

## RESEARCH ARTICLE

# Seismic sensitivity and bone conduction mechanisms enable extratympanic hearing in salamanders

G. Capshaw<sup>1,\*</sup>, D. Soares<sup>2</sup>, J. Christensen-Dalsgaard<sup>3</sup> and C. E. Carr<sup>1</sup>

## ABSTRACT

The tympanic middle ear is an adaptive sensory novelty that evolved multiple times in all the major terrestrial tetrapod groups to overcome the impedance mismatch generated when aerial sound encounters the air–skin boundary. Many extant tetrapod species have lost their tympanic middle ears, yet they retain the ability to detect airborne sound. In the absence of a functional tympanic ear, extratympanic hearing may occur via the resonant qualities of air-filled body cavities, sensitivity to seismic vibration, and/or bone conduction pathways to transmit sound from the environment to the ear. We used auditory brainstem response recording and laser vibrometry to assess the contributions of these extratympanic pathways for airborne sound in atympanic salamanders. We measured auditory sensitivity thresholds in eight species and observed sensitivity to low-frequency sound and vibration from 0.05–1.2 kHz and 0.02–1.2 kHz, respectively. We determined that sensitivity to airborne sound is not facilitated by the vibrational responsiveness of the lungs or mouth cavity. We further observed that, although seismic sensitivity probably contributes to sound detection under naturalistic scenarios, airborne sound stimuli presented under experimental conditions did not produce vibrations detectable to the salamander ear. Instead, threshold-level sound pressure is sufficient to generate translational movements in the salamander head, and these sound-induced head vibrations are detectable by the acoustic sensors of the inner ear. This extratympanic hearing mechanism mediates low-frequency sensitivity in vertebrate ears that are unspecialized for the detection of aerial sound pressure, and may represent a common mechanism for terrestrial hearing across atympanic tetrapods.

**KEY WORDS:** Amphibian, Auditory, Evoked potentials, Sound, Vibration detection

## INTRODUCTION

Hearing mediates the detection, discrimination and localization of acoustic cues emitted by proximate and distant sources within the surrounding environment. For aquatic vertebrates, tissue density is nearly equivalent to that of the surrounding water and permits direct transmission of sound energy to the ear as particle motion. In contrast, in a terrestrial environment sound traveling in air encounters an impedance mismatch at the boundary of the skin, resulting in the majority of acoustic energy being reflected off an

animal. The tympanic middle ear evolved as a key sensory novelty to convert airborne sound pressure into particle motion within the inner ear by mechanically coupling a sound receptive surface (the tympanic membrane or tympanum) to the inner ear via the middle ear ossicles. Fossil evidence supports multiple independent origins of the tympanic middle ear in all terrestrial tetrapod lineages, indicating its significance for the perception of airborne sound (Christensen-Dalsgaard and Carr, 2008; Clack, 1997, 2002; Kitazawa et al., 2015); however, several vertebrate species lack, or have strongly reduced, tympanic middle ears including snakes and many other lizard species, ‘earless’ frogs, caecilians and salamanders (Wever, 1978a, 1985). These species rely on extratympanic pathways for sound transmission from the environment to the inner ear in order to detect sound in a terrestrial environment. Here, we use salamanders as a model to investigate the sensitivity of the atympanic ear to airborne sound pressure and seismic vibration and to assess the mechanisms underlying extratympanic sound transmission to the inner ear.


The amphibian tympanic middle ear consists of a flexible tympanum coupled to the inner ear by middle ear ossicles that span an air-filled middle ear cavity (Wever, 1985). The salamander ear is highly reduced compared with most anuran amphibians, and completely lacks the tympanum and middle ear cavity but retains a middle ear ossicle that occupies part of the oval window of the otic capsule, the stapes or columella (Wever, 1985). An additional middle ear element, the cartilaginous operculum, covers the remainder of the oval window (Wever, 1985). The salamander inner ear encloses several patches of acoustically sensitive sensory epithelia, including the saccular macula, an otolithic end organ sensitive to low-frequency sound and vibration, and two papillar end organs, the amphibian and basilar papillae, that are sensitive to low- and high-frequency sounds, respectively (Wever, 1985). Although the atympanic salamander ear is generally considered insensitive to airborne sound, salamanders are highly sensitive to seismic vibration and are capable of detecting high-energy, low-frequency sound pressure (Christensen et al., 2015a; Zeyl and Johnston, 2016, 2017). Several extratympanic mechanisms may play a role in the transmission of airborne sound energy from the environment to the inner ear in atympanic vertebrates such as salamanders, including close coupling of the inner ear to air-filled cavities that act as resonant chambers for the amplification of acoustic energy in a pathway analogous to the swim bladder of auditory specialist fish, bone conduction of sound, and detection of sound-induced seismic vibrations in the substrate.

In amphibians, air-filled cavities such as the lungs and mouth have been implicated as key components of extratympanic hearing pathways. When ensonified, these cavities may act as resonators that vibrate with maximal amplitude at a characteristic frequency determined by the enclosed volume. In several frog and salamander species, the body wall overlying the lungs was found to be responsive to sound pressure with peak vibratory amplitude

<sup>1</sup>Department of Biology, University of Maryland, College Park, MD 20742, USA.

<sup>2</sup>Department of Biological Sciences, New Jersey Institute of Technology, Newark, NJ 07102, USA. <sup>3</sup>Department of Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.

