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Research Article

Reciprocal Matched Filtering in the Inner Ear of the African Clawed Frog (*Xenopus laevis*)

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ABSTRACT

Anurans (frogs and toads) are the most vocal amphibians. In most species, only males produce advertisement calls for defending territories and attracting mates. Female vocalizations are the exceptions among frogs, however in the African clawed frog (*Xenopus laevis*) both males and females produce distinct vocalizations. The matched filter hypothesis predicts a correspondence between peripheral auditory tuning of receivers and properties of species-specific acoustic signals, but few studies have assessed this relationship between the sexes. Measuring hearing sensitivity with a binaural recording of distortion product otoacoustic emissions, we have found that the ears of the males of this species are tuned to the dominant frequency of the female's calls, whereas the ears of the females are tuned close to the dominant frequency of the male's calls. Our findings provide support for the matched filter hypothesis extended to include male-female calling. This unique example of reciprocal matched filtering ensures that males and females communicate effectively in high levels of background noise, each sex being most sensitive to the frequencies of the other sex's calls.

Keywords: DPOAEs, coupled ears, amphibian papilla, basilar papilla, sexual dimorphism, hearing

INTRODUCTION

Reproductive success in anurans generally relies on their ability to detect, recognize, and localize sound. In

the usual case, vocally active males advertise their species identity, sex, and reproductive fitness (Bogert 1960; Littlejohn 1977; Wells 2007). Most anuran females are the silent partners that choose their mate based on the acoustic information provided by males. However, in a few anuran species, females also produce mating vocalizations that elicit distinctive behaviors in the males (Given 1987; Marquez and Verrell 1991; Emerson 1992; Tobias et al. 1998; Shen et al. 2008). In species with sexually dimorphic vocalizations, one might predict that selection would favor specialized mechanisms in individuals of one sex for detecting vocalizations of the other sex (reciprocal tuning).

In the South African clawed frog, *Xenopus laevis* (Pipidae), receptive partners engage in courtship duets: females ready to oviposit swim to advertising males and produce fertility advertisement calls, rapping, that elicit both males' answer calls and approach (Tobias et al. 1998). A study of the auditory-evoked potentials in four species of *Xenopus* (including *X. laevis*) showed that the sensitivity to the spectral components of the advertisement calls are enhanced in females relative to males (Hall et al. 2016). Beyond the expected female matched sensitivity to the mating call of males, the courtship duet in *X. laevis* suggests that the auditory periphery of this species exhibits a reciprocal matched filtering that facilitates the detection of the opposite sex's vocalizations. This is consistent with not only the robust female responses to male calls but male behaviors (phonotaxis and changes in calling) displayed in response to female vocalizations (Tobias et al. 1998; Wang et al. 2010).

Males and females of *X. laevis* socially interact with a rich repertoire of calls that consists of trills with different patterns of click rates (Kelley and Tobias 1999; Zornik and Kelley 2011). Males produce six call types with inter-click intervals (ICI) ranging from

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about 10 (growling) to 230 ms (ticking; Tobias et al. 2004). Receptive females rap with ICI of 80 ms, whereas non-ovulating unreceptive females tick with ICI of 229 ms (Tobias et al. 1998). Male and female vocalizations are also distinguished by the frequency spectra of their clicks. Female rapping and ticking calls exhibit a dominant carrier frequency of 1.2 kHz. Most male calls—advertisement, answer, chirping, and ticking calls—have peak frequencies between 1.7 and 2.3 kHz, whereas amplectant and growling calls also exhibit a spectral peak at about 1 kHz (Tobias et al. 1998; Tobias et al. 2004). Behavioral studies with *X. laevis* suggest that temporal features suffice for sexual identity recognition, and that spectral features convey female attractiveness evoking a full variety of male courtship vocal responses (Elliott and Kelley 2007; Vignal and Kelley 2007).

The middle ear of *X. laevis*, consists, in part, of a tympanic disk that communicates vibrations to the inner ear, and exhibits a pronounced sexual dimorphism in that the males' tympanic disk is significantly larger than that of the female relative to body size (Mason et al. 2009). Given these anatomical differences and the spectral differences found between sex-specific vocalizations, we tested the hypothesis that *X. laevis* exhibits sexual differences in the inner ear sensitivities that enhance reception of the spectral call features of relevance to each sex by recording distortion product otoacoustic emissions (DPOAEs).

DPOAEs are a by-product of nonlinear ear mechanics that appear when the ear is stimulated simultaneously with two tones (Kemp 1979; Probst et al. 1991; Shera and Abdala 2012). They provide a noninvasive and efficient assessment of sensitivity and tuning of the inner ear. In frogs, DPOAEs display characteristics similar to those recorded from mammals' ears, including maximum DPOAE levels at frequencies of biological significance (Vassilakis et al. 2004; Meenderink et al. 2010), non-monotonic growth functions that are indicative of nonlinear compression (Meenderink and Van Dijk 2005), and vulnerability to physiological insult (Van Dijk et al. 2003). The bimodal dependence on the stimulus frequency of DPOAEs recorded in anurans is considered a result of DPOAE generation in the two papillae with high sensitivity to airborne sound: the amphibian papilla (AP) and the basilar papilla (BP; Van Dijk and Manley 2001; Van Dijk et al. 2002). Since the AP is most sensitive to the low- and mid-frequencies within the frog hearing range and the BP is most sensitive to the highest frequencies, it is assumed that lower-frequency DPOAEs originate from the AP, while higher-frequency DPOAEs are generated in the BP. Here, we use DPOAEs to explore sexual dimorphism in an anuran auditory system.

