

Habitat-related differences in auditory processing of complex tones and vocal signal properties in four songbirds

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Abstract We examined temporal processing of harmonic tone complexes in two woodland species (tufted titmice and white-breasted nuthatches) and two open-habitat species (house sparrows and white-crowned sparrows). Envelope and fine-structure processing were quantified using the envelope following response (EFR) and frequency following response (FFR). We predicted stronger EFRs in the open-habitat species based on broader auditory filters and greater amplitude modulation of vocal signals in this group. We predicted stronger FFRs in woodland species based on narrower auditory filters. As predicted, EFR amplitude was generally greatest in the open habitat species. FFR amplitude, in contrast, was greatest in white-crowned sparrows with no clear difference between habitats. This result cannot be fully explained by species differences in audiogram shape and might instead reflect greater acoustic complexity of songs in the white-crowned sparrow. Finally, we observed stronger FFRs in woodland species when tones

were broadcast with the next higher harmonic in the complex. Thus, species such as nuthatches that have songs with strong harmonics may process these sounds using enhanced spectral processing instead of enhanced amplitude-envelope processing. The results suggest coevolution between signal design and temporal processing of complex signals and underscore the need to study auditory processing with a diversity of signals.

Keywords Hearing · Auditory evoked potential · Amplitude envelope · Phase-locking · Audiogram

Abbreviations

ABR Auditory brainstem response
AEP Auditory evoked potential
EFR Envelope following response
FFR Frequency following rate
AM Amplitude modulation

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Introduction

Auditory processing mechanisms are expected to evolve in parallel with vocal signals based on the sender–receiver matching hypothesis (Dooling et al. 2000; Gall et al. 2012a). Accordingly, studies on hearing sensitivity across a range of taxa reveal a close match between the frequency range present in conspecific signals and the frequency regions of highest sensitivity in the auditory periphery (e.g., Konishi 1970; Dooling 1982; Szymanski et al. 1999; Sisneros and Bass 2003; Ramsier et al. 2012). However, vocalizations often contain complex patterns of spectral and temporal acoustic structure (Nelson and Marler 1990). It remains unclear whether species differ in auditory processing of multiple dimensions of complex stimuli and

whether processing capabilities correlate with species-specific vocal structure.

Sounds comprising more than one frequency component have an inherent gross temporal structure resulting from the interaction between components (Bradbury and Vehrencamp 2011). The auditory system encodes this temporal structure through neural phase locking (i.e., synchronization of action potentials to the temporal structure of the signal; Viemeister and Plack 1993). For instance, the amplitude envelope of complex tones (i.e., sounds consisting of harmonically related frequency components) is modulated at the fundamental frequency of the complex tone (Moore 1993; Viemeister and Plack 1993). The extent to which the gross (i.e., envelope fluctuations) and fine (i.e., faster oscillations of the “carrier” signal) temporal structures of complex tones are processed through phase locking partly depends on properties of the peripheral auditory system. For example, the spectral analysis of sounds results in a tradeoff between frequency and temporal resolution that depends on auditory-filter bandwidth (Fletcher 1940; Moore 1993). Auditory filters with narrow bandwidths provide greater frequency resolution because sounds of similar frequencies are more likely to be processed by different channels. However, enhanced frequency selectivity requires signal integration over relatively long periods of time, compromising the ability of the auditory system to follow changes in amplitude. In contrast, auditory filters with broad bandwidths may provide reduced response to the temporal fine structure of tonal signals due to a decrease in resonance (i.e., diminished “ringing”; de Boer and Kruidenier 1990), but they also provide enhanced temporal resolution. Therefore, auditory systems with narrow auditory filters should excel in the resolution of temporal fine structure of complex stimuli while auditory systems with broad filters should show stronger responses to the amplitude envelope of complex stimuli.

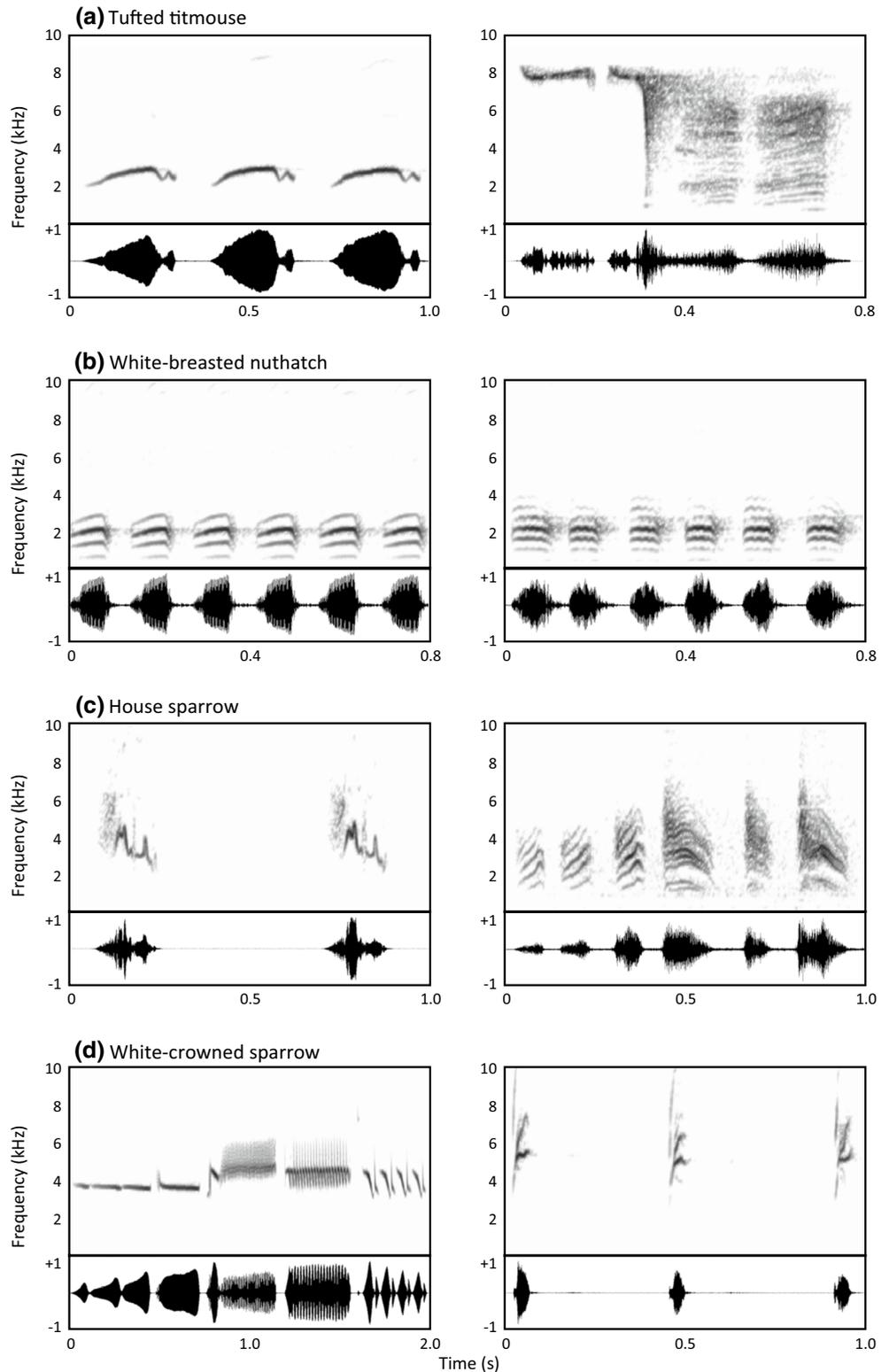
Auditory-filter bandwidth is correlated with habitat-related differences in the acoustic structure of vocalizations. In songbirds, house sparrows (*Passer domesticus*) and white-crowned sparrows (*Zonotrichia leucophrys*) have broader auditory filters than Eastern tufted titmice (*Baeolophus bicolor*) and white-breasted nuthatches (*Sitta carolinensis*; Henry and Lucas 2010). Titmice and nuthatches communicate in forested habitats where reverberations degrade temporal aspects of vocal signals. This degradation selects for tonal vocalizations with slow frequency and amplitude modulations—properties that are relatively unaffected by the effects of reverberation (Fig. 1; Morton 1975; Wiley 1991). House sparrows and white-crowned sparrows communicate in more open habitats, where fewer reverberations and slow amplitude modulations imposed by wind favor the evolution of high-frequency vocalizations with rapid amplitude and/or frequency modulations (Fig. 1;

Morton 1975; Wiley 1991). Species that communicate in open habitats may benefit from broader auditory filters to better process rapid amplitude fluctuations in the signals, while species that communicate in forested habitats may benefit from narrow filters to better process tonal signal components. This interpretation assumes that the peripheral auditory system of these species undergoes a tradeoff between temporal and spectral resolution. Indeed, Henry et al. (2011) showed that house sparrows have greater temporal resolution than white-breasted nuthatches. In addition, individual Carolina chickadees (*Poecile carolinensis*) with narrow filters had poorer temporal resolution than individuals with wider filters (Henry et al. 2011). Thus, species differences in auditory-filter bandwidth, and the inherent tradeoff between temporal and spectral resolution, could lead to differences in the way species process temporal and spectral properties of complex acoustic stimuli.

