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The paradox of hearing at the lek: auditory sensitivity increases after breeding in female gray treefrogs (*Hyla chrysoscelis*)

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Abstract

Both behavioral receptivity and neural sensitivity to acoustic mate attraction signals vary across the reproductive cycle, particularly in seasonally breeding animals. Across a variety of taxa receptivity to signals increases, as does peripheral auditory sensitivity, as females transition from a non-breeding to breeding condition. We recently documented decreases in receptivity to acoustic mate attraction signals and circulating hormone levels, but an increase in peripheral auditory sensitivity to call-like stimuli following oviposition in Cope's gray treefrogs (*Hyla chrysoscelis*). However, it is not known if changes in auditory sensitivity are confined to the frequency range of calls, or if they result from more generalized changes in the auditory periphery. Here, we used auditory brainstem responses (ABRs) to evaluate peripheral frequency sensitivity in female Cope's gray treefrogs before and after oviposition. We found lower ABR thresholds, greater ABR amplitudes, and shorter ABR latencies following oviposition. Changes were most pronounced and consistent at lower frequencies associated with the amphibian papilla, but were also detectable at higher frequencies corresponding to the tuning of the basilar papilla. Furthermore, only ABR latencies were correlated with circulating steroid hormones (testosterone). Changes in peripheral processing may result from changes in metabolic function or sensorineural adaptation to chorus noise.

Keywords Auditory brainstem response · Corticosterone · Estradiol · Oviposition · Testosterone

Introduction

Many animals use acoustic communication signals for mate attraction and courtship (Bradbury and Vehrencamp 2011). In seasonally reproducing animals, the production of acoustic signals is also seasonal, with courtship signal

production typically coinciding with periods of peak reproductive readiness. This is thought to occur, because the benefits of acoustic signal production during the non-breeding season are low, while the costs of signal production are high [e.g., opportunity costs (Thomas et al. 2003), energetic costs (Prestwich 1994; Ophir et al. 2010), and eavesdropping (Tuttle and Ryan 1981; Zuk and Kolluru 1998)]. Changes in signal production are mirrored by hormonally mediated physiological and anatomical changes in the neuromuscular substrates of signal production (Kelley 1980; Brenowitz 2004; Leary 2009; Forlano et al. 2015).

Similarly, the responsiveness of receivers to mate attraction and courtship signals shows seasonal plasticity, with strong behavioral receptivity to courtship signals during the breeding season and diminished behavioral receptivity to these signals outside of the breeding season (Lea et al. 2000; Lynch et al. 2005; Cummings et al. 2008). These behavioral changes are generally attributed to hormonally mediated changes in motivation (Adkins-Regan 1998; Leary 2009), although there is increasing recognition that plasticity in sensory mechanisms contributes to seasonal changes in signal processing and its behavioral consequences (Caras and

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Remage-Healey 2016; Forlano et al. 2016; Wilczynski and Burmeister 2016). Much of the work on sensory plasticity in seasonally reproductive animals has either focused on documenting reproductively related seasonal changes in auditory processing (e.g., comparing individuals in the fall vs. spring) or has experimentally induced (either through changes in photoperiod or hormone manipulation) breeding-like conditions in individuals that had been in non-breeding condition (Caras and Remage-Healey 2016; Forlano et al. 2016; Wilczynski and Burmeister 2016).

One of the most dramatic changes in receptivity to acoustic mate attraction signals occurs not in the seasonal lead-up to breeding, but immediately following oviposition, particularly in species with limited parental care (Lynch et al. 2005; Gall et al. 2019). The changes in behavioral receptivity are usually accompanied by dramatic changes in circulating steroid hormone levels (Lynch and Wilczynski 2005; Gall et al. 2019). In a previous study of female Cope's gray treefrogs (*Hyla chrysoscelis*), we found that behavioral receptivity (positive phonotaxis to sexual advertisement calls) and circulating steroid hormone levels drop precipitously following oviposition, while peripheral auditory processing of call-like stimuli improved (Gall et al. 2019). This was the first report of oviposition-related auditory plasticity, and thus, the scope of oviposition-related auditory plasticity is not yet clear. Therefore, we investigated whether plasticity in auditory processing was confined to frequencies emphasized in conspecific vocalizations or whether there is a more generalized oviposition-related plasticity in auditory sensitivity.

We used auditory-evoked potentials (AEPs), a minimally invasive electrophysiological measure, to assess the auditory sensitivity of females prior to and following oviposition. Amphibians are unique among vertebrates in having two inner ear sensory papillae that transduce airborne sound frequencies: the tonotopically organized amphibian papilla transduces relatively lower frequencies than the basilar papilla, which lacks tonotopy and is broadly tuned to higher frequencies (Simmons et al. 2007). In Cope's gray treefrog, the amphibian papilla is sensitive to frequencies below about 1.75 kHz, with its greatest sensitivity to the low-frequency spectral peak of the conspecific advertisement call (approximately 1.25 kHz), whereas the basilar papilla is sensitive to frequencies above 1.5 kHz, with its greatest sensitivity to the high-frequency spectral peak of the advertisement call (approximately 2.5 kHz) (Schrode et al. 2012, 2014; Ward et al. 2013). Our primary objective was to compare the auditory brainstem responses (ABRs) of pre-oviposition and post-oviposition females to tone bursts with frequencies ranging from 0.5 to 4 kHz, which spans most of the hearing range of the species (Schrode et al. 2014) and encompasses the two spectral components present in conspecific advertisement calls. We hypothesized that the previously documented change in responsiveness to call-like stimuli could

either reflect a generalized increase in auditory processing following oviposition (e.g., driven by metabolic or endocrine changes) or be constrained to frequencies represented in conspecific vocalizations (e.g., resulting from mechanisms such as sensorineural adaptation to chorus noise). Gonadal and adrenal steroids are known to modulate hearing in a variety of vertebrates (Caras and Remage-Healey 2016; Forlano et al. 2016; Hall et al. 2016; Wilczynski and Burmeister 2016); therefore, a secondary objective was to determine whether circulating concentrations of three steroids (testosterone, estradiol, and corticosterone) could help to explain any observed differences in auditory sensitivity.

Methods

Animals and experimental design

All experiments were conducted on the St. Paul Campus of the University of Minnesota and were approved by the University of Minnesota's Institutional Animal Care and Use Committee (protocol#1701-34456A). In total, we measured AEPs in 30 females ($N_{\text{pre}} = 15$; $N_{\text{post}} = 15$) of the western genetic lineage of Cope's gray treefrog (Ptacek et al. 1994). These females were also used in additional evoked potential experiments that took place during a single AEP recording session (reported in Gall et al. 2019). We have also previously reported that circulating steroid hormone levels (testosterone, corticosterone, and estradiol) for these same individuals were significantly lower when measured post-oviposition compared with pre-oviposition (Gall et al. 2019). Mated pairs were collected in June of 2017, from ponds in the Carver Park Reserve (Carver County, MN), the Crow-Hassan Park Reserve (Hennepin County, MN), and Hyland Lake Park Reserve (Hennepin County, MN). Collecting females in amplexus and testing them prior to oviposition ensures that they are reproductively ready and the fact that they have already selected a mate does not appear to influence their choosiness (Murphy and Gerhardt 1996). Each pair was placed in a small plastic container holding shallow pond water for transport to the lab, where pond water was then exchanged for aged tap water. All animals were held at approximately 4 °C until the following day when the animals were assigned to either pre-oviposition or post-oviposition AEP testing.

