at Purdue University prior to auditory testing. The light–dark cycle of the aviary was set to local conditions and the temperature was held constant at 22 °C. Subjects were provided with sunflower seeds, two to three mealworms, and vitamin-treated water each day. Auditory tests were conducted within 3 days of capture, and subjects were released at their capture site 1–2 days after testing. Protocols were approved by the Purdue Animal Care and Use Committee (No. 05–058).

General Auditory Test Procedure and Equipment

Auditory tests were conducted in a sound-attenuating test chamber (1.2 m tall by 1.4 m wide by 1.2 m deep) lined with 7.7 cm thick Sonex foam (Acoustic Solutions, Richmond, VA, U.S.A.). Subjects were anaesthetized with an injection of ketamine (75 mg/kg) and midazolam (7.5 mg/kg) into the breast muscle and placed on a preheated pad (Pet Supply Imports, South Holland, IL, U.S.A.) with their right side facing upwards. We maintained the temperature between the subject and heating pad at 38 ± 2 °C by adding or removing layers of towel. Needle electrodes (Nicolet Biomedical, Fitchburg, WI, U.S.A.) were inserted subdermally at the vertex of the skull (noninverting), near the base of the skull where the occipital crest intersects the midline (inverting), and midway down the back (common ground) to record ABRs. One to two supplemental injections of ketamine and midazolam at half the initial dose were administered every 20–40 min to maintain anaesthesia for 60–80 min, the approximate duration of the auditory experiments. We completed the auditory sensitivity experiment in 11 subjects (6 males, 5 females), and the auditory filter experiment in 12 subjects at 2 kHz (7 males, 5 females), 12 subjects at 3 kHz (8 males, 4 females), and 13 subjects at 4 kHz (8 males, 5 females).

Stimulus presentation, response acquisition and data storage were coordinated by a Tucker Davis Technologies system II modular rack-mount system (TDT, Gainesville, FL, U.S.A.) and Dell computer running SigGen32 and BioSig32 TDT software. Stimuli consisted of periodically presented tone bursts (51.1 tone bursts/s) and, for the notched noise experiments, continuous notched masking noise. Tone bursts were generated digitally on a signal-processing card installed in the computer (TDT model AP2) and converted to analogue (TDT model DA3-4 converter). Notched noise was generated by two analogue waveform generators (TDT model WG1) programmed to produce white noise. The first noise source was routed through a high-pass filter and the second through a low-pass filter (TDT model PF1 programmable filters). Corner frequencies were set to the upper and lower frequencies of the notch, respectively, and roll-off rates were set to 26 poles (approximately 156 dB per octave; see Fig. 3b). The filtered noise signals were passed through two attenuators (TDT model PA4) to a signal mixer (TDT model SM3) for combination with the tone burst stimuli. Output from the mixer was routed through a 31-band equalizer (Behringer Ultragraph model FBQ6200, Bethel, WA, U.S.A.) to a Crown D75 amplifier (Elkhart, IN, U.S.A.) and finally, an electromagnetically shielded dynamic loudspeaker (RCA model 40-5000; Indianapolis, IN, U.S.A.) suspended 30 cm above the test subject for stimulus presentation.

We calibrated the frequency response of the system by generating pure tones of constant amplitude (1 V) on the AP2 card of the computer. Tones were played at the centre frequency of each third-octave band from 0.4 to 10 kHz (15 bands), and the sound pressure level of the tone was adjusted to 65 ± 1 dB SPL at the location of the bird's ear using the gain controls of the equalizer. Sound pressure level was measured with a Bruel & Kjaer model 1613 precision sound level meter (Norcross, GA, U.S.A.) and model 4131 2.6 cm condenser microphone. Sound pressure levels differed from an earlier calibration of the system without the equalizer made using a different sound level meter. ABR threshold levels from Henry & Lucas (2008) were adjusted by −9.3, −5.6, −4.3, −6.6, −6 and −9.2 dB at 0.8, 1.4, 2.2, 3.2, 4.2 and 6.4 kHz, respectively, to account for the difference in calibration (note that the stimulus frequencies of Henry & Lucas (2008) were selected based on similar sound pressure level of the unequalized system for a given input voltage).

Response waveforms to individual tone bursts were conducted from the subject to a headstage (TDT model HS4) through electrode leads, and from the headstage to a biological amplifier (TDT model DB4) through fibre-optic cables. The headstage and biological amplifier band-pass filtered responses from 100 to3000 Hz and amplified responses by 100K. Response waveforms were digitized by a converter (TDT model AD2) and sampled by the signal-processing card for 12 ms starting 1.2 ms prior to the onset of each tone burst stimulus at a sampling rate of 20 kHz. Responses exceeding ±40 µV (40% of the maximum possible input) were rejected as potential artifacts. The 1000 digitized responses were averaged together to generate each ABR waveform stored on the hard disk of the computer.

Auditory Sensitivity Experiment

ABRs were recorded in response to tone bursts of variable frequency and intensity under quiet conditions. Frequencies of 0.5, 1, 2, 3, 4, 6 and 8 kHz were presented in random order. At each frequency, seven intensity levels were presented in decreasing 8 dB steps. Intensity levels decreased from 64 to 16 dB SPL at 2, 3 and 4 kHz, 72–24 dB SPL at 1 and 6 kHz, and 80–32 dB SPL at 0.5 and 8 kHz. Tone bursts were 5 ms in duration with 1 ms $\cos^2$ onset/offset ramps. Each ABR was the average response to 1000 periodically presented tone bursts (51.1 stimuli/s) of alternating polarity.

We measured the amplitude and latency of the first ABR peak. Amplitude was measured relative to the subsequent trough, whereas latency was measured relative to the time of stimulus onset (Fig. 2a). We determined the ABR thresholds of each subject using a cross-correlation technique described previously (e.g. Henry & Lucas 2008, 2009, 2010). Cross-correlation involves cross-multiplying two waveforms as the first waveform is shifted in time relative to the second. The maximum cross-product of the cross-correlation provides a measure of similarity between the waveforms. A separate analysis was conducted at each frequency in each subject using a custom script in PRAAT (Boersma & Weenink 2009). The analysis involved (1) generating an ABR template, (2) determining the *Z* score of each ABR in the data set by cross-correlation, (3) removing nonsignificant ABRs and (4) calculating the ABR threshold from the ABR *Z* score by stimulus intensity function.

(1) A 7 ms ABR template (Fig. 2a, dashed box) was generated by deleting the first 2.3 ms and last 2.7 ms from the ABR of greatest amplitude.

(2) We calculated the *Z* score of each ABR waveform in the data set as the maximum cross-product between the waveform and the ABR template divided by the standard deviation of a null distribution of cross-product values. The null distribution was determined by cross-correlating the ABR template with 1 s of electrophysiological background noise. Null distributions were approximately normal with a mean of zero. ABR *Z* scores greater than 1.645 are greater than 0 at the 95% confidence level, and ABR *Z* scores greater than 2.56 are greater than zero at the 99.5% confidence level.

(3) ABRs were removed from the data set if the timing of the maximum cross-product was inconsistent with the timing of cross-products observed at higher stimulus intensity levels or if the *Z* score was not greater than 1.645. Time lags were expected to increase by 0.1–0.4 ms for each 8 dB decrease in stimulus intensity due to increasing latency of ABR peaks.

(4) ABR thresholds were estimated by fitting a weighted regression model to the ABR *Z* score data (Fig. 2b; GLM procedure; SAS Institute, Inc., Cary, NC, v. 9.1). Weighting was assigned based on *Z* score (i.e. (maximum *Z* score – *Z* score)/maximum *Z* score). Hence, ABRs with lower *Z* scores carried greater weight. The model included stimulus intensity as a continuous independent variable. The ABR threshold was defined as the predicted stimulus intensity level necessary to produce a *Z* score of 2.56 (i.e. the intersection between the weighted regression line and dashed line in Fig. 2b). The weighting procedure ensured that ABR thresholds were not overly influenced by data points far above the threshold level (i.e. far above the dashed line in Fig. 2b).