In the present study of two woodland species (tufted titmice and white-breasted nuthatches) and two species that occupy more open habitat (house sparrows and white-crowned sparrows), we first used acoustic analyses to test whether the vocalizations of the study species conform to the acoustic adaptation hypothesis (Morton 1975). We measured minimum, maximum, and peak frequencies of vocalizations, Wiener entropy, and the rates and strength of envelope fluctuation, all of which are predicted to be greater in the open habitat species. Next, we used auditory evoked potentials (AEPs) to quantify auditory processing of 2- or 3-tone complexes with a fundamental frequency of 600 Hz. AEPs are voltage changes, measured with surface electrodes on the scalp, resulting from hair cell (i.e., cochlear) or neural (i.e., auditory nerve, brainstem, and possibly midbrain) activity caused by acoustic input (Hall 2007). AEPs can be divided into transient and sustained responses. The most important transient response is the auditory brainstem response (ABR), which is an onset response consisting of a series of positive and negative peaks that represent neural activity at specific sites in the auditory system (Brown-Borg et al. 1987; Hall 2007). Sustained responses include phase-locking to the fine structure of sounds (called the frequency following response or FFR) and phase-locking to the rate of modulation of the amplitude envelope of sounds (called the envelope following response or EFR; Boston and Møller 1985; Hall 2007). The characterization of EFR and FFR in birds has been understudied, particularly in non-model species (see Lucas et al. 2007; Gall et al. 2012b). This is particularly true of auditory processing of sounds generated from harmonic stacks; these sounds are fairly common in the vocal repertoire of a number of species (e.g., mammals, Smith et al. 2002; birds, Henry and Lucas 2008; fish, McIver et al. 2014).

We asked whether habitat-based differences in vocal acoustic structure and associated differences in peripheral

Fig. 1 Spectrograms (*top panel*) and waveforms (*bottom panel*) of representative songs (*left*) and calls (*right*) of **a** tufted titmice, **b** white-breasted nuthatches, **c** house sparrows, and **d** white-crowned sparrows. Spectrograms were generated using Hanning windows of 512 points with 75 % window overlap (Sampling rate = 44.1 kHz). Recordings were normalized to 85 % of maximum amplitude and the waveforms plotted as the normalized amplitude (between 1 and -1)



auditory filtering across the study species could account for differential processing of complex tones. Given differences in the physical properties of the vocalizations and the trade-off between spectral and temporal resolution associated

with auditory filter bandwidth, we predicted stronger EFRs in open-habitat species (house sparrows and white-crowned sparrows) that have relatively broad auditory filters. We also predicted stronger FFRs to the frequency components

of the complex tones in woodland species (titmice and nuthatches) with narrower auditory filters. Because the strength of the response to different frequency components may depend on audiogram shape, we used ABRs to characterize sensitivity over a broad range of frequencies.

Materials and methods

Signal analysis: spectral and temporal properties of species-specific vocalizations

We analyzed 46 vocalizations of titmice (34 calls and 12 songs) from ten recordings, 31 of nuthatches (10 calls and 21 songs) from six recordings, 40 of house sparrows (29 calls and 11 songs) from seven recordings, and 24 of white-crowned sparrows (18 calls and 6 songs) from nine recordings. The recordings we analyzed were acquired from the Cornell Lab of Ornithology ($n = 23$), from Elliot et al. (1997; $n = 4$), and from recordings from our own field sites recorded with a Marantz PMD-660 digital recorder and a Sennheiser ME-66 microphone ($n = 5$). None of these recordings were from known individuals. Therefore, we treated all songs measured from the same recording as if they were from the same individual to ensure no pseudoreplication in our statistical analyses (see below). We characterized temporal and spectral features of digital song and call recordings using Sound Analysis Pro v.2011.104 (Tchernichovski et al. 2000) and custom-written PRAAT scripts (v4.6 Boersma and Weenink 2014). Recordings were transformed to mono, resampled at 44,100 Hz with 16-bit resolution in Adobe Audition v4.0.

Spectral properties

We measured minimum frequency, frequency of peak amplitude, maximum frequency, and spectral entropy of the vocalizations. To determine minimum, peak, and maximum frequencies, we first generated a long-term power spectrum of each vocal signal using Fast Fourier Transform in PRAAT (see Supplemental Material Fig S1 for an example). We then smoothed the frequency spectrum with a Loess regression using an AICC (Akaike information criterion with a correction for finite sample sizes) criterion for choosing the smoothing parameter (SAS Software 9.2, Proc Loess) and normalized to its maximum value. We obtained the frequency (in Hz) of peak amplitude as the frequency with highest amplitude in the smoothed spectrum. The minimum and maximum frequencies were calculated as the lowest and highest frequencies with amplitudes 25 dB below that of the peak frequency. Sound Analysis Pro was used to measure Wiener entropy, which is a measure of the width and uniformity of the power spectrum. Wiener

entropy is dimensionless and ranges from 0 for white noise to minus infinity for pure tones.

Temporal properties

To determine amplitude modulation (AM) rates, we first extracted the band-pass filtered (200–2,000 Hz) Hilbert envelope (see Smith et al. 2002) of each element of the vocal signals using a custom-written PRAAT script (see Supplemental Material Fig S2 for an example). We then generated a power spectrum of the envelope of each element and measured the frequency and amplitude of the dominant peak. We used the amplitude of the dominant peak as an estimate of AM strength. The amplitude of the dominant peak was measured relative to an index of the noise floor. The noise floor index was generated by estimating the intensities of the power spectrum in 20-Hz bins over a range from 200 to 2,000 Hz and then taking the upper 90th percentile of the distribution of intensity values. We chose the 90 % criterion (as opposed to 50 %) to ensure a conservative designation of the dominant AM peak.

We used univariate repeated-measures ANOVAs (Proc MIXED in SAS) to investigate the influence of habitat, species within each habitat, and type of vocalization (song or call) on signal properties. The Kenward–Roger method was used to calculate denominator degrees of freedom and we used a compound symmetric variance–covariance matrix. We first included all interaction terms between independent variables; non-significant terms were excluded in order of decreasing F value. Normality of residuals and homogeneity of variances were tested using Proc UNIVARIATE (SAS); all analyses met the assumptions for parametric testing. We used LSMEANS (Proc MIXED) to estimate Least Squares Means (lsmeans) and post hoc tests for pairwise comparisons (LSMEANS/diff).

Study sites and subjects for auditory experiments

The experimental design was approved by the Purdue University Institutional Animal Care and Use Committee (IACUC no. 05-058). The study was conducted between July and February; sampling was restricted to the non-breeding season to avoid confounding effects of seasonal changes in AEPs (Lucas et al. 2002, 2007). We caught birds in the morning with seed-baited treadle traps at the Purdue Wildlife Area (40°26′30″N, 87°03′30″W), the Ross Biological Reserve (40°24′30″N, 87°04′30″W) and at two private residences in Lafayette, IN. Birds were brought to an indoor aviary at Purdue University, housed individually in 1 m³ stainless steel-mesh cages, and provided with ad lib water, seed, mealworms, and grit. We tested 10 tufted titmice (5 males, 5 females), 8 white-breasted nuthatches (7 males, 1 female), 14 house sparrows (9 males,

5 females), and 8 white-crowned sparrows (all males). Sex was determined using plumage patterns in nuthatches and house sparrows, and wing chord in titmice [males ≥ 80 mm (Lucas et al. 1993)]. We took a tissue sample from an outer rectrix of the white-crowned sparrows and used the protocol of Griffiths et al. (1998) for genetic sexing (also see Vélez et al. 2015). Only adults were tested. Juvenile status was determined using outer rectrix shape in titmice, plumage color in house sparrows and white-crowned sparrows, and mouth color in house sparrows, titmice, and nuthatches (Pyle 1997). Average (\pm SD) body mass at the time of capture was 21.0 (\pm 1.1) g in titmice females, 21.6 (\pm 0.7) g in titmice males, 19.6 g in nuthatch females, 20.9 (\pm 1.5) g in nuthatch males, 25.3 (\pm 2.6) g in house sparrow females, 27.6 (\pm 2.6) g in house sparrow males, and 28.4 (\pm 3.9) g in white-crowned sparrows. Auditory tests were conducted within a day of capture and subjects were released at their capture location within 2 days after testing.