Prior to beginning our experiments, pairs were allowed to reach body temperatures of 20 ± 2 °C over the course of 30–40 min inside a temperature-controlled incubator. During this time, pairs did not begin to oviposit, but did resume amplexus. After reaching the correct body temperature, females were weighed, reunited with their mates, and assigned randomly to the pre-oviposition or post-oviposition treatments. Females assigned to the pre-oviposition

treatment were then tested immediately. Females assigned to the post-oviposition treatment were given an additional 24 h to complete oviposition before their AEPs were measured. During this time, these females were housed with their mate in a plastic terrarium (32 cm × 18 cm × 22 cm, W × H × D) placed on a 20° incline and filled to a depth of 8 cm at one end with aged tap water. Oviposition was confirmed by assessing the presence of eggs in the terraria and by assessing female mass following the 24 h oviposition period. Following auditory-evoked potential testing, we took blood samples via cardiac puncture for hormone analysis.

Auditory-evoked potentials

AEP experiments generally followed procedures described previously (Buerkle et al. 2014; Schrode et al. 2014; Gall et al. 2019) and were performed in a small acoustic chamber (MAC-3, Industrial Acoustics Corporation, Winchester, United Kingdom; inside dimensions: 81.3 cm × 61 cm × 61 cm, W × H × D) lined with 2" pyramidal acoustic foam. The temperature in the chamber was maintained at 21 ± 2 °C and monitored by a digital thermometer placed adjacent to the animal.

We used single-frequency tone bursts to assess frequency sensitivity and suprathreshold responses before and after oviposition. Tone bursts were 5 ms in duration with a 1 ms Blackman onset/offset ramp. Tone bursts were presented in third octave band intervals from 0.5 to 4 kHz (i.e., 0.50, 0.63, 0.80, 1.0, 1.25, 1.6, 2.0, 2.5, 3.15, and 4.0 kHz) at sound pressure levels ranging from 45 to 90 dB SPL in 5 dB steps at a rate of $31.1 \text{ stimuli s}^{-1}$. The selection of third octave band intervals included 1.25 and 2.5 kHz, which reflect the population averages of the two spectral components of *H. chrysoscelis* advertisement calls (Schrode et al. 2012; Ward et al. 2013). In addition, broadband clicks were presented at 100 dB SPL every 10–20 min to monitor the physiological stability of the subject.

All stimuli were generated in SiGenRP and presentation was coordinated with BioSigRP [Tucker Davis Technologies (TDT); Alachua, FL, USA]. The stimuli were passed to TDT RP2 digital processor (sampling rate = 48.8 kHz), attenuated with a TDT PA5 programmable attenuator, and amplified by a Crown XLS 202 amplifier (Crown Audio, Inc., Elkhart, IN, USA) before presentation through an Orb Mod 1 speaker (Orb Audio, New York, NY, USA). The speaker was placed inside of a copper mesh Faraday cage that was connected to the building ground. Prior to the experiments, we calibrated the frequency response of the speaker by playing 5-s tones in third octave bands between 0.5 and 4 kHz and recording the speaker output with a Larson Davis System 831 sound-level meter (Larson Davis Inc., Depew, NY, USA). The peak-to-peak amplitude of each stimulus was subsequently adjusted using a TDT PA5 programmable attenuator.

Females were immobilized with an intramuscular injection of succinylcholine chloride in frog ringer's solution (mean \pm SD = $3.4 \pm 0.8 \text{ } \mu\text{g/g}$). For the animals that were tested prior to oviposition, the dose did not differ significantly when calculated using their pre-oviposition (mean \pm SD = $3.2 \pm 0.8 \text{ } \mu\text{g/g}$) and post-oviposition (mean \pm SD = $3.3 \pm 1.2 \text{ } \mu\text{g/g}$) weight ($t_{15} = 2.15$, $p = 0.25$), nor did the doses differ for animals tested pre-oviposition (mean \pm SD = $3.3 \pm 1.2 \text{ } \mu\text{g/g}$) and post-oviposition (mean \pm SD = $3.5 \pm 0.6 \text{ } \mu\text{g/g}$) when calculated using only the post-oviposition weight ($t_{15} = 2.04$, $p = 0.55$). Hence, the effects reported below are not due to weight-dependent differences in the amount of immobilizing agent received by pre-oviposition versus post-oviposition females. After injection, females were placed on an acoustically transparent pedestal that was 30 cm from the speaker. The female was loosely draped with moist surgical gauze to facilitate cutaneous respiration. We applied a 2.5% lidocaine solution to the head of the female and then inserted three subdermal needle electrodes (Grass F-E2; West Warwick, RI, USA): a reference electrode adjacent to the left tympanum, an inverting electrode at the apex of the skull between the two eyes, and a ground electrode adjacent to the right tympanum. The impedance between the electrodes was maintained between 1 and 5 k Ω , although impedance was usually below 3 k Ω .

The electrodes fed into a TDT RA4LI low-impedance headstage and TDT RA4PA pre-amp (Gain = 20). Digitized responses were then passed through a fiber optic cable to a TDT RZ5 biological signal processor and then a computer running BioSigRP software. All responses were high-pass filtered at 10 Hz, low-pass filtered at 3 kHz, and notch-filtered at 60 Hz. Following recording, the responses were analyzed to determine ABR thresholds, as well as the amplitude and latency of each response. Two trained observers used the visual detection method to determine ABR thresholds (Beatini et al. 2018). We determined ABR amplitude (from the first positive to first negative peak) and ABR latency (of the first positive peak) of each waveform (Fig. 1) with Praat version 6.0.29 (Wong and Gall 2015; Boersma and Weenink 2017). ABR amplitudes and ABR latencies were only measured at stimulus amplitudes above the highest individual threshold for each frequency to ensure that there were an equal number of responses at each frequency and stimulus amplitude combination for the two reproductive conditions.

Blood sampling and hormone analysis

Our methods for blood sampling and hormone analysis have been reported previously (Bastien et al. 2018; Gall et al. 2019). Briefly, we rapidly collected ca. 50 μL of blood with a 30-gauge insulin syringe (BD Micro-fine U-100, 0.3 mL, Wokingham, UK) pre-rinsed with heparin within 20 min of completion of the AEP testing. Plasma was separated via

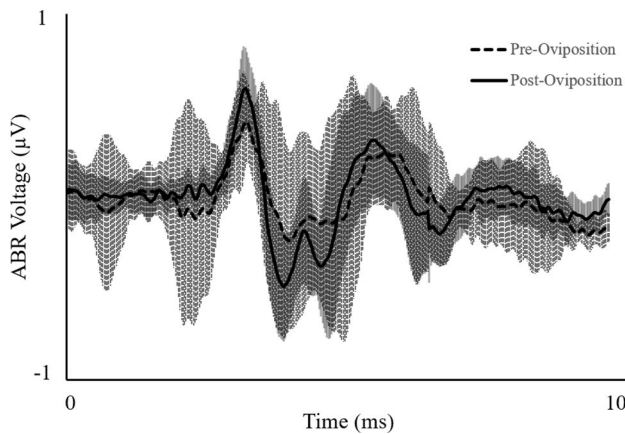


Fig. 1 Average auditory brainstem responses of pre-oviposition ($N=15$) and post-oviposition ($N=15$) females evoked by the 2.5 kHz tone burst at 90 dB SPL. Standard deviation of the responses is shown with hatched error bars (pre-oviposition) and dark gray error bars (post-oviposition)

refrigerated centrifugation and stored at -20°C for 3 weeks, shipped on dry ice to Swarthmore College, and then held at -80°C for 6 months until assayed.