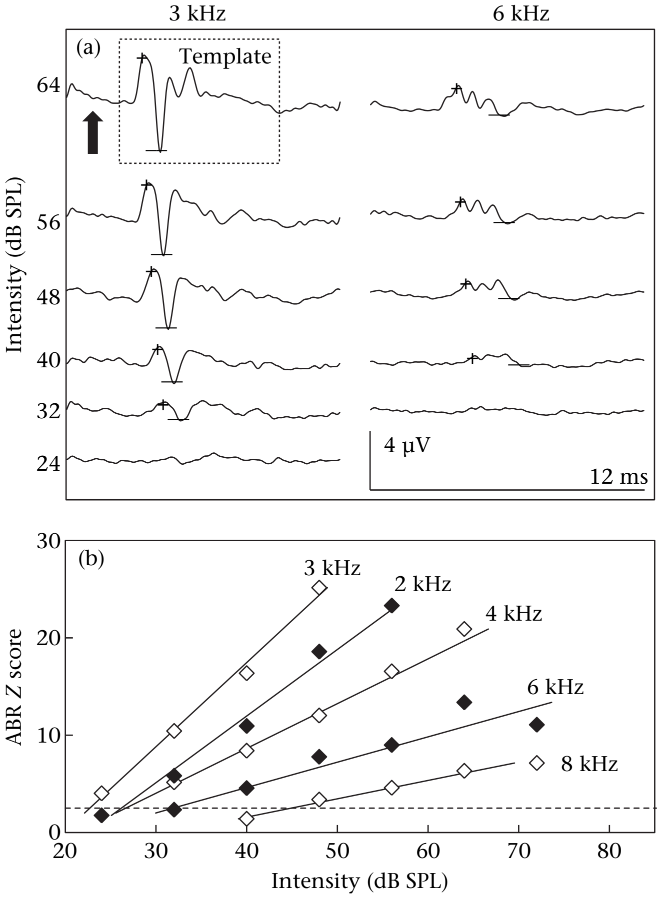

Figure 2. (a) Auditory brainstem responses (ABRs) to tone bursts of variable frequency (labels above) and intensity (labels left), from a single chickadee (GXCX). ABR amplitude and latency of the first ABR peak (+) were measured relative to the subsequent trough (–) and time of stimulus onset (arrow), respectively. The waveform scale is indicated in the lower right. The dashed box indicates the ABR template used in the cross-correlation analysis to determine the ABR threshold. (b) ABR *Z* score as a function of stimulus intensity at five stimulus frequencies. Stimulus frequency is indicated at the top right of each function. The dashed horizontal line indicates a *Z* score significantly greater than zero at the 99.5% confidence level. The ABR threshold is the intensity level at the intersection between the weighted regression line and dashed line.

Auditory Filter Experiment

ABRs were recorded in response to tone bursts of variable frequency and intensity in notched masking noise. Frequencies of 2, 3 and 4 kHz were tested in random order. At each frequency, notch bandwidths of 0, 0.2, 0.4, 0.6 and 0.8 times the test frequency were presented in random order. At each notch bandwidth, seven intensity levels were presented in decreasing 8 dB steps. Intensity levels decreased from 64 to 16 dB SPL at notch bandwidths of 0.6 and 0.8 times the test frequency, 72–24 dB SPL at the notch bandwidth of 0.4 times the test frequency, and 80–32 dB SPL at notch bandwidths of 0 and 0.2 times the test frequency. Tone bursts were 5 ms in duration with 1 ms $\cos^2$ onset/offset ramps. Each ABR was the average response to 1000 periodically presented tone bursts (51.1 stimuli/s) of alternating polarity.

We used a cross-correlation technique to determine the ABR thresholds of each subject at each combination of frequency and notch bandwidth. A separate analysis was conducted at each frequency for each subject using a custom PRAAT script. The method was similar to the cross-correlation technique described for the auditory sensitivity experiment, but procedures for

(1) generating the ABR template and (2) calculating ABR thresholds were slightly different.

(1) The ABR template was generated by selecting the ABR of greatest amplitude at each level of notch bandwidth. The selected ABR waveforms were averaged together to form the template. We accounted for slight differences in ABR latency by subtracting a short segment from the beginning of each waveform prior to averaging. The duration of the cropped segment was determined by cross-correlation with the shortest latency response. The first 2.3 ms and last 2.7 ms of the average waveform were deleted to form the final, 7 ms template.

(2) ABR thresholds were estimated by fitting a weighted general linear model to the ABR *Z* score data (Fig. 3a; GLM procedure; SAS). Weighting was assigned based on *Z* score as in the previous cross-correlation analysis. The model included notch bandwidth as a categorical variable, stimulus intensity as a continuous variable, and an interaction between notch bandwidth and stimulus intensity. The ABR threshold at each notch bandwidth was defined as the predicted stimulus intensity necessary to produce an ABR *Z* score of 2.56 (i.e. the intersection between the weighted regression line and dashed line in Fig. 3a).

The auditory filter parameters of interest included %ERB, defined previously, and K', which is the signal-to-noise ratio of the filter at threshold expressed in dB. We determined %ERB and K' by fitting a two-parameter, rounded exponential filter model to the ABR threshold by notch bandwidth function of each subject at each frequency. The procedure for deriving rounded exponential filter shapes from notched noise threshold data has been described previously (e.g. Glasberg & Moore 1990). In short, the masked threshold for detecting a tone, P_s, in Pa^2 is assumed to equal

$$P_s = K\int_0^\infty N(f)W(f)\mathrm{d}f \tag{1}$$

where K is the signal-to-noise ratio of the auditory filter necessary for signal detection, f is frequency in Hz, $N(f)$ is the long-term average power spectrum of the masking noise in Pa^2/Hz, and $W(f)$ is the weighting function of the auditory filter ranging from 0 to 1.

We measured $N(f)$ directly from recordings of the notched noise stimuli made using a Marantz PMD690 digital recorder and Sennheiser K6-ME62 omnidirectional microphone (2 s duration; 48 kHz sampling frequency). Long-term power spectra of the recordings were generated in PRAAT with a bin width of 25 Hz (Fig. 3b, dashed lines). We solved for K and $W(f)$ (Fig. 3b, solid line) using an iterative Gauss–Newton polynomial fitting procedure (NLIN procedure; SAS). The auditory filter was assumed to be symmetric around its centre frequency. Expressed as a function of g, the normalized deviation in frequency from the centre frequency (frequency deviation/centre frequency), the weight of the auditory filter is given by,

$$W(g) = (1-r)(1+pg)e^{-pg} + r \tag{2}$$

The p parameter indicates the slope of the function near its centre frequency and defines the %ERB of the auditory filter provided that the value of r is small (i.e. %ERB = $400/p$). The r parameter limits the dynamic range of the auditory filter function by imposing a minimum value on the weighting function. Equations (1) and (2) were combined into the following form to solve for K, p and r:

$$P_s(\mathrm{nw})' = K' + 10 \times \log_{10}\sum_{i=1}^{N}\frac{\mathrm{PSD}_{\mathrm{nw}(i)} \times \int_i \mathrm{roex}(p,r)\mathrm{d}f}{4\times10^{-10}} \tag{3}$$

where nw is the masking condition (e.g. 0.0, 0.2, 0.4, 0.6 or 0.8 times the test frequency), $P_s(\mathrm{nw})'$ is the ABR threshold in dB SPL under masking condition nw, K' is efficiency in dB, N is the number of 25 Hz bins between 0.2 and 1.8 times the test frequency, $\mathrm{PSD}_{\mathrm{nw}(i)}$ is the power spectral density of notched noise under masking condition nw in bin i in Pa^2/Hz, and $\int_i \mathrm{roex}(p,r)$ is the integral of the roex(p,r) function evaluated over bin i. The integral of the roex(p,r) function over bin i is given by

$$-(1-r)p^{-1}(2+pa)e^{-pa} + (1-r)p^{-1}(2+pb)e^{-pb} + r(a-b) \tag{4}$$

where a and b are the frequency limits of the bin i in terms of g, and a is the greater value of the two.