Auditory test equipment and procedure

Auditory tests were conducted inside a $1.2 \times 1.4 \times 1.2$ m box lined with 7.2-cm thick Sonex foam (Acoustic Solutions; Richmond, VA, USA). Subjects were weighed and anesthetized with 50–60 mg ketamine/kg and 10–12 mg xylazine/kg into the breast muscle. About 30 min into testing, subjects were given one or two supplemental injections of ketamine (15–20 mg/kg) and xylazine (2–3 mg/kg) to complete the entire set of tests (approximately 80 min). Subjects were placed on a pre-warmed heating pad (52 °C ‘Snuggle-Safe’ pad) covered with towels. The temperature between the subject’s body and the heating pad was maintained at 38 ± 2 °C by adding or removing layers of towel; internal body temperature was not measured directly. Subjects were positioned centrally in the chamber with the lights off and their right ear facing upwards. Subdermal needle electrodes (Nicolet Biomedical, Fitchburg, WI) were inserted just under the skin. The positive electrode was placed at the crown directly above and midway between the eyes. The negative electrode was placed directly posterior to the right auditory meatus and the ground electrode at the nape of the neck.

Stimulus presentation, AEP acquisition, and data storage were coordinated by a modular rack-mount Tucker Davis Technologies System II (TDT; Gainesville, FL) and a Dell PC running TDT SigGen32/BioSig32 software. Digital stimuli passed through a TDT-DA1 digital-to-analogue converter and Crown-D75 power amplifier through an electromagnetically shielded overhead speaker (RCA model 40–5,000; 140–20,000 Hz frequency response) suspended 30 cm above the subject. We calibrated stimuli within ± 1 dB SPL (sound pressure level; re. 20 μ Pa) using a Brüel and Kjær model 1613 Precision Sound-Level Meter and model 4131 2.6 cm condenser microphone placed at the

approximate position of a subject’s ear. Complex tones (“Experiment 1”) were presented at a rate of 13.1 stimuli/sec; audiogram stimuli (“Experiment 2”) were broadcast at 31.1 stimuli/sec. AEPs were fed into a TDT-HS4 headstage and amplified with a TDT-DB4 biological amplifier before passing through an AD1 analogue-to-digital converter to the computer for storage. We checked electrode placement and integrity by measuring inter-electrode impedance; tests were run only when impedance was < 7 kOhms. AEPs were sampled at 40 kHz with a response amplification of 200 k, high-pass filtered at 100 Hz, and low-pass filtered at 10 kHz with a 60-Hz notch filter. AEP waveforms were based on averages of 500 stimulus presentations with two replicates for each stimulus.

We began and ended each auditory test with a standard 90 dB SPL click to ensure the bird’s auditory system did not change over the course of the test. The stereotypical response to click standards (Lucas et al. 2002) makes them useful for identifying birds with abnormal auditory systems and as an additional check for electrode placement. We rejected data when the ABR amplitude (see Lucas et al. 2002) measured at the end of our experiment dropped below 95 % of the ABR amplitude measured at the beginning of the experiment. One house sparrow with deficient hearing was identified using click standards and was excluded from the data set.

Experiment 1: AEPs to complex tones

Acoustic stimuli

We created four complex tones in PRAAT using the “create sound from formula” option. Complex tones were 30-ms long with 3 ms \cos^2 rise/fall times and consisted of either three phase-locked sinusoids with frequencies of 1.2 + 1.8 + 2.4 kHz, or two phase-locked sinusoids with frequencies of 1.2 + 1.8 kHz, 1.2 + 2.4 kHz, or 1.8 + 2.4 kHz. Figure 2a presents a spectrogram and waveform view of the stimuli. These three tones are particularly common in calls and song of white-breasted nuthatches (Henry and Lucas 2008), although the 600-Hz AM rate is found in the calls of all of the species tested in this study (see “Results”). Stimuli were presented at 80 dB SPL in all tests. We chose this level because previous results on clicks and pure tones indicated that AEPs to 80 dB stimuli are robust, not overly contaminated by a cochlear microphonic signal, and species relationships are generally unaffected when lower level stimuli are used (Lucas et al. 2002, 2007). Nonetheless, we acknowledge that species-specific differences in sensation level may exist with the stimuli used in our study, so the results should be viewed within the limited scope of a fixed sound level. Note: Experiment 2 includes an explicit analysis of frequency-specific sensitivity levels.

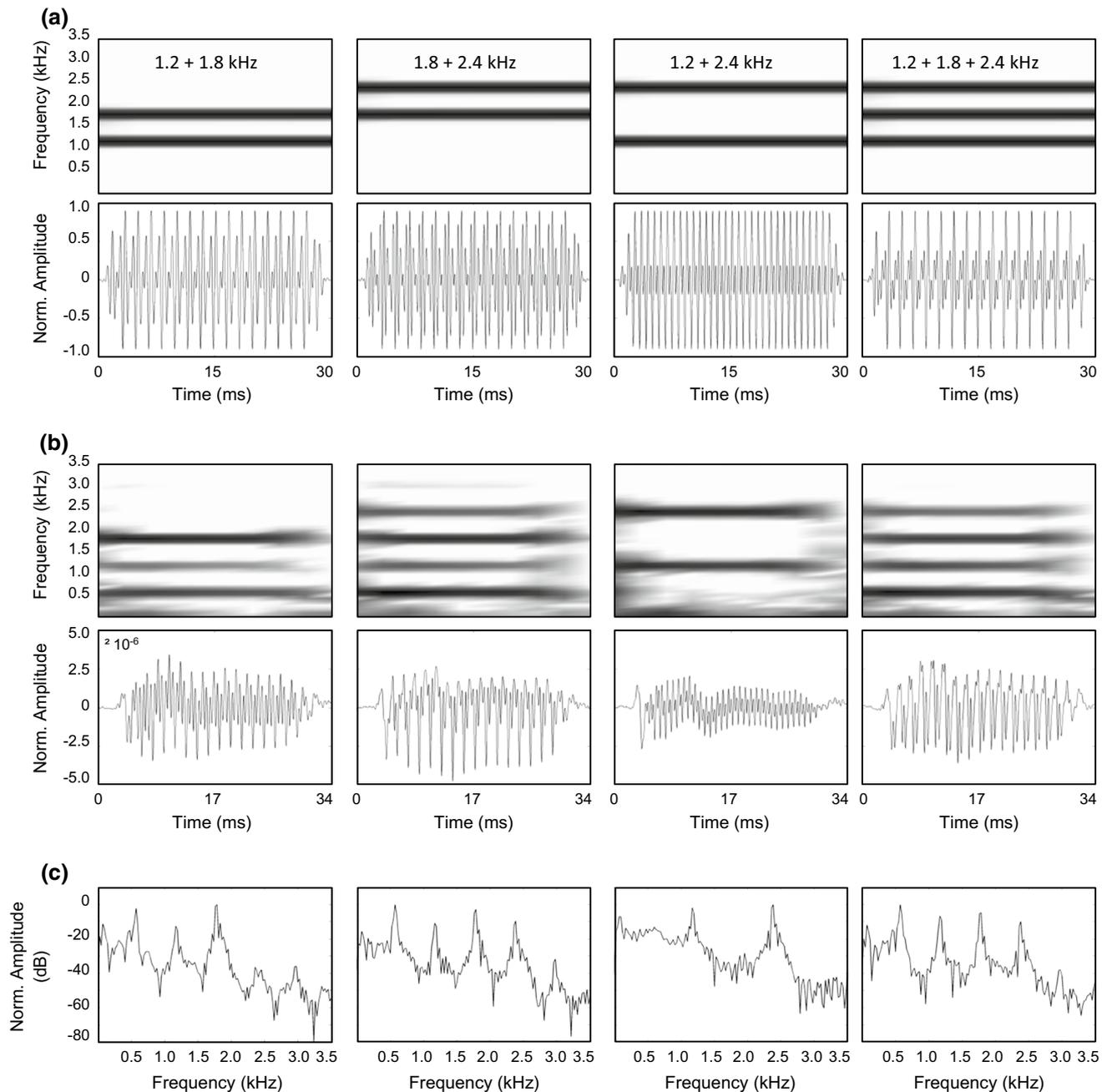


Fig. 2 Spectrograms (*top panel*) and waveforms (*bottom panel*) of **a** the acoustic stimuli and **b** the average AEP of white-crowned sparrows for complex tones, and **c** normalized long-term frequency spectrum of the corresponding average AEP. The *columns* represent tones with frequencies of 1.2 + 1.8 kHz (*far left*), 1.8 + 2.4 kHz (*middle*

left), 1.2 + 2.4 kHz (*middle right*), and 1.2 + 1.8 + 2.4 kHz (*far right*). Spectrograms and frequency spectra were generated with 512-point Hanning windows with 75 % overlap in Matlab R2012a. See Electronic Supplementary Material 1 for average AEPs for tufted titmice, white-breasted nuthatches, and house sparrows