Prior to processing our experimental samples, we validated our extraction and quantification methods for each steroid (see Gall et al. 2019). We used a double liquid diethyl ether extraction method. First, we vortexed plasma and then added subsamples (CORT: $5\text{ }\mu\text{L}$; E_2 : $8\text{ }\mu\text{L}$; T : $10\text{ }\mu\text{L}$) to borosilicate vials containing $200\text{ }\mu\text{L}$ of Type 1 RO water. We then added 2 mL of diethyl ether to each vial and thoroughly vortexed. We twice froze the aqueous layer in a methanol-dry ice slurry, decanting the organic layer into a new vial. We then dried the ether extracts using a vacuum centrifuge at 37°C (Thermo Fisher Savant Speedvac SPD1010, Waltham, MA). These dried extracts were reconstituted in assay buffer (supplied by kit) overnight at 4°C .

Commercial EIA kits (DetectX[®] kits, Arbor Assays, Ann Arbor, MI) were used to assay plasma levels of corticosterone (CORT), 17-beta estradiol (E_2), and testosterone (T). All samples were assayed in duplicate. Briefly, $50\text{ }\mu\text{L}$ of sample or standard for the CORT and T plates and $100\text{ }\mu\text{L}$ for the E_2 plates were plated into wells along with conjugate and antibody. Plates were then placed on an orbital shaker (500 RPM) at room temperature for 1 h (CORT) or 2 h (T , E_2), and then washed four times with wash buffer (supplied by kit). Substrate was then added and the plate was incubated at room temperature for 30 min without shaking. The reaction was stopped and optical densities were read at 450 nm on a Versa_{max} microplate reader with SoftMax Pro software using a four-parameter curve fitting equation (Molecular Devices, Sunnyvale, CA). We accepted the average of duplicate wells unless a CV between duplicates exceeded 15%, in which case the sample was re-assayed until this criterion

was met. Intra- and inter-assay coefficients of variation (CV) for the assays were estimated by including three stripped and spiked samples per plate, thereby incorporating cumulative technical error during extraction and assaying (see Baugh et al. 2018). The assays have detection limits and sensitivities, respectively, of 16.9 pg mL^{-1} and 18.6 pg mL^{-1} for CORT, 2.05 pg mL^{-1} and 2.21 pg mL^{-1} for E_2 , and 9.92 pg mL^{-1} and 30.6 pg mL^{-1} for T . The cross reactivity of the antiserum for each kit is as follows: CORT: 100% for corticosterone, 12.3% for desoxycorticosterone, 0.62% for aldosterone, 0.38% for cortisol; E_2 : 100% for E_2 , 3.2% for estrone sulfate, and 2.5% for estrone; T : 100% for T , 56.8% for 5α -dihydrotestosterone, and 0.27% for androstenedione. Because it has previously been shown using HPLC–MS that plasma DHT concentrations are low or undetectable in females of the related eastern gray treefrog (*Hyla versicolor*; Bastien et al. 2018) and female túngara frogs (*Physalaemus pustulosus*; Baugh et al. 2018), we refer to these estimates as testosterone and not ‘androgens’ more generally.

Statistics

All AEP data were analyzed with repeated-measures mixed models (PROC MIXED) in SAS v. 9.3 after normality and homogeneity of variance were verified (PROC UNIVARIATE). Hormone levels were significantly correlated with one another (Table S1); therefore, we took three approaches to data analysis. First, we constructed single mixed models for each of the three dependent variables (ABR threshold, ABR amplitude, and ABR latency) with independent variables including all hormones, stimulus frequency (and stimulus amplitude for ABR amplitude and ABR latency models), reproductive status (pre-oviposition vs. post-oviposition), and their interactions. Second, we constructed separate models for each of the dependent variables for each of the hormones with stimulus frequency (and stimulus amplitude for ABR amplitude and ABR latency models), reproductive status, and their interactions as additional independent variables. Finally, we performed a PCA (PROC FACTOR) with the three hormone levels and constructed a mixed model for each dependent variable containing the first principal component to represent the hormone levels, stimulus frequency (and stimulus amplitude for ABR amplitude and ABR latency models), reproductive status, and their interactions. The first principal component explained 64% of the variance and was positively correlated with the log transformed CORT ($r=0.34$), T ($r=0.45$) and E_2 ($r=0.45$) levels. The results of the three approaches were qualitatively similar. We present the results of the first approach here and those of the second and third approaches in the supplemental materials (Fig. S1, Table S2–S4).

We analyzed each dependent variable separately. In models for ABR threshold, the between-subjects factor was

reproductive status and the within-subject factors were stimulus frequency and \log_{10} hormone levels (CORT, T , E_2). The models for ABR amplitude and ABR latency were similar, but also included a within-subjects factor of stimulus amplitude. In all models, individual was included as a random factor. Non-significant interactions were removed according to p value and the resulting AIC values for the model. Significant interaction effects that included only class variables were explored using the lsmeans statement with the diffs option. Significant interaction effects that included continuous variables were explored with Pearson's correlations.

Results

Auditory brainstem response [ABR]: threshold

The reproductive status of the animals had a significant influence on ABR thresholds ($F_{1,38} = 7.71$, $p = 0.008$); interestingly, pre-oviposition females had higher thresholds than post-oviposition animals (Fig. 2a). Although we did not find a significant interaction between reproductive status and stimulus frequency ($F_{9,242} = 0.94$, $p = 0.49$), differences in threshold between pre-oviposition and post-oviposition females were typically larger (mean difference \pm SD = 6.3 ± 0.9 dB SPL) and statistically significant ($F_{1,108} > 4.9$, $p < 0.029$) for stimulus frequencies between 0.5 and 1.25 kHz (Fig. 2a), while the differences were typically smaller (mean difference \pm SD = 2.5 ± 1.7 dB SPL) and not statistically significant ($F_{1,108} < 2.9$, $p > 0.089$) for stimulus frequencies between 1.6 and 4 kHz (Fig. 2a). Despite differences in threshold between the two reproductive statuses, we did not find any significant main effects of hormone concentrations [CORT ($F_{1,36} = 1.72$, $p = 0.19$), T ($F_{1,36} = 0.0$, $p = 0.99$), or E_2 ($F_{1,36} = 0.12$, $p = 0.73$)], nor any significant interactions that included hormone concentrations

($F_{9,242} < 0.94$, $p > 0.49$, Fig. 3a–c). We also confirmed that ABR thresholds were significantly influenced by stimulus frequency ($F_{9,242} = 54.3$, $p < 0.001$), as has been previously found in this species (Schrode et al. 2014). The ABR-derived audiogram had the characteristic “W” shape of the gray treefrog audiogram, which results from the different peak sensitivities of the amphibian and basilar papillae (Fig. 2a).