MATERIALS AND METHODS

Experimental Animals

Nine adult *Xenopus laevis* obtained from Nasco (Fort Atkinson, WI) were used in this study. Body weights were 60–75 g ($n=4$, mean 67 g, males) and 182–204 g ($n=5$, mean 194 g, females). Frogs were same-sex group-housed in polycarbonate aquaria in the vivarium of the Division of Laboratory and Animal Medicine, UCLA. They were maintained under a 12-h light/dark cycle at 16–18 °C and fed frog brittle (Nasco) twice weekly. All animal procedures were performed in accordance with the published guidelines in the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication revised 2011). The experimental protocol (#1994–086-71) was approved by UCLA's institutional animal care and use committee.

DPOAE Recording and Analysis

Animals were anesthetized with an intramuscular administration of a pentobarbital sodium solution (Nembutal, Ovation Pharmaceuticals, Inc., 50 mg/ml: ~1–1.2 μ l/g body mass) in one of the hind limbs. During the experiments, frogs were covered by wet gauze to prevent desiccation and to facilitate cutaneous respiration. If movement artifacts appeared in the acoustic recordings, small maintenance doses of anesthetic were administered (0.3 μ l/g, 50 mg/ml).

DPOAE measurements were performed with the frog placed on a vibration-isolation table (Newport VH IsoStation) inside a single-walled sound-attenuating chamber with a room temperature between 18 and 20 °C. The tympanic disk position was estimated by palpation over the skin caudal to the eye where slight changes of texture reveal the presence of the underlying rigid plaque. Moreover, the coloration pattern of the skin was inspected under the microscope to define visual landmarks that later helped in placing the probe.

DPOAEs were simultaneously recorded from both ears, but sound stimulation was delivered exclusively to the right ear (Fig. 1a). The binaural DPOAE recording was especially useful in *X. laevis* in which the ear shows clear structural adaptations for underwater hearing. Probably due to poor impedance matching of the tympanic disk to the air, the ear sensitivity to sound intensity in this species is 30 dB lower in air than in water (Christensen-Dalsgaard and Elepfandt 1995), and the velocity response of the tympanic disk is ca. 25 dB lower than that of the tympanic membrane of the bullfrog (Mason et al. 2009). This reduced sensitivity is typically overcome by using high-level stimulus tones (≥ 80 dB SPL) in most studies that stimulate the ear of *X. laevis* with airborne sound (Van Dijk et al. 2002; Mason et al. 2009;

Hall et al. 2016). However, high-level stimulation imposes two challenges for DPOAE recordings: 1. It precludes studying the DPOAEs originating in the amphibian papilla by the active amplification of low sound pressure stimuli, previously described in ranid frogs (Van Dijk et al. 2003; Meenderink and Van Dijk 2004); 2. It runs the risk of generating system distortion products that appear in response to high sound pressure levels. To avoid these problems, we took advantage of another unique feature of *Xenopus*—the two middle ear cavities and the larynx are connected directly by an air-filled recess (Christensen-Dalsgaard and Elepfandt 1995). The stimuli presented in the right (ipsilateral) ear are transmitted through this recess to the left (contralateral) ear, where the DPOAEs can be recorded without being contaminated by system-generated distortion as a consequence of high-level stimulation in the small space (0.4 cc) of the right coupler.

DPOAEs were binaurally recorded with two low-noise probe systems (ER-10C, Etymotic Research). Each probe included a sensitive microphone that was attached to an eartip (ER-10C-14A) and inserted into a short piece of tygon tubing with a diameter slightly larger than that of the tympanic disk. The built-in sound sources of the Etymotic system were not used due to their limitations in generating low frequency (<1 kHz) primary tone levels above 85 dB SPL. For stimulus generation, the probe at the right ear also included tubing connections to two headphone loudspeakers [Beyerdynamic DT 48 A.O, enclosed in a specially-constructed brass housing (Narins 1987)]. Both probes were carefully sealed against the skin surrounding the tympanic disks with high-vacuum grease (Dow Corning Corporation).

The stimulus tones were generated in two separate D/A channels of a real-time processor (RP2.1, Tucker-Davis Technologies) and the level of each tone was adjusted with a separate programmable attenuator (PA5, Tucker-Davis Technologies). A sampling rate of 25 kHz was used for stimulus generation and microphone recording. The output of each microphone was amplified by 20 dB with a low-noise preamplifier (ER-10C, Etymotic Research) and recorded on computer disk using two separate A/D channels (RP2.1, Tucker-Davis Technologies). Stimulus generation, data acquisition, and data analysis were performed using custom software written in Matlab R2009 (The MathWorks, Inc.) and RPDvsEx (Tucker-Davis Technologies).