AEP analysis

We exported AEP waveforms from BioSig RP (version 4.4.1, Tucker Davis Technologies) as text files and analyzed them using PRAAT. Figure 2b presents a spectrogram and waveform view of the average AEP for each

stimulus recorded in white-crowned sparrows (see Supplemental Material Fig S3-S5 for data from the other species in our study). FFR amplitude was measured by calculating the frequency spectrum of the AEP with a Fast Fourier Transform (sampling rate = 40 kHz; FFT size = 2,048 points; frequency resolution = 19.53 Hz; see Fig. 2c). We

measured the amplitude of the spectrum (in dBnV) at each frequency in the stimulus. The 600-Hz separation between the frequency components of the stimuli (except for the 1.2 + 2.4 kHz complex tone) generates a 600-Hz AM signal (Viemeister and Plack 1993). Because the auditory system will also phase-lock to amplitude modulations in the stimulus (Simmons and Buxbaum 1996; Henry 1997; Gall et al. 2012b), we also measured the amplitude of the EFR at 600 Hz in the spectrum for all of the stimuli except the 1.2 + 2.4 kHz complex tone. All FFR and EFR amplitudes are absolute measures derived from spectra, not amplitudes relative to the noise floor.

We first explored the amplitude of the FFR to each frequency in the stimulus with repeated measures MANOVAs (Proc GLM in SAS) to test for habitat, species within habitat, sex, and stimulus effects because the dependent variables were multivariate. Then, we used univariate repeated-measures ANOVAs (Proc MIXED in SAS) to identify specific patterns for each frequency component of the complex tones. We specified the Kenward–Roger method to calculate denominator degrees of freedom and a compound symmetric variance–covariance matrix. Interaction terms between independent variables were included in the models, and non-significant terms were deleted in order of decreasing F value. We used ‘LSMEANS’ within Proc MIXED to estimate lsmeans and post hoc tests for pairwise comparisons (LSMEANS/diff). Proc UNIVARIATE confirmed that the assumptions for parametric testing were met.

Experiment 2: auditory sensitivity as a function of frequency

We reanalyzed data from audiograms for nuthatches, house sparrows, and titmice (Henry and Lucas 2008). All audiograms were generated using ABRs to measure auditory sensitivity to tones of different frequencies. Here, we measured ABR intensities for white-crowned sparrows following the same experimental protocol on the same equipment used by Henry and Lucas (2008).

Acoustic stimuli

We used 5-ms tone bursts with 1 ms \cos^2 rise/fall times and frequencies of 0.8, 1.4, 2.2, 3.2, 4.2, and 6.4 kHz. Stimuli were presented with alternating phase values of 0.5π and 1.5π radians, and frequencies were presented in random order. For each frequency, we recorded ABRs at eight levels, starting at 80 dB SPL down to 24 dB SPL in 8 dB steps.

AEP analysis

We averaged the two replicates of each ABR at each frequency and intensity; thus ABRs were constructed from

the average response to 1,000 stimulus repetitions. We used visual detection (e.g., Brittan-Powell et al. 2002; Brittan-Powell and Dooling 2004) to determine ABR thresholds for each species. Henry and Lucas (2008) used a cross-correlation method to generate the audiogram. However, the visual detection method is preferable because it is more strongly correlated with behavior-derived audiograms (Gall et al. 2011). For each frequency, we plotted the average ABRs in order of descending stimulus level and operationally defined the ABR threshold as the mean of the lowest stimulus level at which an ABR waveform was visible and the highest level at which it was not. Stimulus level was varied in 8-dB steps. Therefore, ABR threshold was taken as a sound intensity 4 dB (one-half step) below the lowest stimulus level at which a response could be visually detected.

We investigated the effect of habitat, species within each habitat, sex, and frequency on ABR thresholds using repeated-measures ANOVA (Proc MIXED in SAS). We used the Kenward–Roger method to calculate denominator degrees of freedom and a compound symmetric variance–covariance matrix. All interaction terms were included in the models and non-significant terms were deleted in order of decreasing F value. We used the command LSMEANS to estimate lsmeans and post hoc tests for pairwise comparisons (LSMEANS/diff). We used Proc UNIVARIATE to test for normality of residuals and homogeneity of variances.

Results

Signal analyses: spectral and temporal properties of species-specific vocalizations

Spectral properties

We found similar patterns across the three tone properties measured for each song and call (minimum frequency, frequency of peak amplitude, and maximum frequency; see “Materials and methods” for definitions) (Fig. 3a–c). Open-habitat species have higher frequency song than woodland species (habitat effect: minimum frequency, $F_{1,45} = 4.5$, $P < 0.0001$; peak frequency, $F_{1,45} = 3.1$, $P < 0.0001$; maximum frequency, $F_{1,45} = 11.4$, $P < 0.0001$). The pattern is quite different for call elements (Fig. 3a–c), as indicated by significant vocalization-type \times habitat interactions for peak frequency and maximum frequency (minimum frequency, $F_{1,98} = 2.2$, $P = 0.14$; peak frequency, $F_{1,98} = 18.5$, $P < 0.0001$; maximum frequency, $F_{1,98} = 8.9$, $P = 0.004$) and vocalization-type \times species within habitat interactions (minimum frequency, $F_{2,98} = 21.5$, $P < 0.0001$; peak frequency, $F_{2,98} = 61.9$, $P < 0.0001$; maximum frequency, $F_{2,98} = 40.0$, $P < 0.0001$). In general, titmice and