Auditory brainstem response [ABR]: amplitude

We found a significant main effect of reproductive status ($F_{1,115} = 7.7$, $p = 0.006$; Fig. 2b) and a significant interaction between reproductive status and stimulus frequency ($F_{9,1251} = 2.5$, $p = 0.007$) on ABR amplitude. Pre-oviposition animals had lower ABR amplitudes than post-oviposition animals (Fig. 2b), but only at frequencies equal to ($F_{1,217} = 3.9$, $p = 0.04$) or less than 1.25 kHz ($F_{1,217} > 8.5$, $p < 0.004$) and at 2.5 kHz ($t_{205} > 6.1$, $p = 0.01$). Despite differences in ABR amplitude between the two reproductive statuses, we did not find any significant main effects of hormone concentrations [CORT ($F_{1,113} = 0.24$, $p = 0.62$), T ($F_{1,113} = 2.9$, $p = 0.09$), or E_2 ($F_{1,113} = 2.3$, $p = 0.13$), Fig. 3d–f], nor any significant interactions that included hormone concentrations. We confirmed that ABR amplitude was affected by both stimulus amplitude ($F_{8,2096} = 461.9$, $p < 0.001$) and stimulus frequency ($F_{9,1150} = 54.3$, $p < 0.001$; Fig. 2b). ABR amplitude increased with increasing stimulus amplitude and was greatest near the frequencies of best sensitivity, as has previously been shown in this species (Schrode et al. 2014).

Auditory brainstem response [ABR]: latency

There was a significant main effect of reproductive status ($F_{1,116} = 31.4$, $p < 0.001$; Fig. 2c) and a significant interaction between reproductive status and stimulus frequency

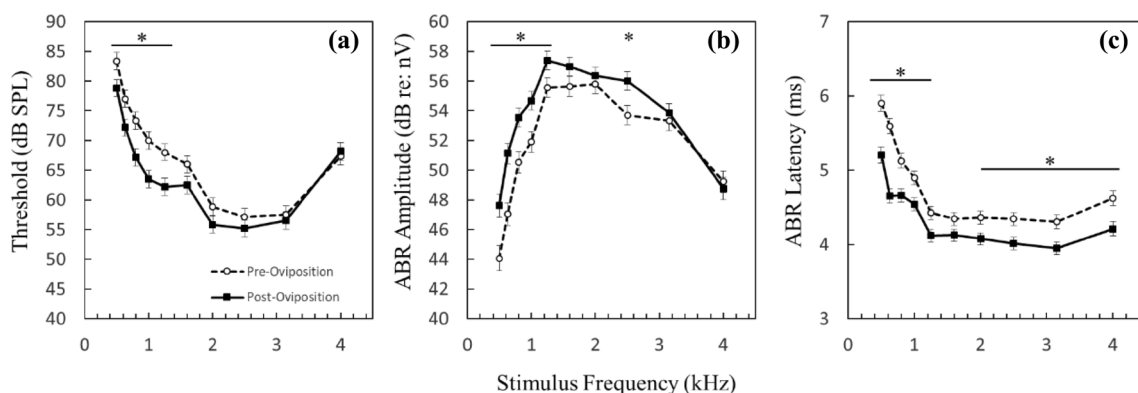


Fig. 2 ABR threshold (a), amplitude (b), and latency (c) (LSMEANS \pm SE) of pre-oviposition and post-oviposition females as a function of stimulus frequency. Asterisks indicate frequencies

at which there was a significant difference in response between pre-oviposition and post-oviposition females

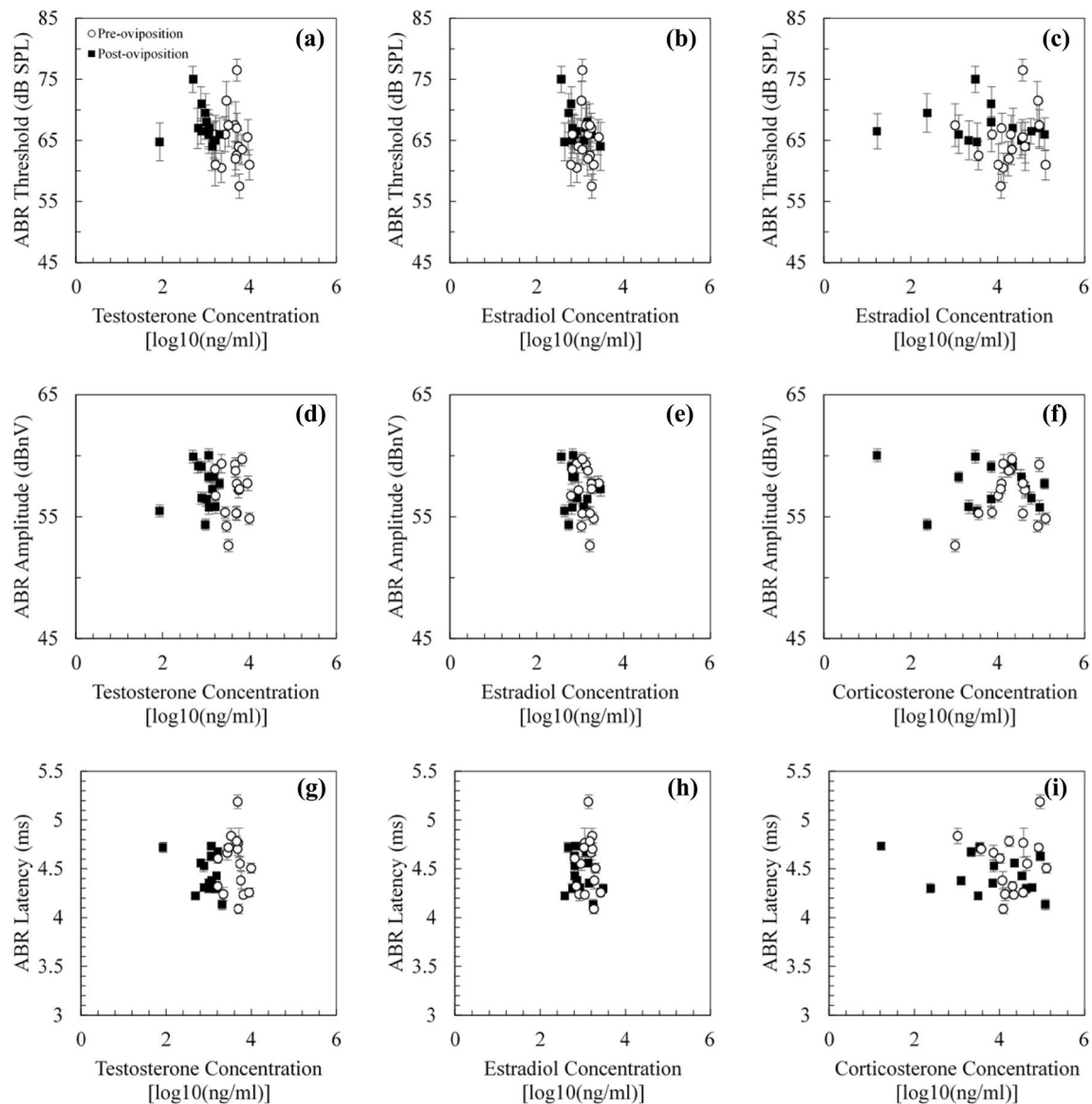


Fig. 3 Three measures of peripheral auditory sensitivity [first row: ABR thresholds (**a–c**), second row: ABR amplitude (**d–f**), and third row: ABR latency (**g–i**)] as a function of circulating hormone levels. Each data point represents a mean \pm SE for an individual averaged across replicates, stimulus frequency, and (for ABR amplitude and