The sound delivery system was calibrated in situ using band-pass filtered (0.2–10 kHz) white noise. Sound pressure levels used in the present study are expressed in dB SPL (dB re 20 μ Pa). The distortion produced by the acoustic driver system was assessed by performing control measurements with the probe pressed against a hard, inanimate surface. Additional-

ly, in two animals that failed to recover from the anesthesia (>5 h following the hearing evaluation), a complete series of control measurements was repeated after confirming their death. These recordings allowed us to evaluate distortions at the ipsilateral and the contralateral ear, and to compare responses between live and dead animals.

The stimuli consisted of two separate, simultaneous pure tones (primaries) of 5-s duration followed by a silent period of 1 s, for a stimulus repetition period of 6 s. The stimulus frequencies were chosen such that they were periodic over the same integer number of sample points. The frequencies and levels of the primaries are denoted as f_1 , f_2 , L_1 , and L_2 , respectively. Amplitudes of the spectral peaks were obtained by performing an FFT on a time-domain-averaged ($n=15$) signal using 8192 points. Only the amplitude of the distortion product at $2f_1-f_2$ ($2f_1-f_2$ DP) was systematically recorded. Background noise level was calculated as the mean amplitude of 5 FFT bins on either side of and 25 Hz from the $2f_1-f_2$ frequency.

We evaluated the attenuation of the contralateral input due to acoustical coupling for all the frequency-level combinations used as stimuli. The difference (in dB) between the L_1 level recorded at the ipsilateral and the contralateral microphone was computed. Individuals' mean transfer functions were grouped by sex, then averaged. Since we used closed-field configuration in both sides, we assume that the direct sound transmission from the ipsilateral (stimulated) ear to the contralateral (non-stimulated) ear was insignificant; thus, sound propagation between ears depended on filtering by middle ears and transmission via internal pathways.

At the start of each session, a frequency sweep was presented to verify the presence of DPOAEs in both ears. If no DPOAE was detected, the probes were repositioned, and the calibration procedure repeated. Stimuli consisted of f_2 frequencies from 0.4–4 kHz in steps of 0.1 kHz and levels $L_1 = L_2 = 90$ dB SPL. A fixed f_2/f_1 ratio of 1.14 was selected using the following guidelines: (a) this is the ratio of the two dominant frequencies present in the advertisement call of the male *X. laevis* (Hall et al. 2016), and (b) this value falls in the range of f_2/f_1 ratios that evokes the highest amplitude $2f_1-f_2$ distortion product in amphibians (Vassilakis et al. 2004; Meenderink et al. 2005). The plots of the recorded $2f_1-f_2$ DP amplitude as a function of f_1 are herein identified as DPOAE audiograms. Replication of this recording at the end of the experimental session (which typically lasted 150 min), allowed confirmation of the physiological stability of our study animals over this period.

For measuring the DPOAE frequency response, a matrix of 864 frequency-level combinations (36 frequencies \times 24 levels) was pseudo-randomly presented with f_2