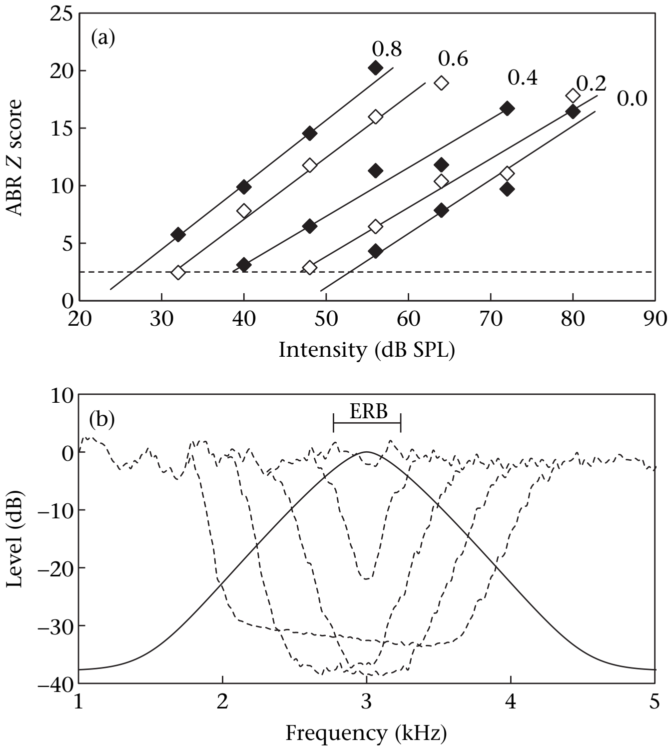

Figure 3. (a) Functions plotting auditory brainstem response (ABR) *Z* scores by stimulus intensity under five masking conditions, from a single subject (GXCX). The stimulus frequency is 3 kHz. The bandwidth of the notched noise, expressed as a proportion of centre frequency, is indicated at the top right of each function. The dashed horizontal line indicates a *Z* score significantly greater than zero at the 99.5% confidence level. The ABR threshold is the intensity level at the intersection between the weighted regression line and dashed line. (b) Rounded exponential auditory filter function derived from the ABR thresholds above (solid line), and long-term average power spectra of notched noise stimuli (dashed lines). The auditory filter function is expressed in dB relative to maximum output. Equivalent rectangular bandwidth (ERB) is indicated at the top of the panel. Power spectra are expressed in dB relative to 15.3 dB/Hz re. 20 μPa^2, which was the average spectrum level of the masking noise.

Statistical Analysis

We used repeated measures mixed models (MIXED Procedure; SAS) to analyse ABR data. The dependent variables for the auditory sensitivity experiment were ABR threshold, ABR amplitude and ABR latency. The analysis of ABR thresholds included frequency and sex as categorical independent variables and the frequency×sex interaction. The analyses of ABR amplitude and latency included frequency and sex as categorical independent variables and intensity as a continuous independent variable. Furthermore, the analyses included intensity^2, all possible two-way interactions, and frequency×sex×intensity. The dependent variables for the auditory filter experiment included masked ABR threshold, %ERB and K'. The analysis of masked ABR thresholds included frequency, notch bandwidth and sex as categorical independent variables and all possible interactions. The analyses of %ERB and K' included frequency and sex as categorical independent variables and frequency × sex.

Dependent variables were transformed in some cases to ensure normality and constant variance of residual values. Unmasked ABR thresholds were transformed using the Box–Cox procedure (TRANSREG Procedure; SAS) and a lambda value of −0.25. ABR amplitude was log transformed. We selected between a variety of possible within-subject covariance structures including unstructured, compound symmetry, first-order autoregressive, and first-order autoregressive with a random subject effect based on minimum Bayesian Information Criterion (Littell et al. 2006). First-order autoregressive covariance with a random subject effect provided the best fit for analyses of ABR amplitude, ABR latency and masked ABR thresholds, while compound symmetry provided the best fit for analyses of unmasked ABR thresholds, %ERB and K'. Nonsignificant interactions ($P > 0.05$) were dropped from the model. The remaining effects were explored using comparisons of least squares (LS) means and tests of simple effects. Denominator degrees of freedom were calculated using the Kenward–Roger method. LS means ± SE are presented throughout.

RESULTS

Auditory Sensitivity

Audiograms plotting ABR thresholds as a function of frequency were broadly similar between Carolina chickadees and three other songbird species tested previously using the same methodology (from Henry & Lucas 2008; Fig. 4a). The frequency range of sensitive ABR thresholds (i.e. ABR thresholds within 10 dB of the best ABR threshold; Fig. 4a, dotted horizontal line) extended from 0.8 to 5.6 kHz in chickadees. The high-frequency limit of sensitivity was similar between chickadees and tufted titmice, *Baeolophus bicolor* (5.6 kHz in both species), and greater in chickadees and titmice than in white-breasted nuthatches, *Sitta carolinensis* (4.2 kHz) or house sparrows, *Passer domesticus* (4.6 kHz). The low-frequency limit of sensitivity, in contrast, was similar among species (chickadee: 0.8 kHz; titmouse: 0.85 kHz; house sparrow: 0.7 kHz; nuthatch: 0.9 kHz).

The analysis of ABR thresholds in chickadees revealed a significant effect of frequency ($F_{6,56.7} = 75.20$, $P < 0.001$) and marginal sex effect (sex: $F_{1,9.1} = 4.82$, $P = 0.055$; Fig. 4b). ABR thresholds were lower from 1 to 4 kHz than at higher and lower frequencies, and lower in males than females by 1.1–6.4 dB depending on stimulus frequency (LS mean difference: −3.8 ± 1.7 dB).

The analysis of ABR amplitude in chickadees found significant effects of frequency and intensity (frequency: $F_{6,56} = 2.19$, $P = 0.058$; intensity: $F_{1,330} = 48.07$, $P < 0.001$; intensity^2: $F_{1,330} = 30.30$, $P < 0.001$; frequency × intensity: $F_{6,330} = 4.16$, $P = 0.001$; frequency × intensity^2: $F_{6,330} = 4.71$, $P < 0.001$; Fig. 5, left). Amplitude was greater from 2 to 4 kHz than at higher and lower frequencies, and increased with increasing intensity level. The analysis also revealed significant variation between sexes (sex: $F_{1,10} = 4.78$, $P = 0.054$; frequency × sex: $F_{6,56} = 4.31$, $P = 0.001$). Males had greater ABR amplitude than females at 0.5, 2, 3 and 6 kHz but not at 1, 4 or 8 kHz (Table 2).

The analysis of ABR latency found significant effects of frequency and intensity (frequency: $F_{6,56} = 21.39$, $P < 0.001$; intensity: $F_{1,334} = 134.08$, $P < 0.001$; intensity^2: $F_{1,334} = 49.04$, $P < 0.001$; frequency × intensity: $F_{6,334} = 11.00$, $P < 0.001$; Fig. 5, right). Latency was shortest at frequencies from 2 to 4 kHz and decreased with increasing intensity level. The analysis also revealed variation between sexes (sex: $F_{1,10} = 1.46$, $P = 0.25$; frequency × sex: $F_{6,56} = 3.33$, $P = 0.007$). Females had lower ABR latency than males at 1 kHz (Table 2).