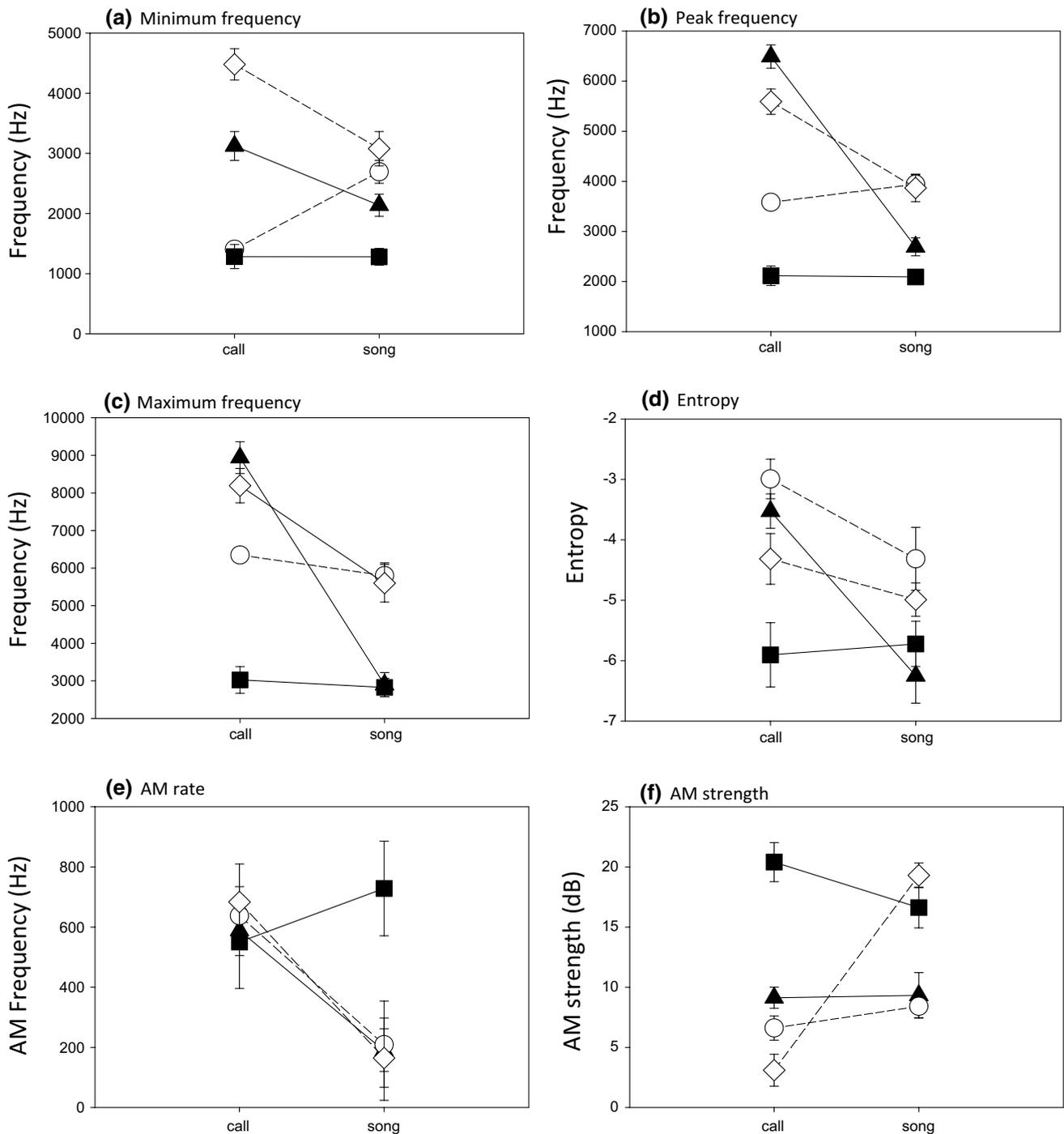


Fig. 3 Acoustic properties of calls and songs of titmice (*triangles*), nuthatches (*squares*), house sparrows (*circles*), and white-crowned sparrows (*diamonds*). LSmean (\pm SE) **a** minimum frequency, **b** peak

frequency, **c** maximum frequency, **d** entropy, **e** peak amplitude modulation rate, and **f** strength of peak amplitude modulation rate. Woodland species: *closed symbols* and *continuous lines*; open-habitat species: *open symbols* and *dashed lines*

white-crowned sparrows had higher frequency calls than house sparrows and nuthatches (all *t* tests for 2-way comparisons: $P < 0.001$). Nuthatches had the lowest minimum, peak, and maximum frequencies of the four species (*t* test for 2-way comparisons: house sparrow vs. nuthatch call

minimum frequency, $P = 0.7$; all other comparisons with nuthatches, $P < 0.01$).

Wiener entropy varied between habitats ($F_{1,26.5} = 18.4$, $P = 0.0002$), types of vocalizations ($F_{1,26.5} = 16.7$, $P = 0.0004$), and species within habitats ($F_{2,26.5} = 6.0$,

$P = 0.007$; Fig. 3d). Entropy was generally higher in the vocalizations of open-habitat species compared to woodland species ($t_{26.5} = 18.4, P = 0.0002$) and in calls compared to songs ($t_{26.5} = 16.7, P = 0.0004$). Within habitats, entropy of vocalizations was higher in titmice than in nuthatches ($t_{27.6} = 2.3, P = 0.030$) and in house sparrows compared to white-crowned sparrows ($t_{25.4} = 5.0, P < 0.0001$). We also found a significant vocalization-type \times species within habitat interaction ($F_{3,26.7} = 5.0, P = 0.007$). There was little variation in entropy between songs and calls of nuthatches ($t_{26.2} = 0.3, P = 0.78$) and white-crowned sparrows ($t_{26.9} = 1.4, P = 0.18$). In contrast, entropy was higher in calls than in songs in titmice ($t_{29.8} = 5.3, P < 0.0001$) and house sparrows ($t_{24.5} = 2.2, P = 0.035$).

Temporal properties

All of the peak amplitude modulation rates were about 200 or 600 Hz (Fig. 3e), and AM rate was generally higher in calls than in songs (ANOVA: $F_{1,27.5} = 16.8, P = 0.0003$; $t_{27.5} = 4.1, P = 0.0003$). We found no significant main effect of habitat on AM rate ($F_{1,27.6} = 1.3, P = 0.27$) nor an effect of species within habitat ($F_{2,27.1} = 1.3, P = 0.28$).

AM strength differed between types of vocalization ($F_{1,28.3} = 14.3, P = 0.0007$), habitats ($F_{1,28.3} = 22.4, P < 0.0001$), and across species within habitat ($F_{2,26.7} = 22.3, P < 0.0001$; Fig. 3f). Within habitat, AM strength is higher in nuthatches than in titmice ($t_{31.1} = 5.9, P < 0.0001$) and in white-crowned sparrows than in house sparrows ($t_{31.1} = 3.4, P = 0.0023$). On average, AM strength is higher in songs than in calls ($t_{28.3} = 3.8, P = 0.0007$), a result due to the strong amplitude fluctuations in the song of nuthatches and the trill and buzz elements of the song of white-crowned sparrows. Contrary to predictions from the acoustic adaptation hypothesis, AM strength was higher in vocalizations of woodland species ($t_{28.3} = 4.7, P < 0.0001$) due to the strong amplitude fluctuations in songs and calls of nuthatches. We also found significant vocalization-type \times habitat ($F_{1,28.3} = 32.0,$

$P < 0.0001$) and vocalization-type \times species within habitat ($F_{2,26.7} = 23.0, P < 0.0001$) interactions. While AM strength varies little between calls and songs in woodland species ($t_{31.1} = 1.2, P = 0.27$), AM strength is higher in songs than in calls in open-habitat species ($t_{23.3} = 8.3, P < 0.0001$). In woodland species, nuthatches have stronger amplitude modulations than titmice in calls ($t_{24.8} = 6.1, P < 0.0001$) and songs ($t_{35.4} = -2.9, P = 0.0068$). In open-habitat species, AM strength of calls is higher in house sparrows than in white-crowned sparrows ($t_{22.7} = 2.1, P = 0.046$), but AM strength of songs is higher in white-crowned sparrows than in house sparrows ($t_{24.3} = 7.9, P < 0.0001$).

Experiment 1: AEPs to complex tones

We measured the EFR amplitude to the 600-Hz amplitude modulations and the FFR amplitude to the frequency components of complex tones with frequencies of 1.2 + 1.8 + 2.4 kHz, 1.2 + 1.8 kHz, 1.2 + 2.4 kHz, and 1.8 + 2.4 kHz. We found significant effects of habitat on EFR and FFR amplitudes (repeated measures MANOVA: Wilk’s Lambda = 0.45, $F_{9,27} = 3.7, P = 0.0042$) and significant differences between species within habitat (Wilk’s Lambda = 0.12, $F_{18,54} = 5.7, P < 0.0001$), but not between sexes (Wilk’s Lambda = 0.60, $F_{9,27} = 2.0, P = 0.082$). These results were supported by univariate repeated-measures ANOVAs (Table 1). We now describe EFR amplitude in response to the 600-Hz AM rate and FFR amplitude to each frequency in the complex tones.

EFR amplitude to the 600-Hz amplitude modulation rate

As predicted, the amplitude of the 600-Hz EFR was higher in open-habitat species than in woodland species (Fig. 4a; $F_{1,35} = 6.2, P = 0.018$). EFR amplitude also differed across stimuli ($F_{2,72} = 17.6, P < 0.0001$) and was generally lower in the 1.2 + 1.8 kHz complex than in the other two complex tones (all $t_{35} > 4.1, all P < 0.001$). Within habitats, a significant species \times stimulus interaction ($F_{4,72} = 5.1,$

Table 1 Results of univariate repeated measures ANOVAs for EFR amplitude at 600 Hz envelope fluctuations and FFR amplitude at each frequency component of the complex tones

Frequency	Species		Habitat		Stimulus		Sex		Species \times stimulus		Habitat \times stimulus	
	$F_{2,35}$	P	$F_{1,35}$	P	$F_{2,72}$	P	$F_{1,35}$	P	$F_{4,72}$	P	$F_{2,72}$	P
EFR, 600 Hz	0.81	0.4545	6.2	0.0177	17.62	<0.0001	0.32	0.5724	5.11	0.0011	N.S.	N.S.
FFR, 1.2 kHz	0.9	0.4167	1.05	0.3132	9.26	0.0003	1.08	0.3066	2.75	0.0345	5.78	0.0047
FFR, 1.8 kHz	5.57	0.008	3.97	0.0542	7.29	0.0013	0.38	0.5391	5.18	0.001	14.82	<0.0001
FFR, 2.4 kHz	6.1	0.0054	6.98	0.0122	17.94	<0.0001	0.01	0.9271	N.S	N.S	N.S.	N.S.