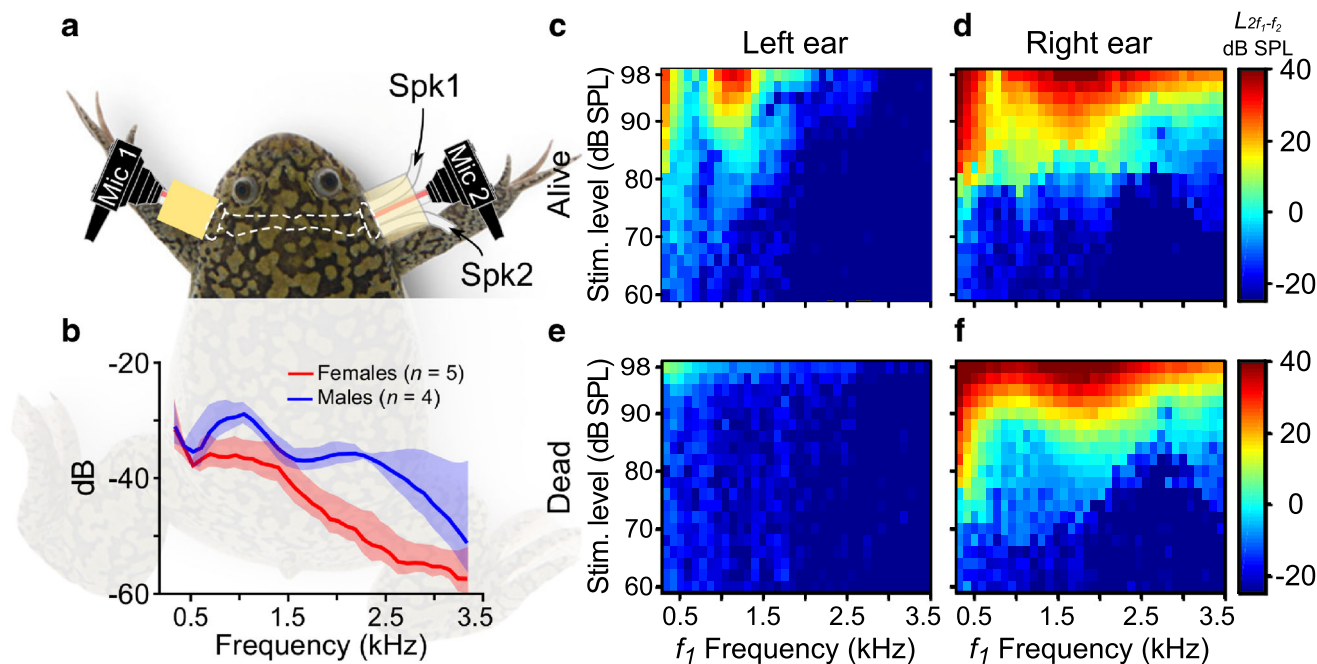


FIG. 1. Binaural DPOAE recording in *Xenopus laevis*. **a** Sketch of the DPOAE recording setup: two speakers delivered stimuli exclusively to the right ear; two microphones simultaneously recorded distortion products from both ears. The dashed lines represent the air-filled recess that connects the two middle ear cavities and follows the diagram from Christensen-Dalsgaard and Elepfandt (1995). **b** Measurements of sound transmission via the air-filled interaural pathway. The sound pressure level of monaurally presented stimuli was measured by the two DPOAE probe microphones. Sound

pressure levels are plotted in dB relative to the pressure level measured in the ipsilateral ear. Mean values (solid lines) and standard deviations (shaded areas) are shown. **(c–f)** Color maps representing the amplitude of the distortion product $2f_1-f_2$ simultaneously recorded at both ears. Data from one frog **(c, d)** alive and **(e, f)** after death recorded at **(c, e)** the left contralateral ear and **(d, f)** the right ipsilateral ear. The maps illustrate both DPOAE responses and system-generated distortion.

values from 0.4–4 kHz (in 0.1 kHz steps), and L_2 values from 50 to 98 dB SPL (in 2 dB steps). The amplitude of $2f_1-f_2$ DP for each stimulus frequency-level combination was represented in a color map. Threshold of DPOAE detection was defined as the levels of the primary-tones necessary to elicit $2f_1-f_2$ DP levels equivalent to -12 dB SPL. This criterion is, on average, equivalent to a $2f_1-f_2$ DP reaching a level of two standard deviations above the noise level near the $2f_1-f_2$ frequency. Considering DPOAEs arise in both papillae (Van Dijk and Manley 2001; Vassilakis et al. 2004), we evaluated the frequency of maximum sensitivity and the minimum threshold for low- and high-frequency primary-tone combinations corresponding to the amphibian and basilar papillae, respectively. Measurements from DPOAE audiograms and DPOAE threshold curves corresponding to both sexes were compared using a Mann-Whitney U -test ($p < 0.05$).

RESULTS

Distortion products were recorded in all ears investigated, ipsilateral, and contralateral to the acoustic stimulation. Sound transmission via the interaural recess was more efficient at lower frequencies and exhibited sex

differences (Fig. 1b). In males, frequencies below 2.2 kHz were less attenuated (<36 dB) than in females, and a minimum of attenuation (29 ± 2 dB) appeared around $f_1 = 1$ kHz. In females, attenuation was between 36 and 38 dB for frequencies below 1.5 kHz and increased monotonically at higher frequencies. Considering that sound transmitted from one coupler to the other was filtered through the tympanic disks covered by skin and fatty tissue, transfer functions calculated from the microphone recordings overestimate the ipsi-contralateral differences between the sounds arriving at the two inner ears where the DPOAEs originate. However, transfer functions calculated from dead animals yield similar attenuation levels (30–40 dB), and distortion products were indistinguishable from system noise at the contralateral ear, thus suggesting that system-generated distortions were sufficiently attenuated through the interaural recess and did not stimulate the contralateral ear. We cannot rule out that the emissions generated by amplification mechanisms in the right inner ear could reach the contralateral ear and contribute to stimulation along with attenuated primaries.

Distortion products recorded at the left (contralateral) ear of a live frog were not detected in the same ear after the animals' death, suggesting that

these distortion products are of physiological origin, i.e., DPOAEs (Fig. 1c, e). System-generated distortions were measurable only for primary tones above 94 dB SPL at frequencies below 0.7 kHz (Fig. 1e). Conversely, most of the distortion products recorded in live frogs at the ipsilateral ear were also present in the recordings made in dead animals, except for a small band of frequencies between 0.8–1.4 kHz (Fig. 1d, f). Control recordings with the probe placed against a hard, inanimate surface generated a similar color map to those recorded in two dead animals, thus verifying the pattern of system distortion. Therefore, further analyses are based on the contralateral DPOAE recordings.