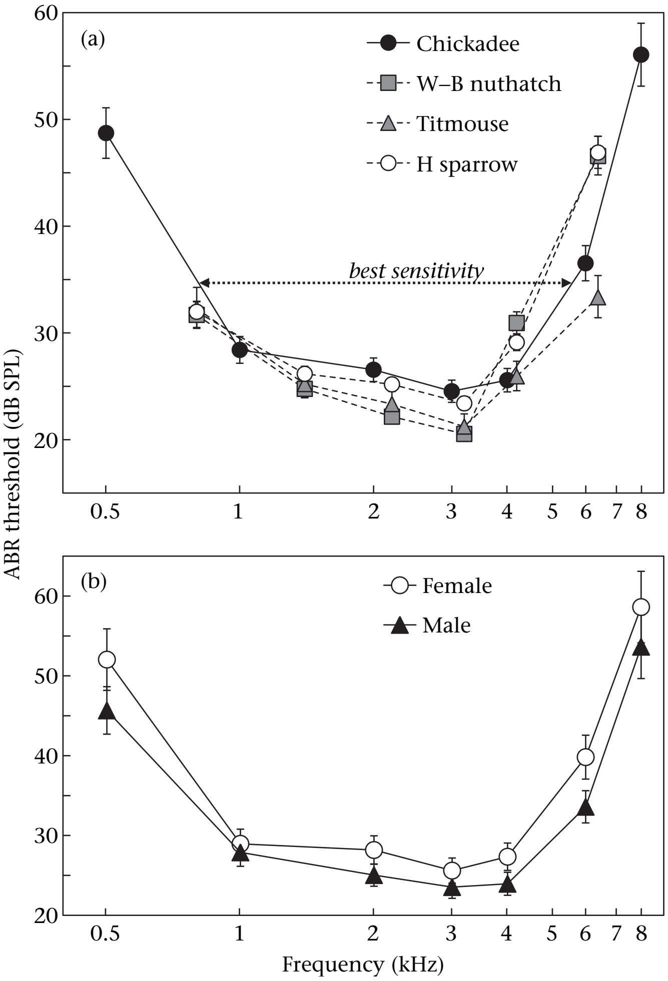

Figure 4. Audiograms plotting auditory brainstem response (ABR) thresholds (LS mean ± SE) as a function of frequency. (a) Comparison between Carolina chickadees and three other songbirds (white-breasted nuthatch, tufted titmouse, house sparrow) tested using the same method (i.e. from Henry & Lucas 2008). Note that the other songbirds were tested at different stimulus frequencies. The dotted horizontal line indicates the frequency range of ABR thresholds within 10 dB of best sensitivity in chickadees. (b) ABR audiograms of male and female chickadees.

Auditory Filters

The analysis of ABR thresholds in notched noise revealed significant effects of frequency and notch bandwidth (frequency: $F_{2,62.8} = 11.64$, $P < 0.001$; notch bandwidth: $F_{4,114} = 567.52$, $P < 0.001$; frequency × notch bandwidth: $F_{8,114} = 6.65$, $P < 0.001$; Fig. 6). ABR thresholds decreased with increasing notch bandwidth, and the rate of decrease was greater at 3 and 4 kHz than at 2 kHz. The pattern suggests that the percentage of frequency selectivity is greater at 3 and 4 kHz than at 2 kHz. Furthermore, threshold functions varied between sexes (sex: $F_{1,11.7} = 3.25$, $P = 0.097$; frequency × sex: $F_{2,62.8} = 2.72$, $P = 0.074$; notch bandwidth × sex: $F_{4,114} = 6.07$, $P < 0.001$; frequency × notch bandwidth × sex: $F_{8,114} = 3.00$, $P = 0.004$). Females had higher ABR thresholds than males at 2 kHz (LS mean difference: 3.57 ±1.32 dB; $t_{10} = 2.70$, $P = 0.022$), and the slope of the threshold by notch bandwidth function was more negative in females than in males at 3 kHz (notch bandwidth × sex: $F_{4,35.5} = 5.15$, $P = 0.002$) and 4 kHz

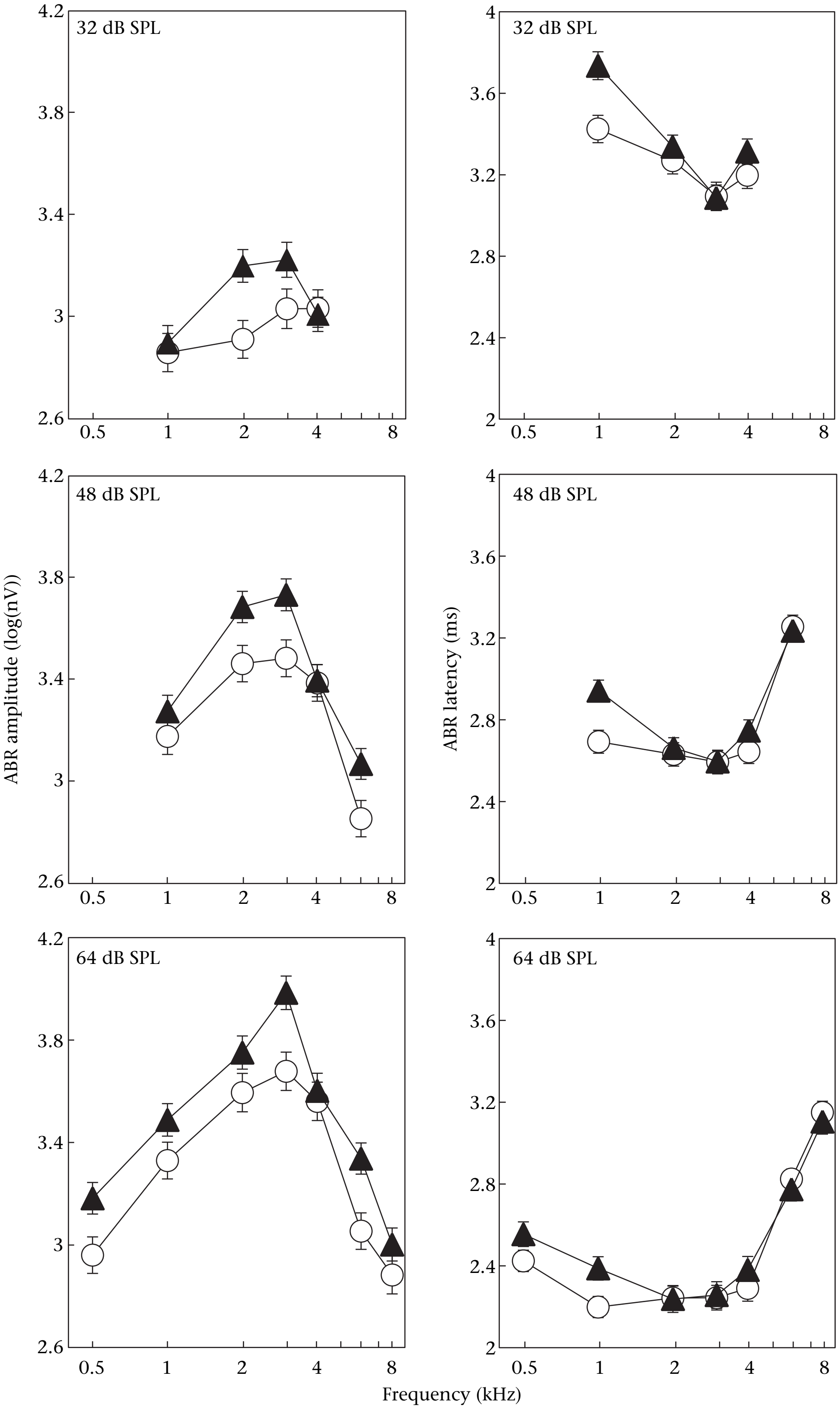

Figure 5. Auditory brainstem response (ABR) amplitude (log transformed; left) and ABR latency (right) of Carolina chickadees as a function of frequency. Stimulus intensity is indicated at the top left of each panel. LS means ± SE for males (solid triangles) and females (open circles) are plotted separately.