Values in italics highlight significant results. Non-significant interaction terms (N.S.) were removed from the model in order of decreasing F value (see text for details)

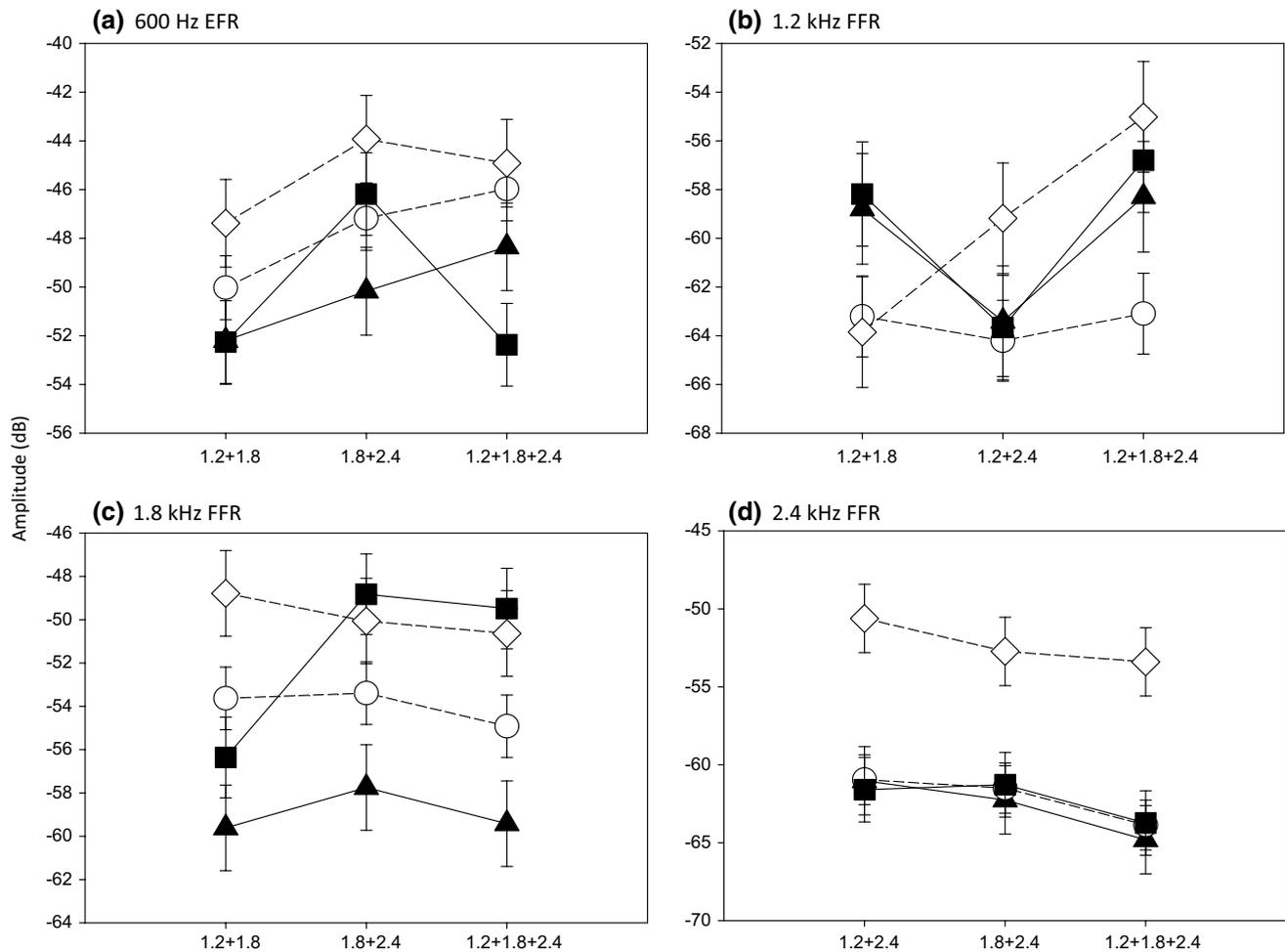


Fig. 4 Amplitude (lsmeans \pm SE) of **a** the EFR to the 600-Hz envelope fluctuations, and **b** the FFR to the frequency components at 1.2 kHz, **c** 1.8 kHz, and **d** 2.4 kHz as a function of input stimulus. *Triangles* titmice, *Squares* nuthatches, *Circles* house sparrows, and

Diamonds white-crowned sparrows. Woodland species: *closed symbols* and *continuous lines*; open-habitat species: *open symbols* and *dashed lines*

$P = 0.0011$) was mainly driven by differences between woodland species; EFR amplitude was higher for the 1.2 + 1.8 + 2.4 kHz complex tone in titmice ($t_{72} = 2.85$, $P = 0.0057$), but highest in the 1.8 + 2.4 kHz complex tone in nuthatches ($t_{72} > 4.5$, $P < 0.01$ in both cases).

FFR amplitude to the 1.2-kHz component of the complex tones

For both woodland species, the 1.2-kHz FFR amplitude was larger by about 5.5 dB when a 1.8-kHz tone was present in the complex tone compared to when the 1.8-kHz tone was absent (Fig. 4b; differences in lsmeans \pm SE: 5.0 ± 1.5 dB for 1.2 + 1.8 vs. 1.2 + 2.4 kHz tones; 6.0 ± 1.5 dB for 1.2 + 2.4 vs. 1.2 + 1.8 + 2.4 kHz tones; 0.9 ± 1.5 dB for 1.2 + 1.8 vs. 1.2 + 1.8 + 2.4 kHz tones).

In contrast, the 1.2-kHz FFR for the house sparrow was weak for all three complex tones and strong for the white-crowned sparrow when coupled with a 2.4-kHz tone element, but weak without that element (Fig. 4b). The result of these patterns is a significant stimulus \times habitat interaction ($F_{2,72} = 2.8$, $P = 0.0047$), and within habitats, a significant species \times stimulus interaction ($F_{4,72} = 2.8$, $P = 0.035$). We tested these patterns further with habitat- or species-specific ANOVAs. The woodland species tested alone showed a significant stimulus effect ($F_{2,30} = 10.7$, $P = 0.0003$) but no significant species ($F_{1,14} = 0.0$, $P = 0.96$), or species \times stimulus effect ($F_{2,30} = 0.2$, $P = 0.82$). An ANOVA run with only house sparrow data indicated no significant stimulus effect ($F_{2,28} = 0.2$, $P = 0.83$), and an ANOVA run with only white-crowned sparrow data indicated a significant stimulus effect ($F_{2,14} = 32.6$, $P = 0.0001$).

FFR amplitude to the 1.8-kHz component of the complex tones

There were two general patterns for the FFR in response to the 1.8-kHz tone (Fig. 4c). As with the 1.2-kHz tone, 1.8 kHz FFR amplitude in nuthatches was about 7 dB stronger when the next highest harmonic (2.4 kHz) was present compared to a tone complex that did not include this higher harmonic (differences in l smeans \pm SE: 7.5 ± 1.1 dB for 1.2 + 1.8 vs. 1.8 + 2.4 comparison; 6.9 ± 1.1 dB for 1.2 + 1.8 vs. 1.2 + 1.8 + 2.4 comparison; 0.7 ± 1.1 dB for 1.8 + 2.4 vs. 1.2 + 1.8 + 2.4 comparison). In contrast, there was no significant change in FFR amplitude for the 1.8-kHz tone over the three different complex tones for any of the other three species. These patterns resulted in a significant stimulus \times habitat interaction ($F_{2,72} = 14.8$, $P < 0.0001$) and within habitats, a significant species \times stimulus interaction ($F_{4,72} = 5.2$, $P = 0.001$). When data from each species were tested separately, nuthatches showed a significant effect of stimulus type on 1.8 kHz FFR amplitude ($F_{2,16} = 40.3$, $P = 0.0001$), but no other species showed a significant effect of stimulus type (titmice: $F_{2,14} = 0.7$, $P = 0.49$; house sparrow: $F_{2,28} = 2.11$, $P = 0.14$; white-crowned sparrow: $F_{2,14} = 2.0$, $P = 0.17$). In addition, testing all but nuthatches, there was a significant species effect on the 1.8-kHz FFR amplitude ($F_{2,28} = 5.1$, $P = 0.013$), but no significant stimulus ($F_{2,56} = 2.0$, $P = 0.14$) or species \times stimulus effect ($F_{4,56} = 0.9$, $P = 0.49$). Multiple comparisons indicated that the 1.8-kHz FFR amplitude of titmice was overall significantly less than that of the white-crowned sparrows ($t_{28} = 3.2$, $P = 0.004$), but house sparrows were not significantly different from titmice ($t_{28} = 2.0$, $P = 0.06$) or from white-crowned sparrows ($t_{28} = 1.7$, $P = 0.11$).