latency) stimulus level. Open circles indicate individuals that were tested prior to oviposition and closed squares indicate individuals that were tested following oviposition. It is important to note that these data do not entirely represent the output of our repeated-measures statistical model and are shown only for descriptive purposes

($F_{9,1152} = 4.4$, $p < 0.001$) on ABR latency. Pre-oviposition animals had longer ABR latencies than post-oviposition animals (Fig. 2c) at all frequencies ($F_{1,137} > 4.9$, $p < 0.03$) except 1.6 kHz ($F_{1,138} = 3.0$, $p = 0.09$). We did not find significant main effects of CORT ($F_{1,114} = 1.6$, $p = 0.21$) or E_2 ($F_{1,115} = 0.2$, $p = 0.65$), nor any significant interactions that included hormone concentrations (Fig. 3g–i). However, we did find a significant main effect of T ($F_{1,114} = 7.9$, $p = 0.006$) in which ABR latency decreased with increasing circulating levels of T [slope \pm SE = -0.30 ± 0.11 ms, $\log_{10}(\text{ng mL}^{-1})$]. We confirmed that ABR latency was affected by both the amplitude

($F_{8,2054} = 191.8$, $p < 0.001$) and frequency ($F_{9,793} = 24.7$, $p < 0.001$; Fig. 2c) of the stimulus, as well as their interaction ($F_{48,2131} = 4.9$, $p < 0.001$). ABR latency decreased with increasing stimulus amplitude and was shortest near the frequencies of best sensitivity, as has previously been shown in this species (Schrode et al. 2014).

Discussion

Our data indicate that greater auditory sensitivity following oviposition extends across the range of hearing. Specifically, we found that greater peripheral sensitivity in post-oviposition females, as measured by ABR thresholds, was observed at lower frequencies transduced by the amphibian papilla. The changes in ABR amplitude were also found primarily in the range of frequencies transduced by the amphibian papilla (up to 1.25 kHz), although there was also a significant increase in ABR amplitude at 2.5 kHz that was associated with the post-oviposition reproductive status. Shorter ABR latencies were found at all frequencies tested except 1.6 kHz in post-oviposition females.

In our previous work, we found that post-oviposition animals had significantly lower levels of estradiol, testosterone, and corticosterone than pre-oviposition animals (Gall et al. 2019). This was true for animals that were used only for hormone assays, animals that were first tested in phonotaxis experiments, and in that animals that were used here for ABR experiments. We have now found that ABR thresholds and amplitudes were not affected by the circulating levels of any hormones. ABR latency, however, was correlated with circulating levels of testosterone. Overall, our results reveal considerable plasticity in peripheral auditory sensitivity associated with reproductive status. Moreover, most differences in auditory sensitivity appear to primarily be constrained to the frequencies processed by the amphibian papilla, although latency changes appear to be more generalized.

Sensory plasticity: patterns and function

Our results were surprising, given both the behavioral and endocrine changes that accompany oviposition in our study animals (previously reported in Gall et al. 2019) and the mounting evidence of reproductively correlated changes in peripheral auditory processing (Caras and Ramage-Healey 2016; Forlano et al. 2016; Wilczynski and Burmeister 2016). In a wide variety of vertebrate taxa, changes in peripheral auditory processing across breeding conditions occur in a manner that is largely consistent with the changes in vocal production and receptivity. Midshipman fish (*Porichthys notatus*), house sparrows (*Passer domesticus*), and Emei music frogs (*Babina daunchina*), for example, show enhanced peripheral sensitivity to frequencies in the range of conspecific vocalizations during times of reproductive readiness (Sisneros et al. 2004; Henry and Lucas 2009; Zhang et al. 2012). In midshipman fish, enhanced sensitivity is thought to result

from hormonally mediated expression of ion channels in hair cells, as well as seasonal additions of hair cells to the saccule (Forlano et al. 2016). In addition, female, but not male, house sparrows show enhanced frequency resolution during the breeding season that coincides with elevated levels of estradiol (Gall et al. 2013). However, this work has primarily focused on the change from a non-reproductive status to a reproductive status or focused on animals separated by much longer time scales (weeks to months) than the 23 h separating our pre-oviposition and post-oviposition animals.

In white-crowned sparrows, experimentally inducing reproductive readiness in non-breeding individuals, through photoperiod manipulation and silastic hormone implants, resulted in elevated ABR thresholds and increased ABR latency (Caras et al. 2010). This finding suggests that exceptionally high levels of circulating steroid hormones can result in decrements in auditory sensitivity. In frogs, circulating levels of steroid hormones increase as females become reproductively ready, likely peaking while females are in amplexus and then decreasing following oviposition (Lynch and Wilczynski 2005). Most of the previous work on hormones and auditory processing in frogs, however, has focused on central auditory processing structures, animals during the breeding season with unknown reproductive condition, or females that have not yet made a mate choice (Wilczynski and Burmeister 2016). In these cases, hormone levels may not yet be at their peak and may have a different relationship with auditory sensitivity than in animals that have recently made a mate choice. It is possible that hormone-hearing relationships are highly non-linear, and at very high concentrations, including at peak reproductive readiness in frogs, there are dampening effects on auditory sensitivity. It is also possible that the timescale of hormonally mediated auditory plasticity may be greater than the rapid change associated with transition from a breeding to non-breeding condition in our frogs. There are few comparable studies investigating the effect of reproductive status and hormones on peripheral auditory processing in treefrogs (see Hall et al. (2016) for an AEP study showing steroid hormones influence sex-specific auditory tuning in African clawed frogs, *Xenopus laevis*). There are, however, relevant studies of the effects of hormones on auditory processing by the treefrog midbrain. For instance, Miranda and Wilczynski (2009a) captured females of the closely related green treefrogs (*Hyla cinerea*) in amplexus (mated) and females that were not in amplexus (unmated) and held females from both groups for 5–8 days prior to testing. They then obtained multi-unit recordings from the auditory midbrain and found that mated females had diminished sensitivity compared to non-mated females. Miranda and Wilczynski (2009b) have also shown that experimentally administered testosterone can increase multi-unit thresholds in the midbrains of female

green treefrogs that were purchased from commercial suppliers and maintained in the laboratory for at least 2 weeks. The methodology and anatomical location of recordings differed between our study (we captured all animals in amplexus and tested them very close to the time of capture, verifying reproductive status by measuring weight changes and making egg mass observations) and that of Miranda and Wilczynski (2009a, b). However, collectively, these studies indicate that testosterone may play a role in modulating the function of both peripheral and central auditory systems in frogs.