Table 2
Tests of simple effects of sex on auditory brainstem response (ABR) amplitude and latency at each frequency

Frequency (Hz)	ABR amplitude			ABR latency		
	ndf, ddf	*F*	*P*	*ndf, ddf*	*F*	*P*
500	1,16.9	6.46	0.021	1,35.6	1.64	0.21
1000	1,16	2.12	0.16	1,28.3	11.32	0.002
2000	1,15.6	7.15	0.017	1,28.1	0.07	0.79
3000	1,16.6	8.11	0.011	1,30.8	0.00	0.95
4000	1,16.5	0.05	0.819	1,30.7	2.22	0.15
6000	1,15.6	8.44	0.011	1,27.6	0.29	0.59
8000	1,17.6	0.65	0.43	1,36.1	0.14	0.72

(notch bandwidth×sex: $F_{4,39.3} = 8.17$, $P < 0.001$), suggesting greater frequency selectivity, but not at 2 kHz (notch bandwidth×sex: $F_{4,35.9} = 0.35$, $P = 0.84$).

The roex(p,r) auditory filter model provided a robust fit to the ABR threshold by notch bandwidth functions (Fig. 6, solid lines). The median R^2 value was 0.985, and the interquartile range of R^2 values ranged from 0.963 to 0.993 ($N = 37$ models). The %ERB of auditory filters in Carolina chickadees decreased between 2 and 4 kHz (Fig. 7a). Compared to five other songbirds tested previously using the same method (from Henry & Lucas 2010), the effect of frequency on %ERB was similar to dark-eyed, *Junco hyemalis*, white-crowned sparrows, *Zonotrichia leucophrys*, and house sparrows. In tufted titmice and white-breasted nuthatches, in contrast, %ERB is relatively constant from 2 to 4 kHz. Differences in frequency selectivity between Carolina chickadees and other songbirds varied with stimulus frequency. Frequency selectivity in chickadees was generally intermediate at 2 and 3 kHz, and relatively high at 4 kHz. Frequency selectivity at 4 kHz was similar between chickadees and dark-eyed juncos, tufted titmice and white-breasted nuthatches, and greater in chickadees than in house sparrows and white-crowned sparrows.

The analysis of %ERB in chickadees revealed a significant effect of frequency ($F_{2,20.7} = 37.86$, $P < 0.001$), and greater frequency selectivity in females than in males (sex: $F_{1,8.7} = 23.75$, $P = 0.001$; Fig. 7b). The LS mean difference in %ERB was -0.9 ± 1.4 at 2 kHz ($t_{28.6} = -0.70$, $P = 0.49$), -3.2 ± 1.4 at 3 kHz ($t_{29.3} = -2.21$, $P = 0.035$), and -4.5 ± 1.3 at 4 kHz ($t_{27.6} = -3.35$, $P = 0.011$).

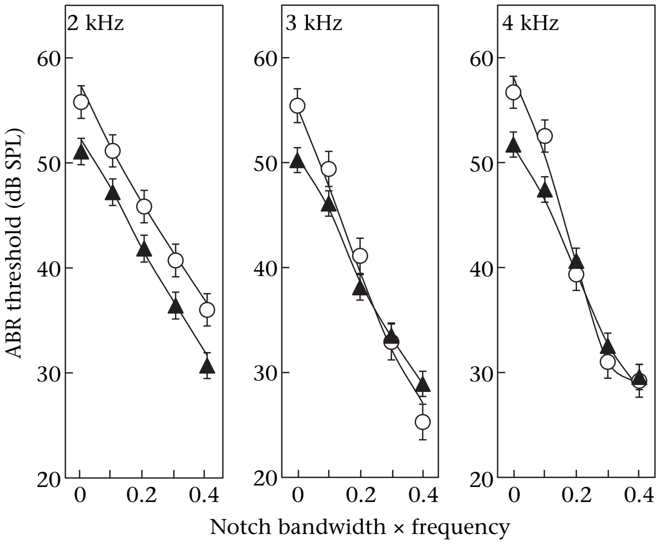

Figure 6. Masked auditory brainstem response (ABR) thresholds (LS mean ± SE) of Carolina chickadees as a function of notch bandwidth. Solid lines represent the fit of the roex(p,r) auditory filter model. Test frequency is indicated at the top of each panel. Thresholds for males (solid triangles) and females (open circles) are plotted separately.

The analysis of K', the signal-to-noise ratio of the auditory filter at threshold in dB, revealed a significant effect of frequency ($F_{2,20.9} = 11.14$, $P = 0.001$). K' was greater at 4 kHz (LS mean: 50.48 ± 1.03) than at 2 and 3 kHz (LS means of 45.06 ± 1.06 and 44.98 ± 1.11 dB, respectively). K' also varied between sexes (sex: $F_{1,11.5} = 11.82$, $P = 0.005$). Females generally had higher K' values than males (LS mean difference: 4.94 ± 1.44 dB), suggesting less efficient signal extraction from noise. Finally, the value of the r parameter was near zero at 2 and 3 kHz and slightly greater than zero at 4 kHz. Furthermore, r tended to be greater in males than in females at 4 kHz (Table 3).

DISCUSSION

Auditory Sensitivity

The observed effects of stimulus frequency on ABR thresholds, amplitude and latency indicate that Carolina chickadees have a large number of neurons tuned to frequencies between 2 and 4 kHz in the cochlea and auditory brainstem. As predicted, the high-frequency limit of sensitive ABR thresholds in chickadees was greater than that of house sparrows and nuthatches, which have a lower maximum frequency of vocalizations (6.5 kHz; Henry & Lucas 2008), and similar to that of tufted titmice, which have a high maximum vocal frequency (9–10 kHz: Lucas & Freeberg

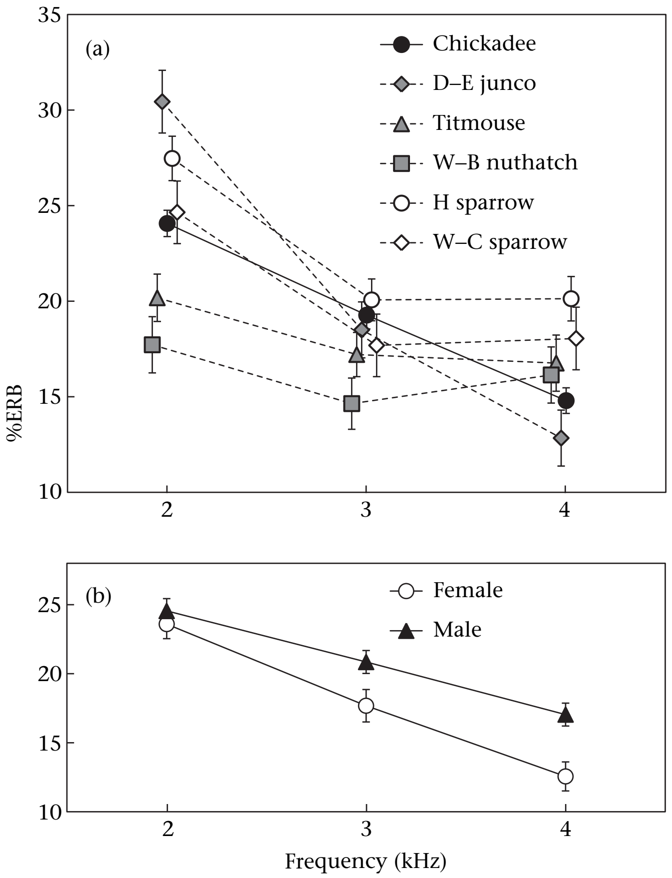

Figure 7. Percentage of equivalent rectangular bandwidth (%ERB) of the auditory filter (LS mean ± SE) as a function of frequency. (a) Comparison between Carolina chickadees and five other songbirds (dark-eyed junco, tufted titmouse, white-breasted nuthatch, house sparrow, white-crowned sparrow) tested using the same method (i.e. from Henry & Lucas 2010). Note that data from the other songbirds are shifted slightly along the horizontal axis (<0.1 kHz) to avoid obscuring the error bars (i.e. all species were tested at 2, 3 and 4 kHz). (b) Comparison between male and female chickadees.