FFR amplitude to the 2.4-kHz component of the complex tones

Overall, the FFR amplitude for the 2.4-kHz tone was about 10 dB higher in white-crowned sparrows than in the other three species (Fig. 4d; differences in l smeans \pm SE: 9.8 ± 2.8 dB for house sparrow; 9.9 ± 2.9 dB for nuthatch; 10.3 ± 3.2 dB for titmice). This resulted in a significant main effect of species on the 2.4-kHz FFR amplitude ($F_{3,36} = 6.1$, $P = 0.0018$), with white-crowned sparrow amplitudes significantly different from the other three species ($t_{36} > 3.5$; $P < 0.0015$ in all cases) and the other three not significantly different from one another ($t_{36} < 0.23$; $P > 0.82$ in all cases). In addition, there was a significant effect of stimulus on 2.4 kHz FFR amplitude although the effect size was < 3 dB in all cases ($F_{2,72} = 17.9$, $P = 0.0001$; differences in l smeans \pm SE: 0.9 ± 0.5 dB for 1.2 + 2.4 vs 1.8 + 2.4 comparison; 2.9 ± 0.5 dB for 1.2 + 2.4 vs

1.2 + 1.8 + 2.4 comparison; 2.0 ± 0.5 dB for 1.8 + 2.4 vs 1.2 + 1.8 + 2.4 comparison). The 2.4-kHz FFR amplitude was significantly lower with the 1.2 + 1.8 + 2.4 kHz tone complex than with the other two complex tones ($t_{72} > 4.1$; $P < 0.0001$ in both cases) and not significantly different between the other two stimuli ($t_{72} = 1.8$; $P < 0.08$). The pattern was similar across species, as indicated by a non-significant species \times stimulus-type interaction ($F_{6,72} = 0.7$, $P = 0.65$).

Experiment 2: auditory sensitivity as a function of frequency

As described above, white-crowned sparrows had strong FFR amplitudes compared to the woodland species, contrary to predictions. This could result from particularly sensitive sound processing in white-crowned sparrows over the range from 1.2 to 2.4 kHz. We test this possibility here. Note that the visual detection method for measuring auditory sensitivity used here (see “Materials and methods”) yielded the same pattern as the cross-correlation method for threshold estimation used previously for nuthatches, titmice, and house sparrows (see Brittan-Powell et al. 2002; Henry and Lucas 2008).

The audiogram of the white-crowned sparrow is not consistent with the prediction that their strong FFRs to our complex tones result from high sensitivity to those tones. Instead, the audiogram shapes were consistent with the relationship between sensitivity and the frequency properties of calls and songs: white-crowned sparrows had an audiogram with enhanced sensitivity shifted to higher frequencies compared to the other three species (Fig. 5). An ANOVA revealed a significant effect on ABR thresholds of frequency ($F_{5,270} = 130.9$, $P < 0.001$), a frequency \times species interaction ($F_{15,270} = 21.7$, $P < 0.001$), and a frequency \times habitat interaction ($F_{5,270} = 18.0$, $P < 0.001$). Compared to the other species, white-crowned sparrows have significantly lower (i.e., ‘better’) ABR thresholds at 4.2 and 6.4 kHz (all pairwise comparisons: $t > 2.6$, $df = 186-192$, $P < 0.01$). However, white-crowned sparrows have significantly higher (i.e., ‘worse’) ABR thresholds than each of the other species at 0.8, 1.4, and 2.2 kHz (all pairwise comparisons: $t > 2.7$, $df = 186-192$, $P < 0.02$). ABR thresholds for titmice, nuthatches, and house sparrows are not significantly different from one another at 0.8, 1.4, and 2.2 kHz (all pairwise comparisons: $t < 1.5$, $df = 195-197$, $P > 0.13$), and titmice ABR thresholds are significantly lower than both house sparrows and nuthatches at 6.4 kHz (titmice vs house sparrows: $t_{197} = 4.8$, $P < 0.0001$; titmice vs nuthatches: $t_{195} = 4.1$, $P < 0.0001$). We found no main effects of species within habitats ($F_{2,53} = 0.9$, $P = 0.40$), habitat ($F_{1,53} = 0.4$, $P = 0.52$), or sex ($F_{1,53} = 0.5$, $P = 0.50$).

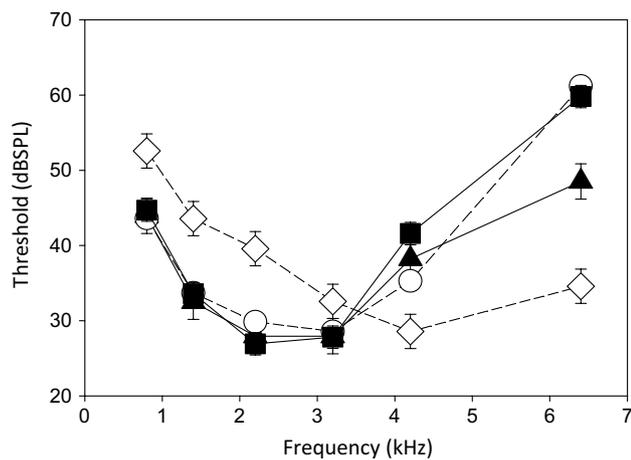


Fig. 5 Auditory thresholds (LS means \pm SE) as a function of stimulus frequency for titmice (*triangles*), nuthatches (*squares*), house sparrows (*circles*), and white-crowned sparrows (*diamonds*). Thresholds were estimated using the visual detection method (see “[Materials and methods](#)”). Woodland species: *closed symbols and continuous lines*; open-habitat species: *open symbols and dashed lines*

Discussion

Our results address three issues related to vocal communication: (1) habitat-specific properties of both song and call signals used by two woodland-adapted and two more open-adapted bird species, (2) how the auditory system processes both the frequency components and the amplitude envelope of harmonic tone complexes, and (3) whether auditory sensitivity across a range of frequencies can help us understand auditory processing of harmonic complexes. (1) With respect to song and call properties, our results provide partial confirmation of the acoustic adaptation hypothesis that open-habitat species should use songs with relatively high entropy levels and relatively high peak and maximal frequencies (Wiley 1991; see Wilkins et al. 2013). (Note: The Wiener entropy gives us an index of the relative tonality of a signal, with low values resulting from pure tones and high values resulting from ‘noisy’ or chaotic signals). However, assuming that a strong AM component is expected in songs of open-habitat species, AM song components do not fit this pattern in our set of species, in part because nuthatches (a woodland species) use strong harmonics in all of their vocalizations (Ritchison 1983) which in turn generate a strong AM signal. In contrast, call properties are not strongly correlated with habitat, but the use of calls in deciduous forests after leaf fall invalidates the assumptions of the acoustic adaptation hypothesis relative to sound propagation. Moreover, calls in these species may be used over shorter distances than songs, invalidating the assumption of the acoustic adaptation hypothesis

that signal distortion over long distances selects for vocal signals that are minimally degraded over distance. Interestingly, all species in our study had calls with peak AM rates at about 600 Hz. AM rates of about 600 Hz generate maximal EFRs in a number of songbird species (Henry and Lucas 2008; Gall et al. 2012b).