The improved sensitivity that we found following oviposition seems counterintuitive given the previous work on reproductively related sensory plasticity. Lower sensitivity prior to oviposition would presumably diminish the ability of females to acoustically locate mates. However, female treefrogs encounter a challenging and noisy acoustic environment during mate choice and amplexus (Bee 2015). In such a noisy environment, enhanced auditory sensitivity may increase the noise in relationship to the signal of interest, making it more difficult to evaluate the details of signals and discriminate among males. In the closely related eastern gray treefrog (*Hyla versicolor*), for example, females approach the lek from adjacent forest and may sit near chorusing males for an hour or more (Schwartz et al. 2004). During this time, females will be exposed to the calls of many different frog species, including conspecific gray treefrogs (Nityananda and Bee 2011). However, females appear to use only acoustic information about conspecifics from the 1–2.5 min immediately prior to mate selection to make their choice (Schwartz et al. 2004). Thus, females likely encounter only a small number of males during their mate decision. Reduced gain, therefore, could improve the ability of females to discriminate more easily among the small number of males that are near her vicinity in the lek.

There are other examples of auditory adaptations found in animals that encounter noisy environments that improve the signal-to-noise ratio. For instance, northern cricket frogs, *Acris crepitans*, that live in more challenging acoustic environments have narrower auditory filters than those that live in less challenging acoustic environments (Witte et al. 2005). Similarly, the rainforest cricket *Paroecanthus podagrosus*, which experiences high acoustic competition, had much narrower auditory filters than two species of European field crickets. Moreover, these narrower auditory filters more effectively reduced background noise (Schmidt et al. 2011). Finally, the Chinese concave-eared torrent frog, *Odorrana tormota*, which encounters background noise from fast moving waters while communicating, is known to modulate the volume and stiffness of their middle ear cavity, which modulates frequency-specific transfer properties in a way that reduces background noise and improves detection of conspecific signals (Gridi-Papp et al. 2008).

Sensory plasticity: physiological mechanisms

Our results suggest that differences in circulating concentrations of three steroid hormones are, for the most part, not mediating the observed differences in auditory sensitivity associated with the difference in reproductive status between pre-oviposition and post-oviposition females. Circulating levels of corticosterone, estradiol, and testosterone were unrelated to ABR thresholds and ABR amplitudes, and corticosterone and estradiol levels were also unrelated to ABR latency, although higher levels of testosterone were associated with shorter ABR latencies. In addition to reproductive status and circulating levels of hormones, individuals also differed in their most recent acoustic exposure, as a result of the additional 24 h that the post-oviposition animals spent in the lab prior to testing. Previous work suggests that exposure to conspecific chorus noise is effective in modulating peripheral auditory sensitivity in treefrogs (Gall and Wilczynski 2015, 2016).

All of the females in this study were captured in amplexus in active breeding choruses. Our best evidence suggests these females are exposed to, at a minimum, 1–2 h of background chorus noise with a long-term average sound pressure level ranging from 60–80 dB SPL (LCeq, Tanner and Bee 2018), with maximum values in some choruses reaching about 72 dB SPL and 84 dB SPL in the third octave bands centered at 1.25 kHz and 2.5 kHz, respectively, (Bee unpublished data). Peak sound pressure levels of male advertisement calls at a distance of 1 m range from 96–104 dB (Gerhardt 1975). Thus, females evaluating several nearby males in an active chorus are likely exposed to much higher sound levels than those due to background chorus noise alone. Exposure to high-intensity sounds can result in a temporary threshold shift (TTS), which corresponds to an increase in hearing thresholds following sound exposure that recovers to pre-exposure levels over time (Ryan et al. 2016). The literature on TTS in anurans is limited to two species and suggests that the phenomenon does exist in anurans, but its effects may not be as pronounced as those in mammals (Finneran 2015; Dobie and Humes 2017). Prolonged exposure to high levels of noise caused temporary reductions in auditory function in bullfrogs, *Rana catesbeiana* (Simmons et al. 2014); however, even at very high levels (> 150 dB), long exposure periods were needed to induce changes in DPOAEs (distortion product otoacoustic emissions, > 12 h) or morphological damage to hair cells (> 4 h). Furthermore, temporary shifts in the thresholds of auditory nerve fibers in the Puerto Rican coqui frog, *Eleutherodactylus coqui*, induced by sound exposure typically lasted less than the duration of the stimulus (e.g., recovery following 3 minutes of exposure at 80–118 dB took less than 3 minutes, Zelick and Narins 1985; Penna and Narins 1989). On the surface, these findings suggest that the levels and duration of sound exposure

in natural choruses may be unlikely to induce TTS. However, these anuran studies used pure tone or narrow band noise to induced threshold shifts, which allows for easy comparisons to mammalian data. At present, it remains unknown whether more biologically relevant stimuli, such as chorus noise and calling males, presented over biologically relevant time periods, such as several hours, are capable of inducing TTS that could persist on the order of hours. Future work should investigate whether TTS occurs in frogs exposed to a noisy lek, as finding TTS would greatly affect our current assumptions that auditory sensitivity should peak during the period in which mate choice occurs. It could suggest, for instance, that sensitivity may increase as a way of offsetting auditory deficits induced by acoustic exposure at the lek.

Long-term damage from noise exposure has also not been found previously in frogs. In fact, Richard Salvi and Robert Capranica reportedly abandoned early experiments on noise-induced hearing loss in bullfrogs when they were unable to detect permanent threshold shifts after exposure to noise levels up to 165 dB (Salvi 2008). The lack of permanent threshold shifts could be due to at least two non-mutually exclusive mechanisms: hair cell regeneration (Salvi 2008) and protective mechanisms (Wever 1985). In mammals, there are two primary acoustic reflexes that are thought to potentially have a protective effect: the medial olivocochlear (MOC) reflex (Guinan 1996) and the middle ear reflex (Moller 1974). The MOC operates at the level of the outer hair cells and reduces the gain provided by the cochlear amplifier (Guinan 1996, 2006). This reduced gain is thought to serve a protective function, although some argue that its primary role is to reduce biologically relevant background noise and is unlikely to have evolved in a protective context (Kirk and Smith 2003). Although it is currently unclear whether there is an MOC-like mechanism in anurans, the presence of otoacoustic emissions suggests that there is an analogous effect to the cochlear amplifier in mammals, which most likely arises from hair bundle motility in anurans (Simmons et al. 2007). However, an MOC-like acoustic reflex would be constrained to the amphibian papilla, as the basilar papilla of anurans lacks efferent innervation (Simmons et al. 2007). The middle ear reflex (i.e., acoustic reflex) of mammals can reduce the transfer of sound from the tympanum to the cochlea when the animal is exposed to high levels of external acoustic energy or prior to vocalizing (Moller 1974). This reflex is thought to protect the cochlea from sound-induced damage and also to play a role in reducing low-frequency masking of communication signals. However, it is not clear whether such an acoustic reflex exists in birds, reptiles, or anurans (Hetherington 1994; Saunders et al. 2000; Mason 2007). In frogs, the opercular muscle has been suggested to be involved in a middle ear acoustic reflex, although the tonic nature of the muscle suggests that it is unable to modulate fast responses to loud sounds (Hetherington 1994; Mason 2007). It is currently unknown whether it

could modulate responsiveness over longer time periods, such as those experienced by chorusing frogs. In mammals, both of these protective mechanisms operate on the order of milliseconds to seconds (Church and Cudahy 1984; Backus and Guinan 2006), suggesting that they may not be responsible for the longer duration effects that would be required to produce the oviposition-related plasticity that we see here.