The response (amplitude of $2f_1-f_2$ DP) matrices to a set of primary-tone frequency-level combinations are plotted as color maps shown in Figs. 1 and 2. From these color maps, frequency-threshold (tuning) curves are obtained by tracing out a fixed response criterion (–12 dB SPL) across the frequency range tested. *Xenopus* ears were sensitive to f_1 frequencies from 0.35–2.79 kHz (range across $n=9$ frogs). The DPOAE response area, as seen in the color matrix, is often limited by the minimum frequency used ($f_1 = 0.35$ kHz, $2f_1-f_2 = 0.3$ kHz), since the microphone noise precluded emission detection for lower frequencies. It is noteworthy that even for the lowest frequency measurable with our system ($2f_1-f_2 = 0.3$ kHz), the amplitude of this distortion product was distinguishable from background noise for low primary-tone levels (about 60 dB SPL), suggesting an extension of the hearing range of *X. laevis* to lower frequencies.

The DPOAE frequency response area exhibited two sensitivity peaks in both females and males (Fig. 2a, b). The analysis of the frequencies at which both peaks appeared suggests a sex-dependent tuning. In females, the best sensitivity in the lower frequency range, presumably corresponding to the AP, was found at a median of 0.73 kHz with a between-subject range of 0.55–0.91 kHz. The second sensitivity peak, presumably corresponding to the BP range, showed the best sensitivity at a median frequency of 1.58 kHz with a between-subject range of 1.49–1.58 kHz. In males, the median AP best sensitivity occurred at 0.45 kHz with a between-subject range of 0.35–0.53 kHz. The BP peak sensitivity for males was 1.14 kHz with a range of 1.09–1.18 kHz. Both the AP and the BP were tuned to significantly higher frequencies in females than in males (AP: $U=0.5$ $p=0.036$; BP: $U=0.000$ $p=0.016$; Fig. 2c).

Males and females did not differ in their minimum threshold of DPOAE detection corresponding to each papilla. However, when both sexes were considered together, we found significant differences between the thresholds of both auditory end-organs ($U=9.000$ $p=0.010$; Fig. 2c). The AP minimum threshold was

between 58 and 76 dB SPL, with a median value of 61 dB SPL. Minimum threshold for the BP was 76 dB SPL with a range of 62–80 dB SPL. These values are consistent with the higher thresholds expected in air relative to water.

The DPOAE audiograms depicting DPOAE levels as a function of the f_1 primary tone confirm the bimodal frequency dependence of the $2f_1-f_2$ emission (Fig. 3). A median response curve for each sex was obtained by determining the median response amplitude for each frequency across all subjects. Relative amplitude maxima below and above 0.88 kHz (range 0.70–0.96 kHz) in females, and 0.61 kHz (range 0.61–0.70 kHz) in males, reflect two spectral filters as derived from the AP and the BP. High level stimulation ($L_1 = L_2 = 90$ dB SPL) allowed recording of robust DPOAEs (>0 dB SPL), possibly generated by passive nonlinear responses in both papillae. The response corresponding to the BP (f_1 above the amplitude notch), reflected a difference in the primary frequency for which the DPOAE level was maximal for the females and males. We compared the frequency corresponding to the highest DPOAE response in the BP with the dominant frequencies in male and female calls. Visual inspection of the DPOAE audiograms shows that females tended to have the highest amplitude DPOAEs at a median frequency of 1.66 kHz, close to the dominant frequency of the male advertisement calls (between 1.7–2.3 kHz, Tobias et al. 2004). In contrast, for males, BP emissions reach a maximum amplitude at a median value of 1.14 kHz, close to the peak frequency of the female call (1.2 kHz, Tobias et al. 1998).

I/O Analysis

We explored the dependence of DPOAE amplitude on stimulus levels for the frequencies of highest sensitivity of both papillae. Examples of typical I/O functions for low- and high-frequency primary tones, corresponding to the AP and the BP respectively, are shown in Fig. 4. DPOAE I/O curves are evaluated by comparing the responses to low- and high-primary-tone levels (Meenderink and Van Dijk 2004; Vassilakis et al. 2004), with the division separating the two subgroups at the notch in the I/O function (typically between 65 and 75 dB SPL). The poor sensitivity of *Xenopus* to airborne sounds shifted this range to higher stimulus levels. Moreover, the inter-individual variability found in sensitivity confounded our ability to select a unique stimulation level as the border between subgroups. We calculated the slope of the DPOAE I/O curve at each data point by fitting a straight line through its two neighbors (i.e., the data points evoked with stimulus levels 2 dB above and below the data point in question). To delimit the two