(2) Auditory processing of the frequency components and amplitude envelope were measured using the FFR and EFR, respectively. We expected FFR amplitudes to follow habitat use for three reasons. First, assuming that song properties are critical drivers of auditory physiology (Konishi 1970; Dooling 1982; Gall et al. 2012a), the woodland species were expected to have higher FFR amplitudes because their minimum and peak frequencies are in the range of frequencies used in our complex tones. In contrast, our open-habitat species had songs with minimum and peak frequencies out of the range of our complex tones. Note that the cause and effect relationship between song properties and auditory physiology could be reversed, with auditory physiology essentially dictating the evolution of song properties. Either interpretation would generate our predictions. In addition, both vocal signals (Catchpole and Slater 1995) and the auditory systems (e.g., Lucas et al. 2002; Gall et al. 2012a, b) are plastic enough for the arrow of causation to point in either direction. Second, the woodland species had more tonal songs (as indexed by lower entropy levels) which may select for stronger FFR amplitudes. Third, the open-habitat species have broader auditory filters than the woodland species (Henry and Lucas 2008, 2010). Broad filters should allow for enhanced temporal resolution and, therefore, AM processing, but decreased processing of temporal fine structure (Viemeister and Plack 1993; Henry et al. 2011).

Our results indicated that AM processing is generally stronger in open-habitat species, as predicted based on auditory-filter properties. In contrast, FFR amplitudes generally failed to match the predicted patterns. White-crowned sparrows (an open-habitat species) have the strongest FFR at all frequencies for almost all combinations of complex tones. Titmice (a woodland species) had amongst the weakest FFR amplitudes at both 1.8 and 2.4 kHz for each of the tone complexes. In addition, there was evidence of enhancement of FFR amplitudes in titmice for 1.2 kHz tones when these tones were broadcast with 1.8 kHz tones. This enhancement was seen with nuthatches for both 1.2 and 1.8 kHz tones broadcast with the next highest harmonic (1.8 and 2.4 kHz, respectively).

(3) A potential mechanism that could explain strong FFRs in white-crowned sparrows would be high sensitivity at the frequencies used in our complex tones. However, white-crowned sparrows have poorer sensitivity than the other three species over the range of frequencies used

in our complex tones. Consistent with the sender–receiver matching hypothesis, the audiogram of white-crowned sparrows shows strong correspondence with its use of frequencies above 3 kHz in both call and song elements (see Konishi 1970; Dooling 1982; Caras et al. 2010). Vocalizations of white-crowned sparrows have higher minimum frequencies than those of the other three species, and white-crowned sparrows show a shift towards higher frequencies in the audiogram. Indeed, only the house sparrow showed a slight mismatch between audiogram shape and song properties with a peak frequency of about 4 kHz in the song but relatively poor sensitivity at 4 kHz (also see Henry and Lucas 2008).

We found no sex effects on the auditory properties that we measured for these species. This result is consistent with other published results showing no or at most weak effects of sex on FFR or EFR amplitude (Lucas et al. 2007; Henry and Lucas 2008). Nonetheless, our sample sizes were not ideal for testing for sex effects and should, therefore, be treated with some caution.

Our results are striking in two respects. First, white-crowned sparrows appear to phase lock strongly to all aspects of our complex tones. Clearly neither the audiogram nor the auditory filters offer insight into the basis of FFR intensity in this species. Second, the amplitude of the harmonic elements in our complex tones is enhanced by the presence of the next higher harmonic in woodland species. We address these in order.

Phase-locking in white-crowned sparrows

White-crowned sparrows showed generally stronger phase-locking to both the fine and gross temporal structures of our complex tones than the other species. One possible explanation for these results is that vocal complexity drives auditory performance in these species. The rationale for this statement is that ecological and social conditions may select for complex song structures (e.g., Catchpole and Slater 1995), and this may in turn select for an auditory system that processes this vocal complexity. As discussed above, both the vocal system and auditory system appear plastic enough for this rationale to be plausible. The songs of white-crowned sparrows are the most complex of our study species, including tonal elements like whistles and warbles, and broadband, amplitude-modulated components like trills and buzzes (Fig. 1; Marler and Tamura 1962). Increased complexity in the vocal repertoire could lead to specializations of the auditory system. While the auditory structures that generate EFRs and FFRs in birds are currently unknown, it is possible that differences in these structures lead to species differences in response strength. Kubke et al. (2004) report that auditory nuclei in

the hindbrain of bird species with auditory specializations (e.g., owls and songbirds) show hyperplasia (i.e., a relative increase in cell number) when compared to those of non-specialists (e.g., falcons and doves). Similarly, species of songbirds vary in the degree of hyperplasia. Whether variation in the relative size of auditory nuclei correlates with vocal complexity in songbirds is still to be determined, but studies on the song control system suggest that is possible. The volume of nuclei in the song control system of songbirds vary within (Nottebohm et al. 1981; Canady et al. 1984; Airey et al. 2000) and between (DeVoogd et al. 1993; Székely et al. 1996) species. Generally, species and individuals with more complex vocal repertoires have relatively larger nuclei. If species with more complex songs also have relatively larger peripheral and brainstem structures, and if relatively larger peripheral and brainstem structures generate stronger responses to acoustic stimuli, the enhanced auditory performance in white-crowned sparrows (compared to the other three species) could be driven by the complexity of the vocal repertoire.

Our results with white-crowned sparrows are important because they suggest that vocal complexity can lead to specializations of the peripheral auditory system and brainstem to process different dimensions of the vocal repertoire. Dooling et al. (2002) described correlations between auditory and vocal performance in zebra finches (*Taeniopygia guttata*), budgerigars (*Melopsittacus undulatus*), and canaries (*Serinus canaria*). The vocal repertoire of zebra finches has the most broadband harmonic structure, followed by that of budgerigars and canaries (Zaan 1984; Farabaugh and Dooling 1996; Dooling et al. 2002). Accordingly, zebra finches were better at discriminating stimuli varying in temporal structure and showed stronger compound action potentials of the eighth nerve to the same temporally structured stimuli. Furthermore, Okanoya and Dooling (1990) showed that each species in the budgerigar/canary/zebra finch study system discriminates their own versus heterospecific calls and that each species is better at distinguishing different calls of their own species compared to different heterospecific calls. Lohr et al. (2003) found a similar pattern in these three species, although enhanced within-species discrimination was only evident at high signal-to-noise ratios. In addition, we have recently shown that auditory sensitivity to high-frequency sounds is better predicted by vocal complexity than by high-frequency vocal content in sparrows (Vélez et al. 2015). Together, these results support the idea that auditory processing mechanisms are correlated with vocal performance. Our results complement these studies by showing that species differences in the extent to which multiple dimensions of acoustic signals are processed correlates with the complexity of the vocal repertoire.

Spectral enhancement in woodland species

Our data show that the two woodland species used in our study have relatively poor EFRs, but they also exhibit spectral enhancement of FFR intensity when some tones were coupled with the next higher harmonic in the series. This is important because both species use harmonics in their vocal repertoire (Ritchison 1983; Owens and Freeberg 2007), with pure harmonics nearly ubiquitous in the vocal signals of nuthatches. Theunissen and Doupe (1998) showed that auditory neurons in the zebra finch forebrain nucleus HVC are much more sensitive to the amplitude envelope of their own song than they are to the spectral properties of their song. Similarly, Lohr and Dooling (1998) suggested that non-spectral cues are more important than spectral cues in zebra finch based on behavioral evidence. The implication of these previous studies was that species with strong harmonic structure in their songs and, therefore, strong AM components, should emphasize processing of envelope cues. However, our results offer an alternative mode of processing of strongly harmonic signals: spectral enhancement of harmonics independent of the envelope itself. Indeed, enhancement has been demonstrated both at the cellular level (Schneider and Woolley 2011; Woolley and Portfors 2013) and in the brainstem as indexed by evoked potentials (Krishnan 2002). Moreover, Vernaleo and Dooling (2011) showed in zebra finches that temporal fine structure alone is sufficient for syllable discrimination and may be more important than previously thought in the processing of vocalizations. We now have evidence of spectral enhancement in the brainstem. In addition, titmice and nuthatches have relatively narrow auditory filters (Henry and Lucas 2010), and this property may provide an index of the relative weighting of temporal versus spectral sound properties, even when the vocal repertoire has strong levels of amplitude modulation.

Conclusions

We show here that species differ in how their auditory system encodes temporal and spectral properties of complex acoustic stimuli. These differences match properties of species-specific vocal signals, implying tight coevolution between signal production and receiver physiology. Our results underscore the contention that the processing of a diversity of signal properties (Nelson and Marler 1990) may be multidimensional. As emphasized by Møller (2006), experimental protocol needs to match this level of diversity. For example, our spectral and temporal analyses of song and call properties of each species' vocal signals illustrate the relevance of the tone complexes we

used in this experiment. The analysis of auditory processing of these important signal components in turn leads us to a deeper understanding of the range of mechanisms that could be used to process complex sounds.

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