Conclusions

In conclusion, we found that there appears to be a generalized oviposition-related auditory plasticity in female Cope's gray treefrogs, although this plasticity is primarily confined to the amphibian papilla rather than basilar papilla. This also suggests that the greater auditory responsiveness to call-like stimuli found following oviposition may be driven primarily by lower frequency components of the calls. Our results suggest that auditory plasticity is driven primarily by non-endocrine mechanisms (or at least, not by circulating hormone levels) and is unlikely to be responsible for the reduction in behavioral responsiveness to conspecific calls following oviposition. However, it is not yet clear what mechanisms may be responsible for the shifts in peripheral auditory function that accompany shifts in reproductive status and future work is warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Animal collections were made under Special Permit 21947 from the State of Minnesota Department of Natural Resources. This study was approved by the Institutional Animal Care and Use Committee at the University of Minnesota (Protocol 1701-34456A, approved 3 March 2017). This article does not contain any studies with human participants performed by any of the authors.

References

- Adkins-Regan E (1998) Hormonal mechanisms of mate choice. *Am Zool* 38:166–178
- Backus BC, Guinan JJ (2006) Time-course of the human medial olivocochlear reflex. *J Acoust Soc Am* 119:2889–2904

- Bastien B, Farley G, Ge F, Malin JS, Simon-Plumb CL, Pulley DM, Stowell N, Yang C, Baugh AT (2018) The waiting and mating game: condition dependent mate sampling in female gray treefrogs (*Hyla versicolor*). *Front Ecol Evol Behav Evol Ecol* 6:140
- Baugh AT, Bastien B, Still M, Stowell N (2018) Validation of water-borne steroid hormones in a tropical frog (*Physalaemus pustulosus*). *Gen Comp Endocrinol* 261:67–80
- Beatini JR, Proudfoot GA, Gall MD (2018) Frequency sensitivity in Northern saw-whet owls (*Aegolius acadicus*). *J Comp Physiol A* 204:145–154
- Bee MA (2015) Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. *Int J Psychophysiol* 95:216–237
- Boersma P, Weenink D (2017) Praat: doing phonetics by computer [Computer program]. Version 6. 0. 29, retrieved 3 June 2018 from <http://www.praat.org/>
- Bradbury JW, Vehrencamp SL (2011) Principles of animal communication, 2nd edn. Sinauer Associates, Sunderland
- Brenowitz EA (2004) Plasticity of the adult avian song control system. *Ann New York Acad Sci* 1016:560–585
- Buerkle NP, Schrode KM, Bee MA (2014) Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). *Comp Biochem Physiol A Mol Integr Physiol* 178:68–81
- Caras ML, Remage-Healey L (2016) Modulation of peripheral and central auditory processing by estrogens in birds. In: Bass A, Sisneros J, Popper A, Fay R (eds) *Hearing and hormones*. Springer handbook of auditory research, vol 57. Springer, Cham
- Caras ML, Brenowitz E, Rubel EW (2010) Peripheral auditory processing changes seasonally in Gambel's white-crowned sparrow. *J Comp Physiol A* 196:581–599
- Church GT, Cudahy EA (1984) The time course of the acoustic reflex. *Ear Hear* 5:235–242
- Cummings ME, Bernal XE, Reynaga R, Rand AS, Ryan MJ (2008) Visual sensitivity to a conspicuous male cue varies by reproductive state in *Physalaemus pustulosus* females. *J Exp Biol* 211:1203–1210
- Dobie RA, Humes LE (2017) Commentary on the regulatory implications of noise-induced cochlear neuropathy. *Int J Audiol* 56(sup1):74–78
- Finneran JJ (2015) Noise-induced hearing loss in marine mammals: a review of temporary threshold shift studies from 1996 to 2015. *J Acoust Soc Amer* 138(3):1702–1726
- Forlano PM, Sisneros JA, Rohmann KN, Bass AH (2015) Neuroendocrine control of seasonal plasticity in the auditory and vocal systems of fish. *Front Neuroendocrinol* 37:129–145
- Forlano PM, Maruska KP, Sisneros JA, Bass AH (2016) Hormone-dependent plasticity of auditory systems in fishes. In: Bass A, Sisneros J, Popper A, Fay R (eds) *Hearing and hormones*. Springer handbook of auditory research, vol 57. Springer, Cham
- Gall MD, Wilczynski W (2015) Hearing conspecific vocal signals alters peripheral auditory sensitivity. *Proc R Soc B* 282:20150749
- Gall MD, Wilczynski W (2016) The effects of call-like masking diminish after nightly exposure to conspecific choruses in green treefrogs (*Hyla cinerea*). *J Exp Biol* 219(1295):1302
- Gall MD, Salameh TS, Lucas JR (2013) Songbird frequency selectivity and temporal resolution vary with sex and season. *Proc R Soc B* 280:20122296
- Gall MD, Bee MA, Baugh AT (2019) The difference a day makes: breeding remodels hearing, hormones and behavior in female Cope's gray treefrogs (*Hyla chrysoscelis*). *Hormones Behav* 108:62–72
- Gerhardt HC (1975) Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *J Comp Physiol A* 102:1–12
- Gridi-Papp M, Feng AS, Shen JX, Yu ZL, Narins PM (2008) Active control of ultrasonic hearing in frogs. *Proc Natl Acad Sci USA* 105:11013–11018
- Guinan JJ (1996) Physiology of olivocochlear efferents. In: Dallos P, Popper AN, Fay RR (eds) *The Cochlea*. Springer handbook of auditory research, vol 8. Springer, Berlin, pp 435–502
- Guinan JJ (2006) Olivocochlear efferents: anatomy, physiology, function, and the measurement of efferent effects in humans. *Ear Hear* 6:589–607
- Hall IC, Woolley SMN, Kwong-Brown U, Kelley DB (2016) Sex differences and endocrine regulation of auditory-evoked, neural responses in African clawed frogs (*Xenopus*). *J Comp Physiol A* 202:17–34
- Henry KS, Lucas JR (2009) Vocally correlated seasonal auditory variation in the house sparrow (*Passer domesticus*). *J Exp Biol* 212:3817–3822
- Hetherington TE (1994) The middle ear muscle of frogs does not modulate tympanic responses to sound. *J Acoust Soc Am* 95:2122–2125
- Kelley DB (1980) Auditory and vocal nuclei in the frog brain concentrate sex hormones. *Science* 207:553–555
- Kirk EC, Smith DW (2003) Protection from acoustic trauma is not a primary function of the medial olivocochlear efferent system. *J Assoc Acoust Res* 4:445–465
- Lea J, Halliday T, Dyson M (2000) Reproductive stage and history affect the phonotactic preferences of female midwife toads, *Alytes muletensis*. *Anim Behav* 60:423–427
- Leary CJ (2009) Hormones and acoustic communication in anuran amphibians. *Int Comp Biol* 49:452–470
- Lynch KS, Wilczynski W (2005) Gonadal steroid fluctuations in a tropically breeding female anuran. *Gen Comp Endocrinol* 43:51–56. <https://doi.org/10.1016/j.ygcen.2005.02.023>
- Lynch KS, Rand AS, Ryan MJ, Wilczynski W (2005) Plasticity in female mate choice associated with changing reproductive states. *Anim Behav* 69:689–699
- Mason MJ (2007) Pathways for sound transmission to the inner ear in amphibians. In: Narins PM, Feng AS, Fay RR, Popper AN (eds) *Hearing and sound communication in amphibians*. Springer handbook of auditory research, vol 28. Springer, New York
- Miranda JA, Wilczynski W (2009a) Female reproductive state influences the auditory midbrain response. *J Comp Physiol A* 195:341–349
- Miranda JA, Wilczynski W (2009b) Sex differences and androgen influences on midbrain auditory thresholds in the green treefrog, *Hyla cinerea*. *Hear Res* 252:79–88
- Moller AR (1974) The acoustic middle ear muscle reflex. In: Keidel WD, Neff WD (eds) *Handbook of sensory physiology*, vol V/1: auditory system. Springer, Berlin, pp 519–548
- Murphy CG, Gerhardt HC (1996) Evaluating the design of mate-choice experiments: the effect of amplexus on mate choice by female barking treefrogs, *Hyla gratiosa*. *Anim Behav* 51:881–890. <https://doi.org/10.1006/anbe.1996.0092>
- Nityananda V, Bee MA (2011) Finding your mate at a cocktail party: frequency separation promote auditory stream segregation of concurrent voices in multi-species frog choruses. *PLoS One* 6:e21191
- Ophir AG, Schrader SB, Gillooly JF (2010) Energetic cost of calling: general constraints and species-specific differences. *J Evol Biol* 23:1564–1569
- Penna M, Narins PM (1989) Effects of acoustic overstimulation on spectral and temporal processing in the amphibian auditory nerve. *J Acoust Soc Am* 85:1617–1629
- Prestwich KN (1994) The energetics of acoustic signaling in anurans and insects. *Am Zool* 34:625–643
- Ptacek MB, Gerhardt HC, Sage RD (1994) Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor*. *Evolution* 48:898–908