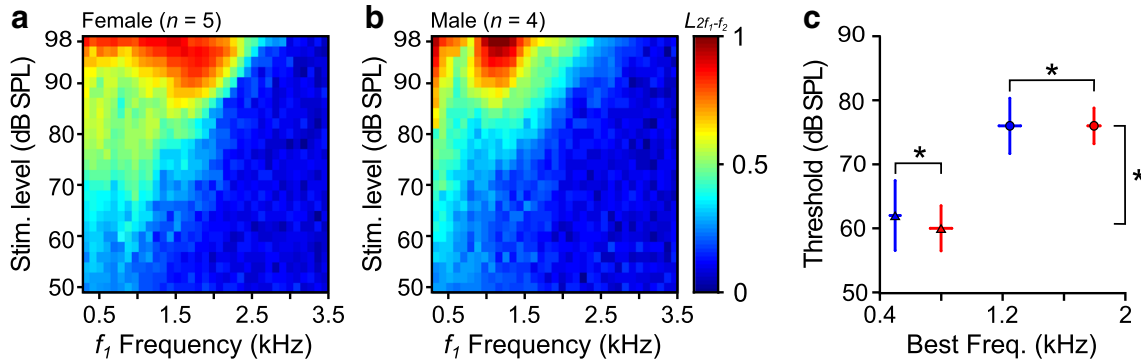


FIG. 2. Sex differences in frequency tuning in the inner ear of *Xenopus laevis*. DPOAE response matrices for (a) females and (b) males constructed with the median amplitude of $2f_1-f_2$ distortion product for each frequency-level combination from the normalized individual matrices. (c) Frequencies of best sensitivity and minimum

thresholds evaluated in the two sensitivity peaks found in the DPOAE response matrices of all individuals. Symbols represent the median values and bars represent the interquartile range. Asterisks denote differences of statistical significance ($p < 0.05$).

subgroups—responses to low- and high-primary tone levels—we identified the stimulus level where the region of decreasing slope values suddenly changed towards steeper, increasing slope values. At low- and intermediate-primary-tone levels (ca. 65–90 dB SPL), I/O functions from the AP exhibited a compressive nonlinearity with an extended plateau region in which the $2f_1-f_2$ amplitude leveled off, followed by a notch. In contrast, the I/O functions from the BP were monotonically increasing, with a narrow or absent plateau, and no notch. The average slope registered with low- and intermediate-stimulus levels was 0.16 dB/dB for the AP and 1.01 dB/dB for the BP. At primary-tone levels exceeding the plateau/notch region (above 90 dB SPL), I/O functions have a much steeper slope (2.45 dB/dB) for both papillae.

Binaural DPOAE recordings were also compared using the I/O functions (see Fig. 4). For the AP frequency range, DPOAEs from both ears had equivalent amplitudes (± 3 dB) for stimulus levels below 80 dB SPL. At higher levels of stimulation, the $2f_1-f_2$ amplitude at the ipsilateral ear approached the system distortion levels measured in control recordings (i.e., in dead animals or with the probe pressed against a

hard, inanimate surface). No consistent correlation was found between the ipsilateral and the contralateral responses at frequencies presumably detected by the BP.

DISCUSSION

According to the auditory matched filter hypothesis (Capranica and Moffat 1983), auditory processing in non-mammalian vertebrates (e.g., fishes, amphibians, reptiles, and to some extent birds) is dependent on extensive peripheral prefiltering. That is, the ears (auditory sensory filters) in these vertebrates are often tuned to signals of biological significance so that less post-processing is required by their central nervous systems. In contrast, mammals have the luxury of “taking in” a wide spectrum of sensory input and relying on cortical processing to extract the meaning from the message. Thus, according to one formulation of the matched filter hypothesis, the optimum receiver strategy for extracting the desired signals from the background noise would be to include a bias

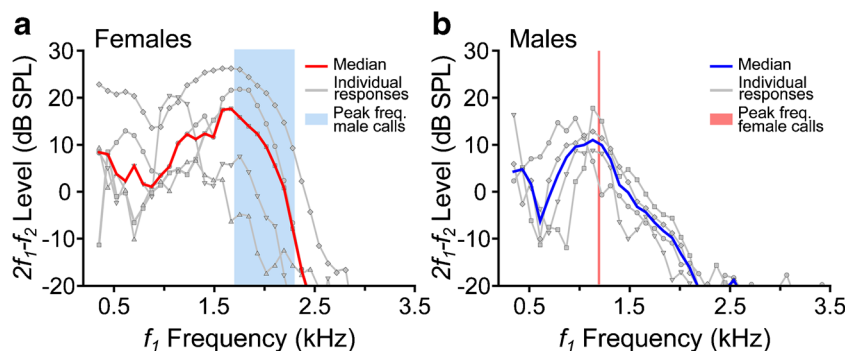


FIG. 3. DPOAE audiograms for (a) females and (b) males. The median $2f_1-f_2$ DP amplitude for each frequency was evaluated across same sex subjects to define the median response curve (solid line: red, females; blue, males). Individual responses are represented in gray. Stimulus parameters: $L_1 = L_2 = 90$ dB SPL and $f_2/f_1 = 1.14$