Table 3
Summary statistics of the *r* parameter (×1000)

Frequency (Hz)	Sex	Median	Interquartile range	Range
2000	F	0.000	0.000–2.081	0.000–2.730
	M	0.000	0.000–0.903	0.000–1.920
3000	F	0.000	0.000–0.788	0.000–1.575
	M	0.573	0.076–2.039	0.000–3.719
4000	F	0.049	0.016–0.078	0.000–1.219
	M	0.361	0.143–0.883	0.000–1.836

2007; Henry & Lucas 2008). However, the low-frequency limit of sensitivity was similar between chickadees and other species despite slight variation in the minimum frequency of vocalizations (2–3 kHz in chickadees versus 1.5–2 kHz in titmice, nuthatches and house sparrows).

In addition to chickadees and titmice, exceptional high-frequency auditory capabilities have been found in other songbirds with high-frequency vocalizations, including the great tit, *Parus major* (Langemann et al. 1998) and six species of emberizid sparrows (Konishi 1969, 1970; Okanoya & Dooling 1988). The combined results indicate a positive correlation between the high-frequency limit of sensitive hearing and the maximum frequency of vocalizations in songbirds that most likely reflects coevolution between these characteristics. Ultimately, the evolutionary history of auditory diversification in songbirds should be explored using a phylogenetic analysis and larger sample of species. Body size must also be considered because it may contribute to species differences in auditory sensitivity in some cases. Smaller birds tend to be more sensitive to high frequencies and less sensitive to low frequencies than larger species, but with considerable auditory variation at any given body size (Konishi 1969, 1970; Dooling et al. 2000). Among six songbirds of similar body mass (17–22 g) the high-frequency limit of moderate sensitivity (i.e. thresholds within 30 dB of the best threshold) varies from 5.6 to 9.2 kHz and the frequency of best sensitivity varies from 2.5 to 4.5 kHz (Gleich et al. 2005; note that auditory variation was random with respect to body mass in this sample).

We predicted insensitive low-frequency hearing in chickadees due to their relatively high minimum frequency of vocalizations, but the low-frequency limit of sensitive ABR thresholds was similar between chickadees and several species with a lower minimum frequency of vocalizations (house sparrows, nuthatches and titmice). Previous studies also found no relationship between the minimum frequency of vocalizations and low-frequency sensitivity (Konishi 1969, 1970), suggesting that these characters may not coevolve. Indeed, most songbirds are sensitive to frequencies well below the frequency range of vocalizations. Sensitive low-frequency hearing may aid in detection of broadband acoustic cues generated by stalking predators, as suggested by Konishi (1970).

The frequency range of auditory sensitivity in chickadees is not ideally tuned the frequency range of vocalizations. Chickadee vocalizations commonly contain frequencies from 2 to 10 kHz, yet ABR thresholds at 4, 6 and 8 kHz are 1.5, 12.0 and 31.6 dB above the best threshold (23.1 dB SPL at 3 kHz), respectively. Similar disparities have been noted in house sparrows based on the ABR (Henry & Lucas 2008), and a number of other songbirds based on single-unit recordings from the cochlear nucleus (Konishi 1969, 1970). The dominant frequency of vocalizations tends to fall slightly above the frequency range of maximum auditory sensitivity in these species. The pattern may reflect constraints on the evolution of high-frequency sensitivity in songbirds potentially related to the morphology of the avian middle ear. Their middle ear contains a single ossicle, the columella, that transfers high-frequency vibration less efficiently than does the three-ossicle system of mammals (Saunders et al. 2000). Finally, note that the threshold estimate at 8 kHz in chickadees may be slightly overestimated by the ABR method. Songbirds generally have few auditory neurons with characteristic frequencies near their high-frequency limit of sensitivity (Konishi 1969, 1970), which should decrease ABR amplitude relative to the amplitude of electrophysiological background noise. Lower ABR amplitude, in turn, may increase the ABR threshold estimate.

Frequency Selectivity of Auditory Filters

The %ERB of auditory filters in Carolina chickadees decreased between 2 and 4 kHz as in three other songbirds with a relatively high minimum frequency of long-range signals (house sparrows, white-crowned sparrows and dark-eyed juncos), and in contrast to two species with a lower minimum frequency of long-range signals (nuthatches and titmice). Note that the minimum frequency of long-range signals is 3–4 kHz in chickadees, house sparrows, white-crowned sparrows and juncos, and 1.5–2 kHz in titmice and nuthatches (Henry & Lucas 2008, 2010). %ERB in chickadees was intermediate compared to other songbirds at 2 and 3 kHz, and relatively narrow at 4 kHz. %ERB at 4 kHz was similar between chickadees and three other woodland species with tonal songs (titmice, nuthatches and juncos), and lower in chickadees than in two open habitat species with rapidly modulated songs (house sparrows and white-crowned sparrows; Henry & Lucas 2010).

Taken together, the results indicate that chickadees and other songbirds generally have high percentage frequency selectivity within their frequency range of vocalizations. The pattern may reflect coevolution between the frequency range of maximum frequency selectivity and the frequency range of vocalizations. Greater frequency selectivity provides several potential benefits including greater frequency resolution of signals and better signal detection in background noise. Relatively narrow auditory filters improve signal detection because they are more likely to separate a narrowband signal, higher-frequency masking sounds and lower-frequency masking sounds (e.g. wind noise) into different neural channels (Brumm & Slabbekoorn 2005). Furthermore, in the case of broadband masking sounds, relatively narrow filters decrease the noise level processed within each filter. Each filter processes a smaller bandwidth of sound, and therefore, a smaller fraction of the total energy contained in the masking noise.

Our results are also consistent with previous findings that woodland songbirds have greater maximum frequency selectivity than species inhabiting open areas (Henry & Lucas 2010). Woodland songbirds tend to communicate with tonal vocal signals that resist degradation due to reverberations (e.g. the fee-bee-fee-bay song of Carolina chickadees), while species inhabiting open areas generally produce faster signal modulations that are less likely to be masked by added modulations from wind (acoustic adaptation hypothesis; Wiley & Richards 1978; Richards & Wiley 1980; Badyaev & Leaf 1997; Boncoraglio & Saino 2007). Relatively narrow auditory filters in chickadees and other woodland species, in turn, may have evolved for greater frequency resolution of tonal signals, while broader auditory filters in open habitat species may have evolved for greater temporal resolution of FM and AM. Alternatively, greater frequency selectivity in woodland songbirds may reflect an adaptation to increase the active space of communication signals in an acoustically challenging environment. The presence of leaves and tree branches increases the rate of signal attenuation, especially for high-frequency signal components, and degrades patterns of FM and AM (Wiley & Richards 1978; Richards & Wiley 1980). Narrower auditory filters, in turn, may offset these effects through greater signal detection in background noise.

Habitat-based differences in auditory filter bandwidth have also been found between two populations of cricket frog, *Acris crepitans* (Witte et al. 2005). Female frogs from a pine forest environment have narrower auditory filters than females from an open habitat. Narrower filters in this case probably reflect an adaptation to increase the active space of vocal signals in an acoustically challenging environment rather than optimization for habitat-based differences in signal structure. Indeed, the auditory system of woodland frogs appears to separate vocal signals from noise more effectively in either habitat.

The maximum frequency selectivity of auditory filters in Carolina chickadees, though relatively high compared to other songbirds, provides relatively coarse frequency resolution of tonal song notes. Neurons tuned to a 4 kHz song note, for example, respond nearly as strongly to tones of 3.7 and 4.3 kHz (i.e. the intensity of stimulation decreases by approximately 6 dB at these points). Frequency selectivity of Carolina chickadees most likely increases at higher levels of auditory processing, at least in some ascending pathways, based on evidence of excellent behavioural frequency discrimination in other songbirds (i.e. 1–2% frequency difference limens; reviewed in Dooling et al. 2000) including black-capped chickadees, *Poecile atricapillus*. Black-capped chickadees transpose tonal song notes up and down over a frequency range of 860 Hz, and territorial males routinely match dominant song frequencies within 50 Hz during aggressive countersinging contests (FitzSimmons et al. 2008). Frequency selectivity may increase at higher levels of auditory processing through a variety of mechanisms including neural phase-locking and lateral inhibition (Suga et al. 1997; Gleich & Manley 2000), while other pathways may retain low-frequency selectivity in order to preserve temporal information. Indeed, neurons of the auditory midbrain and forebrain in zebra finches, *Taeniopygia guttata*, show a broad variety of response characteristics (Woolley et al. 2009). Some neurons of the midbrain appear optimized for preserving modulations of sound intensity, whereas other neurons have high-frequency selectivity (Woolley & Casseday 2004). These studies suggest that specializations for processing of vocal signals may exist throughout the auditory system.