- Ryan AF, Kujawa SG, Hammill T, Le Prell C, Kil J (2016) Temporary and permanent noise-induced threshold shifts: a review of basic and clinical observations. *Otol Neurotol* 37(8):e271–e275
- Salvi RJ (2008) Overview: regeneration and repair. In: Salvi RJ, Popper AN, Fay RR (eds) *Hair cell regeneration, repair, and protection*. Springer handbook of auditory research, vol 33. Springer, New York
- Saunders JC, Duncan RK, Doan DE, Werner YL (2000) The middle ear of reptiles and birds. In: Dooling RJ, Fay RR, Popper AN (eds) *Comparative hearing: birds and reptiles*. Springer handbook of auditory research, vol 13. Springer, New York
- Schmidt AK, Riede K, Römer H (2011) High background noise shapes selective auditory filters in a tropical cricket. *J Exp Biol* 214:1754–1762
- Schrode K, Ward JL, Vélez A, Bee MA (2012) Female preferences for spectral call properties in the western genetic lineage of Cope's gray treefrog (*Hyla chrysoscelis*). *Behav Ecol Sociobiol* 66:1595–1606
- Schrode KM, Buerkle NP, Brittan-Powell EF, Bee MA (2014) Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex, and size. *J Comp Physiol A* 200:221–238
- Schwartz JJ, Huth K, Hutchin T (2004) How long do females really listen? Assessment time for female mate choice in the grey treefrog, *Hyla versicolor*. *Anim Behav* 68:533–540
- Simmons DD, Meenderink SWF, Vassilakis PN (2007) Anatomy, physiology, and function of the auditory end-organs in the frog inner ear. In: Narins PA, Feng AS, Fay RR, Popper AN (eds) *Hearing and sound communication in amphibians*, vol 29. Springer, New York, pp 184–220
- Simmons DD, Lohr R, Wotring H, Burton MD, Hooper RA, Baird RA (2014) Recovery of otoacoustic emissions after high-level noise exposure in the American bullfrog. *J Exp Biol* 217:1626–1636. <https://doi.org/10.1242/jeb.090092>
- Sisneros JA, Forlano PM, Deitcher DL, Bass AH (2004) Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. *Science* 305:404–407
- Tanner JC, Bee MA (2018) Within-individual variation in sexual displays: signal or noise? *Behav Ecol* 1:2. <https://doi.org/10.1093/beheco/ary165>
- Thomas RJ, Cuthill IC, Goldsmith AR, Cosgrove DF, Lidgate HC, Burdett Proctor SL (2003) The trade-off between singing and mass gain in a daytime-singing bird, the European robin. *Behaviour* 40:387–404
- Tuttle MD, Ryan MJ (1981) Bat predation and the evolution of frog vocalizations in the neotropics. *Science* 214:677–678
- Ward JL, Love EK, Vélez A, Buerkle NP, O'Bryan LR, Bee MA (2013) Multitasking males and multiplicative females: dynamic signaling and receiver preferences in Cope's grey treefrog. *Anim Behav* 86:231–243
- Wever EG (1985) *The amphibian ear*. Princeton University Press, Princeton
- Wilczynski W, Burmeister SS (2016) Effects of steroid hormones on hearing and communication in frogs. In: Bass A, Sisneros J, Popper A, Fay R (eds) *Hearing and hormones*. Springer handbook of auditory research, vol 57. Springer, Cham
- Witte K, Farris HE, Ryan MJ, Wilczynski W (2005) How cricket frog females deal with a noisy world: habitat-related differences in auditory tuning. *Behav Ecol* 16:557–571
- Wong A, Gall MD (2015) Frequency sensitivity in the auditory periphery of male and female black-capped chickadees (*Parus atricapillus*). *Zoology* 118:357–363
- Zelick R, Narins PM (1985) Temporary threshold shift, adaptation, and recovery characteristics of frog auditory nerve fibers. *Hear Res* 17:161–176
- Zhang D, Cui J, Tang Y (2012) Plasticity of peripheral auditory frequency sensitivity in Emei music frog. *PLoS One* 7:e45792
- Zuk M, Kolluru GR (1998) Exploitation of sexual signals by predators and parasitoids. *Quart Rev Biol* 73:415–438

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