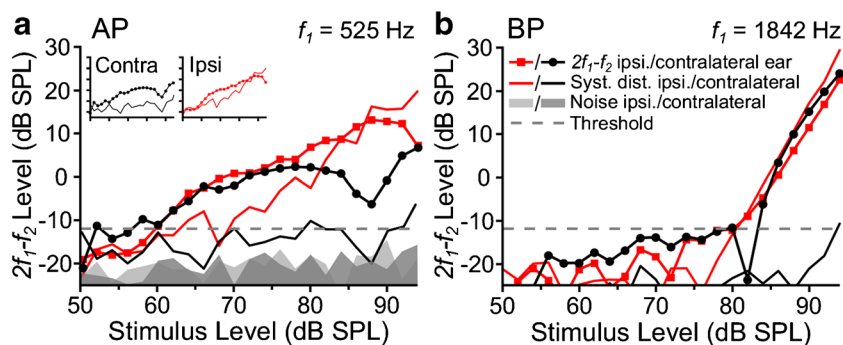


FIG. 4. DPOAE input/output functions representing the amplitude of the $2f_1 - f_2$ distortion product as a function of stimulus levels. Lines with symbols represent data recorded in a live frog for (a) $f_1 = 525$ Hz and (b) $f_1 = 1842$ Hz that correspond to DPOAEs originating from the

amphibian papilla and the basilar papilla, respectively. Solid lines represent system-generated distortions. Threshold of DPOAE detection was defined as $2f_1 - f_2 = -12$ dB SPL.

into the frequency response of the auditory system (Capranica and Moffat 1983; Wehner 1987). Rather than an unbiased, flat frequency response, the receiver's auditory system should have a frequency response that closely matches the envelope of the energy spectrum of the emitter's call (Narins and Clark 2016), a correlation that is well established in frogs (Gerhardt and Schwartz 2001; but see Zhao et al. 2017).

In the present study of *X. laevis*, the matched filter hypothesis is taken one step further—the frequency of highest amplitude of $2f_1 - f_2$ DP in females (1.66 kHz) roughly corresponds to the dominant frequencies in the male's call (1.7–2.3 kHz) whereas the frequency of highest amplitude of $2f_1 - f_2$ DP in males (1.14 kHz) closely approximates the dominant frequency in the female's call (1.2 kHz). In contrast to our findings, extracellular recordings in fibers from the auditory nerve and the dorsal medullary nucleus did not reveal gender differences in frequency sensitivity (Elliott et al. 2007). The neurons selective to the spectral range where sexual differences appeared in the present study, were pooled together with all the fibers with characteristic frequencies above 700 Hz in the study of Elliott et al. (2007). The apparent contrast between studies could be related to this difference in spectral categorization.

DPOAE recordings revealed peak hearing sensitivities in both males and females at frequencies slightly below the dominant frequencies of calls produced by the opposite sex. However, these small mismatches (<5%) should not affect the recognition of vocalizations. Broadband calls, as in the case of *Xenopus* clicks that have approximately 500 Hz bandwidth at -3 dB (Elliott et al. 2007), and broadly tuned auditory fibers such as BP afferent fibers (Manley and Van Dijk 2008), have a wide region of spectral overlap. Discrepancies of 15% or more between auditory tuning and the average dominant frequency in the advertisement call are often found in anuran species (Gerhardt and

Schwartz 2001). These authors suggested that a broad and relatively weak tuning could mediate frequency preferences that represent stabilizing or directional selection. Spectral mismatches found in hearing evaluation could be associated to call-frequency preferences among conspecifics. In female frogs of different species, a tendency to prefer call frequencies that deviate from the mean, specifically lower frequency calls, has been described (Ryan and Keddy-Hector 1992). Similar studies to evaluate call preferences in males would be limited to the few species in which females also vocalize.

Matched filters are ideal for extracting a known signal from background noise, but they require “knowing” something about the spectrum of the expected signal. In this case, evolution has sculpted the male's auditory apparatus to “expect” the female's call and vice-versa. This arrangement is especially useful in low-visibility environments with a high density of signalers, such as the natural habitats of this species during the breeding season (Tobias et al. 1998; Kelley and Tobias 1999). Examples of auditory sexual dimorphism among the invertebrates are relatively numerous, principally among the arthropods (Yager 1990; Bailey and Römer 1991; Hoy and Robert 1996). In contrast, examples of this phenomenon among vertebrates are rare. Narins and Capranica (1976) revealed the first example of a vertebrate (*Eleutherodactylus coqui*) with differences in auditory processing related to sex. They described a sexually dimorphic distribution of the best excitatory frequencies of primary auditory neurons. Sex differences in frequency tuning have been corroborated in a few species of frogs (Wilczynski et al. 1984; Wilczynski et al. 1992; Shen et al. 2011). In *Xenopus*, a study of auditory-evoked potentials described a higher sensitivity in females to the dominant frequencies in the male advertisement calls (Hall et al. 2016). Auditory-evoked potentials (AEPs) represent the overlapped output of synchronized neural activity in the

auditory nerve and brainstem (Loftus-Hills and Johnstone 1970; Schrode et al. 2014), but the relative contribution of these different electrical sources (inner ear hair cells, VIIIth nerve neurons and CNS auditory neurons) to the AEPs has not yet been identified in anurans. The site at which the sexually different spectral cues first arise in *X. laevis* was unclear. DPOAE generation, on the other hand, is associated with the nonlinear mechanics in the auditory hair-cell epithelia in the inner ear. Our results suggest this could be the locus in the auditory periphery where sex differences in hearing emerge. Sexual differences found in frequency tuning of the inner ear of *X. laevis* are in agreement with the data on the sexual dimorphism of the tympanic disk (Mason et al. 2009). Males exhibit larger tympanic disks relative to body size and have higher inner-ear sensitivity than females at lower frequencies.