Sex Differences

As predicted, female Carolina chickadees had greater peripheral auditory frequency selectivity than males. Potentially, greater frequency selectivity may enhance perception of frequency differences between song notes, which may indicate male quality and dominance status. In black-capped chickadees, the frequency difference between song notes indicates male dominance status (Christie et al. 2004), and females mated to dominant males have relatively high reproductive success (Doucet et al. 2005). Differences in ABR amplitude, ABR thresholds and K' values, however, suggest that females are less sensitive than males, at least under quiet conditions. Greater auditory sensitivity in males should improve detection of distant songs, which may be important for territory defence. The functional significance of auditory differences between sexes, however, should be interpreted with caution because auditory sensitivity and frequency selectivity may vary between autumn, when data were collected for the current report, and spring, when fee-bee-fee-bay songs are produced most extensively. ABR amplitude increases in spring in a variety of songbirds including Carolina chickadees (Lucas et al. 2002, 2007; Henry & Lucas 2008), and the volume of the auditory forebrain increases in spring in European starlings (De Groof et al. 2009).

Sex differences in chickadees, and seasonal auditory variation in songbirds and other vertebrates, may reflect a general relationship between sex hormones and auditory processing. Oestrogen receptors are present in the inner ear of zebra finches (Noirot et al. 2009) and a diversity of nonavian species including the plainfin midshipman fish, *Porichthys notatus*, rodents and humans (reviewed in Hultcrantz et al. 2006). Auditory function clearly varies with oestrogen level in the midshipman, humans and rodents, and with testosterone level in the midshipman, potentially through conversion to oestrogen by aromatase (Sisneros et al. 2004; Hultcrantz et al. 2006). The relationship between sex hormones and auditory function in songbirds requires further exploration.

Conclusions

Results of the current study of Carolina chickadees, taken together with studies of other songbirds, point to coevolution between a variety of auditory and vocal characteristics. The correlation between the high-frequency limit of sensitive hearing and maximum frequency of vocalizations represents particularly compelling evidence of coevolution, while correlations between (1) the frequency range of maximum frequency selectivity and frequency range of long-range communication signals and (2) maximum frequency selectivity and maximum signal modulation are more suggestive at this point based on the small number of species sampled. Finally, sex differences observed in the current study of chickadees, together with seasonal differences observed in previous studies of chickadees, other songbirds and other vertebrates, point to a general relationship between sex hormones and auditory function.

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References

Badyaev, A. V. & Leaf, E. S. 1997. Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk*, **114**, 40–46.

Baker, M. C. & Gammon, D. E. 2007. The gargle call of black-capped chickadees: ontogeny, acoustic structure, population patterns, function, and processes leading to sharing of call characteristics. In: *Ecology and Behavior of Chickadees and Titmice* (Ed. by K. Otter), pp. 167–183. Oxford: Oxford University Press.

Boersma, P. & Weenink, D. 2009. *Praat: Doing Phonetics by Computer. Version 5.1.07.* Computer program retrieved May 7, 2009, from: http://www.praat.org/.

Boncoraglio, G. & Saino, N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology*, **21**, 134–142.

Brittan-Powell, E. F. & Dooling, R. J. 2004. Development of auditory sensitivity in budgerigars (*Melopsittacus undulatus*). *Journal of the Acoustical Society of America*, **115**, 3092–3102.

Brittan-Powell, E. F., Dooling, R. J. & Gleich, O. 2002. Auditory brainstem responses (ABR) in adult budgerigars (*Melopsittacus undulatus*). *Journal of the Acoustical Society of America*, **112**, 999–1008.

Brittan-Powell, E. F., Lohr, B., Hahn, D. C. & Dooling, R. J. 2005. Auditory brainstem responses in the eastern screech owl: an estimate of auditory thresholds. *Journal of the Acoustical Society of America*, **118**, 314–321.

Brumm, H. & Slabbekoorn, H. 2005. Acoustic communication in noise. *Advances in the Study of Behavior*, **35**, 151–207.

Christie, P. J., Mennill, D. J. & Ratcliffe, L. M. 2004. Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. *Behavioral Ecology and Sociobiology*, **55**, 341–348.

De Groof, G., Verhoye, M., Poirier, C., Leemans, A., Eens, M., Darras, V. M. & Van der Linden, A. 2009. Structural changes between seasons in the songbird auditory forebrain. *Journal of Neuroscience*, **29**, 13557–13565.

Dooling, R. J., Lohr, B. & Dent, M. L. 2000. Hearing in birds and reptiles. In: *Comparative Hearing: Birds and Reptiles* (Ed. by R. J. Dooling, A. N. Popper & R. R. Fay), pp. 308–359. New York: Springer-Verlag.

Doucet, S. M., Mennill, D. J., Montgomerie, R., Boag, P. T. & Ratcliffe, L. M. 2005. Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. *Behavioral Ecology*, **16**, 218–222.

Elliot, L., Stokes, D. & Stokes, L. 1997. *Stokes Field Guide to Bird Songs: Eastern Region Compact Disc*. New York: Time Warner Audio Books.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist, Supplement*, **139**, S125–S153.

Feng, A. S., Narins, P. M., Xu, C., Lin, W., Yu, Z., Qiu, Q., Xu, Z. & Shen, J. 2006. Ultrasonic communication in frogs. *Nature*, **440**, 333–336.

FitzSimmons, L. P., Foote, J. R., Ratcliffe, L. M. & Mennill, D. J. 2008. Frequency matching, overlapping and movement behaviour in diurnal countersinging interactions of black-capped chickadees. *Animal Behaviour*, **75**, 1913–1920.

Fletcher, H. 1940. Auditory patterns. *Reviews of Modern Physics*, **12**, 47–65.

Glasberg, B. R. & Moore, B. C. J. 1990. Derivation of auditory filter shapes from notched-noise data. *Hearing Research*, **47**, 103–138.

Gleich, O. 1994. Excitation patterns in the starling cochlea: a population study of primary auditory afferents. *Journal of the Acoustical Society of America*, **95**, 401–409.

Gleich, O. & Manley, G. A. 2000. The hearing organ of birds and crocodilian. In: *Comparative Hearing: Birds and Reptiles* (Ed. by R. J. Dooling, R. R. Fay & A. N. Popper), pp. 70–138. New York: Springer.

Gleich, O., Dooling, R. J. & Manley, G. A. 2005. Audiogram, body mass, and basilar papilla length: correlations in birds and predictions for extinct archosaurs. *Naturwissenschaften*, **92**, 595–598.

Hailman, J. P. 1989. The organization of major vocalizations in the Paridae. *Wilson Bulletin*, **101**, 305–343.

Hall, J. 2007. *New Handbook of Auditory Evoked Responses*. Boston: Pearson Education.

Henry, K. S. & Lucas, J. R. 2008. Coevolution of auditory sensitivity and temporal resolution with acoustic signal space in three songbirds. *Animal Behaviour*, **76**, 1659–1671.

Henry, K. S. & Lucas, J. R. 2009. Vocally-correlated seasonal auditory variation in the house sparrow (*Passer domesticus*). *Journal of Experimental Biology*, **212**, 3817–3822.

Henry, K. S. & Lucas, J. R. 2010. Habitat-related differences in the frequency selectivity of auditory filters in songbirds. *Functional Ecology*, **24**, 614–624.