The air-filled recess connecting the two middle ear cavities in *Xenopus* functions as an acoustical low-pass filter. Sound transmission via the interaural recess is more efficient at lower frequencies, and it also exhibits differences between sexes. These differences could be associated with sexual differences in size—males tend to be 20–30% smaller than females. Body size differences could imply variation in the cross-sectional area and length of the air-filled recess between the two ears, which in turn affect sound transmission and resonant frequencies in the air-filled cavity.

In frogs, the range of stimulus frequencies for which DPOAEs can be detected is correlated with the range of inner-ear sensitivity (Van Dijk and Manley 2001; Van Dijk et al. 2002; Meenderink et al. 2005). In *X. laevis*, DPOAEs appeared in a frequency range (0.35–2.79 kHz) coincident with the spectral sensitivity characterized in behavioral and neurophysiological studies (Wever 1985; Elepfandt et al. 2000; Elliott et al. 2011; Hall et al. 2016). DPOAEs were previously evaluated in two males of *X. laevis* (Van Dijk et al. 2002). These authors found emissions in a more limited frequency range (0.61–2.51 kHz), but it is important to note that they recorded ipsilateral to the acoustic stimulation, where the microphone noise and the measuring system distortion limited their ability to detect DPOAEs to levels above 5 dB SPL. In the Van Dijk et al. (2002) study, maximum DPOAE levels appeared at $f_1 = 1.2$ kHz in both males, coinciding with the dominant frequency in the female's call and close to the median value described in the present study (1.14 kHz). Inter-individual variation may have contributed to the slight differences found between the DPOAE studies.

Anurans' DPOAE audiograms illustrate a bimodal dependence on frequency with two amplitude maxima that have been correlated with maximum excitation in either the AP or the BP (Van Dijk and Manley

2001; Van Dijk et al. 2002; Vassilakis et al. 2004). There is a consensus that low-frequency DPOAEs originate from the AP, while higher-frequency DPOAEs are generated in the BP. *Xenopus* DPOAE audiograms have a bimodal shape resembling those of other frog species, e.g., *Hyla cinerea*, *Rana pipiens*, *Rana catesbeiana*. The two maxima in the *Xenopus* DPOAE audiograms appear at roughly the same frequencies as the minimum thresholds of extracellular recordings in auditory nerve fibers and dorsal medullary nucleus cells (Elliott et al. 2007). They are also spectrally coincident with the highest sensitivity calculated from the DPOAE threshold curves (see Figs. 2 and 3). It has been proposed that there is interference between emissions originated in both papillae near the frequency corresponding to the amplitude notch between the two maxima (Meenderink et al. 2005). Considering the frequency where the amplitude notch appeared in *Xenopus* DPOAE audiograms, the spectral selectivity of the AP extends between 0.35–0.88 kHz in females, and between 0.35–0.61 kHz in males. However, we do not rule out that the hearing range of *X. laevis* is extended to frequencies lower than the lowest frequency measurable with our system ($f_1 = 0.35$ kHz). Above the notch, DPOAE amplitude is dominated by emissions from the BP with higher selectivity to frequencies between 0.88–2.28 kHz in females, and 0.61–1.93 kHz in males.

Analysis of DPOAE input/output functions corroborated the generation of DPOAEs from both papillae. I/O functions with a compressive nonlinearity and an extended plateau, typical of the AP (Meenderink and Van Dijk 2004; Vassilakis et al. 2004), were found for stimulus frequencies lower than that corresponding to the amplitude notch in the DPOAE audiogram. For higher frequencies, the I/O functions were monotonically increasing with a narrow or absent plateau, as is expected from the BP response (Meenderink and Van Dijk 2004, 2005).

CONCLUSIONS

In most anuran species, acoustic communication is the primary mediator of sexual behavior. *X. laevis* is one of the few exceptions among frogs where both sexes vocally advertise their reproductive readiness. DPOAE recording in this species revealed a sexually dimorphic auditory tuning, each sex being more sensitive to the frequencies of the other sex's calls. Reciprocal matched filtering in the inner ear of *X. laevis* could allow both male and females to conserve time and energy for locating a receptive individual of the other sex, and thus it is likely to provide a reproductive advantage in noisy habitats.

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COMPLIANCE WITH ETHICAL STANDARDS

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

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