Hultcrantz, M., Simonoska, R. & Stenberg, A. E. 2006. Estrogen and hearing: a summary of recent investigations. *Acta Oto-laryngology*, **126**, 10–14.

Konishi, M. 1969. Hearing, single-unit analysis, and vocalizations in songbirds. *Science*, **166**, 1178–1181.

Konishi, M. 1970. Comparative neurophysiological studies of hearing and vocalizations in songbirds. *Zeitschrift für Vergleichende Physiologie*, **66**, 257–272.

Konishi, M. 1973. How the owl tracks its prey. *American Scientist*, **61**, 414–424.

Lall, A. B., Seliger, H. H., Biggley, W. H. & Lloyd, J. E. 1980. Ecology of colors of firefly bioluminescence. *Science*, **210**, 560–562.

Langemann, U., Gauger, B. & Klump, G. M. 1998. Auditory sensitivity in the great tit: perception of signals in the presence and absence of noise. *Animal Behaviour*, **56**, 763–769.

Lauer, A. M., Dooling, R. J. & Leek, M. R. 2009. Psychophysical evidence of damaged active processing mechanisms in Belgian waterslager canaries. *Journal of Comparative Physiology A*, **195**, 193–202.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. & Schabenberger, O. 2006. *SAS for Mixed Models*, 2nd edn. Cary, North Carolina: SAS.

Lohr, B., Nowicki, S. & Weisman, R. 1991. Pitch production in Carolina chickadee songs. *Condor*, **93**, 197–199.

Lucas, J. R. & Freeberg, T. M. 2007. Information and the chick-a-dee call: communicating with a complex vocal system. In: *Ecology and Behavior of Chickadees and Titmice* (Ed. by K. Otter), pp. 199–213. Oxford: Oxford University Press.

Lucas, J. R., Freeberg, T. M., Krishnan, A. & Long, G. R. 2002. A comparative study of avian auditory brainstem responses: correlations with phylogeny and vocal complexity, and seasonal effects. *Journal of Comparative Physiology A*, **188**, 981–992.

Lucas, J. R., Freebeg, T. M., Egbert, J. & Schwabl, H. 2006. Fecal corticosterone, body mass, and caching rates of Carolina chickadees (*Poecile carolinensis*) from disturbed and undisturbed sites. *Hormones and Behavior*, **49**, 634–643.

Lucas, J. R., Freeberg, T. M., Long, G. R. & Krishnan, A. 2007. Seasonal variation in avian auditory evoked responses to tones: a comparative analysis of Carolina chickadees, tufted titmice, and white-breasted nuthatches. *Journal of Comparative Physiology A*, **192**, 201–215.

Manley, G. A., Gleich, O., Leppelsack, H. J. & Oeckinghaus, H. 1985. Activity patterns of cochlear ganglion neurons in the starling. *Journal of Comparative Physiology A*, **157**, 161–181.

Marean, G. C., Burt, J. M., Beecher, M. D. & Rubel, E. W. 1998. Auditory perception following hair cell regeneration in European starling (*Sturnus vulgaris*): frequency and temporal resolution. *Journal of the Acoustical Society of America*, **103**, 3567–3580.

Monstrom, A. M., Curry, R. L. & Lohr, B. 2002. Carolina chickadee (*Poecile carolinensis*). In: *The Birds of North America Online. No. 636* (Ed. by A. Poole). Ithaca, New York: Cornell Lab of Ornithology, doi:10.2173/bna.636.

Moore, B. C. J. 1993. Frequency analysis and pitch perception. In: *Human Psychophysics* (Ed. by W. A. Yost, A. N. Popper & R. R. Fay), pp. 58–89. New York: Springer-Verlag.

Moore, B. C. J. & Glasberg, B. R. 1983. Suggested formulas for calculating auditory filter bandwidths and excitation patterns. *Journal of the Acoustical Society of America*, **74**, 750–753.

Nava, S. S., Conway, M. & Marins, E. P. 2009. Divergence of visual motion detection in diurnal geckos that inhabit bright and dark habitats. *Functional Ecology*, **23**, 794–799.

Nelson, D. A. & Marler, P. 1990. The perception of birdsong and an ecological concept of signal space. In: *Comparative Perception. Vol. 2: Complex Signals* (Ed. by W. C. Stebbins & M. A. Berkley), pp. 443–478. New York: J. Wiley.

Noirot, I. C., Adler, H. J., Cornil, C. A., Harada, N., Dooling, R. J., Balthazart, J. & Ball, G. F. 2009. Presence of aromatase and estrogen receptor alpha in the inner ear of zebra finches. *Hearing Research*, **252**, 49–55.

Okanoya, K. & Dooling, R. J. 1988. Hearing in the swamp sparrow (*Melospiza georgiana*) and the song sparrow (*Melospiza melodia*). *Animal Behaviour*, **36**, 726–732.

Richards, D. G. & Wiley, R. H. 1980. Reverberations and amplitude fluctuations in the propagation of sound in a forest: implications for animal communication. *American Naturalist*, **115**, 381–399.

Ryan, M. J., Fox, J. H., Wilczynski, W. & Rand, A. S. 1990. Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature*, **343**, 66–67.

Saunders, J. C., Duncan, R. K., Doan, D. E. & Werner, Y. L. 2000. The middle ear of reptiles and birds. In: *Comparative Hearing: Birds and Reptiles* (Ed. by R. J. Dooling, A. N. Popper & R. R. Fay), pp. 308–359. New York: Springer-Verlag.

Sisneros, J. A., Forlano, P. M., Deitcher, D. L. & Bass, A. H. 2004. Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. *Science*, **305**, 404–407.

Smith, S. T. 1972. *Communication and Other Social Behavior in Parus carolinensis*. Cambridge, Massachusetts: Nuttall Ornithological Club.

Soard, C. M. & Ritchison, G. 2009. Chick-a-dee calls of Carolina chickadees convey information about degree of threat posed by avian predators. *Animal Behaviour*, **78**, 1447–1453.

Suga, N., Zhang, Y. F. & Yan, J. 1997. Sharpening of frequency tuning by inhibition in the thalamic auditory nucleus of the mustached bat. *Journal of Neurophysiology*, **77**, 2098–2114.

Thirakhupt, K. 1985. Foraging ecology of sympatric parids: individual and populational responses to winter food scarcity. Ph.D. thesis, Purdue University.

Thyer, N. & Mahar, D. 2006. Discrimination of nonlinear frequency glides. *Journal of the Acoustical Society of America*, **119**, 2929–2936.

Viemeister, N. F. & Plack, C. J. 1993. Time analysis. In: *Human Psychophysics* (Ed. by W. A. Yost, A. N. Popper & R. R. Fay), pp. 116–154. New York: Springer-Verlag.

Wiley, R. H. & Richards, D. G. 1978. Physical constraints on acoustic communication in atmosphere: implications for evolution of animal vocalizations. *Behavioral Ecology and Sociobiology*, **3**, 69–94.

Witte, K., Farris, H. E., Ryan, M. J. & Wilczynski, W. 2005. How cricket frog females deal with a noisy world: habitat-related differences in auditory tuning. *Behavioral Ecology*, **16**, 571–579.

Woolley, S. M. N. & Casseday, J. H. 2004. Response properties of single neurons in the zebra finch auditory midbrain: response patterns, frequency coding, intensity coding, and spike latencies. *Journal of Neurophysiology*, **91**, 136–151.

Woolley, S. M. N. & Rubel, E. W. 1999. High-frequency auditory feedback is not required for adult song maintenance in Bengalese finches. *Journal of Neuroscience*, **19**, 358–371.

Woolley, S. M. N., Wissman, A. M. & Rubel, E. W. 2001. Hair cell regeneration and recovery of auditory thresholds following aminoglycoside ototoxicity in Bengalese finches. *Hearing Research*, **153**, 181–195.

Woolley, S. M. N., Gill, P. R., Fremouw, T. & Theunissen, F. E. 2009. Functional groups in the avian auditory system. *Journal of Neuroscience*, **29**, 2780–2793.