\*Author for correspondence (gcapshaw@umd.edu)

 G.C., 0000-0002-5688-2538; D.S., 0000-0002-8161-0841; J.C., 0000-0002-6075-3819; C.E.C., 0000-0001-5698-6014

comparable to that of the tympanum (Ehret et al., 1990, 1994; Hetherington, 1992, 2001; Hetherington and Lindquist, 1999; Jørgensen, 1991; Jørgensen et al., 1991; Lindquist et al., 1998; Narins et al., 1988). The midbrain response to airborne sound of the earless toad *Bombina orientalis* was reduced by 20–25 dB when the lungs were filled and the body wall was covered with silicone grease (Hetherington and Lindquist, 1999); however, this experiment resulted in a global diminishment of sensitivity thresholds rather than a reduction in sensitivity at a characteristic resonant frequency correlated with the volume of the lungs and/or body wall. The relationship observed between lung volume, body wall responsiveness and auditory sensitivity may have represented the metabolic effects of insufficient respiration on neural activity, as the animal was immobilized and reliant on cutaneous respiration that was probably impeded by covering the skin with grease. An additional study has indicated that removing the resonant responsiveness of the lungs and overlying body wall without influencing respiratory activity has no measurable effect on sensitivity thresholds in salamanders (Zeyl and Johnston, 2017). Similar to the lungs, the air-filled mouth cavity may act as a resonator when ensonified and has been found to influence the frequency response characteristics of the anuran auditory system (Chung et al., 1978). The mouth cavity also provides a more direct route for sound energy transfer to the ears through fewer layers of bone and tissue and, in minute earless frogs, encloses a larger volume of air than the lungs, which enables it to resonate nearer to the dominant frequency of the male advertisement call (Boistel et al., 2013).

Alternatively, extratympanic hearing may rely on bone conduction mechanisms to transfer acoustic energy from the environment to the inner ear. Bone conduction is the direct interaction between sound and the animal in which sound induces vibrations in the skull and body that are detectable by the auditory system. In its simplest form it is direct translation of the animal by the sound wave, but it may involve several pathways to ultimately stimulate the ear, including via inertial forces acting on the middle ear ossicles or on the inner ear fluids, and/or through sound-induced deformations of the skull. In species with mobile middle ear ossicles, vibrations passing through the head can result in relative motion of the ossicles and the adjacent cranial bones, a mechanism that is important for the reception of low-frequency signals in many terrestrial species (Bárány, 1938; Stenfelt, 2013; Tonndorf, 1972). The amphibian opercularis system, a unique anatomical coupling of the operculum of the middle ear to the pectoral girdle by a tonic muscle (Kingsbury and Reed, 1908; Monath, 1965; Wever, 1985), may aid in this pathway if acoustic energy impinging on the animal results in differential movement of the head relative to the body. Sound pressure may also induce compressive and expansive deformation of the otic capsules (Tonndorf, 1962, 1966; von Békésy, 1948), especially at higher frequencies (Stenfelt, 2013). Because the fluid enclosed within the inner ear may be considered incompressible, these vibrations result in fluid displacement through the relatively compliant fenestrations of the otic capsule, leading to particle motion that can stimulate the hair cells of the auditory epithelia.

Finally, vibration sensitivity may facilitate extratympanic hearing through the detection of seismic vibrations generated in the substrate by sound pressure stimuli. The amphibian opercular system has been implicated as an anatomical adaptation enabling the transmission of substrate-borne vibrations from the ground up through the forelimbs to the inner ear via the connection of the operculum to the tonic opercularis muscle (Hetherington et al., 1986; Lombard and Straughan, 1974; Mason, 2007). When the

opercularis muscle was experimentally severed, American bullfrogs (*Lithobates catesbeianus* syn. *Rana catesbeiana*) had a 6.5–13.1 dB reduction in seismic sensitivity (Hetherington, 1985, 1988). Furthermore, the amphibian opercular system has been of great interest as a terrestrial acoustical pathway because it achieves full development after metamorphosis and has not been observed in aquatic larvae or in paedomorphic species. However, the direct connection between the peripheral skeleton and the middle ear ossicles conferred by the opercular system may not be required for vibration-based acoustic sensitivity: larval axolotls that lack an opercularis system have comparable sound and vibration sensitivity thresholds to those measured in post-metamorphic individuals under the same experimental conditions (Christensen et al., 2015a). Vibration sensitivity has been shown to enable atympanic species to detect airborne sound pressure as sound-induced head vibrations (Christensen et al., 2012, 2015b), and a similar vibration detection mechanism may contribute to extratympanic detection of sound in air for salamanders.

Here, we used auditory brainstem response (ABR) recordings to measure the sensitivity of the atympanic salamander ear to airborne sound pressure. We employed a comparative approach to investigate potential extratympanic pathways for sound transmission to the inner ear, including the air-filled lungs and mouth cavity. We incorporated salamander species with lungs (family Ambystomatidae) and without (family Plethodontidae) as a natural comparison for the contribution of the lungs to extratympanic hearing, and tested sound pressure sensitivity before and after the resonant capacity of the mouth was experimentally obstructed. Additionally, we used ABR recordings to determine seismic vibration sensitivity and measured sound-induced substrate vibration to assess the contribution of seismic vibration detection to sound pressure sensitivity thresholds. Finally, we used laser Doppler vibrometry to examine the efficacy of sound pressure stimuli to induce detectable vibrations in the salamander skull as evidence for the role of bone conduction in the extratympanic detection of airborne sound.

## MATERIALS AND METHODS

We recorded ABRs in response to airborne sound pressure and seismic vibration stimuli in two ambystomatid species [*Ambystoma opacum* ( $N=7$ ) and *Ambystoma tigrinum* ( $N=8$ )] and six plethodontid (lungless) species [*Desmognathus fuscus* sp. ( $N=10$ ), *Eurycea cirrigera* ( $N=10$ ), *Eurycea lucifuga* ( $N=11$ ), *Gyrinophilus porphyriticus* ( $N=10$ ), *Plethodon cinereus* ( $N=24$ ) and *Plethodon glutinosus* ( $N=8$ )]. Plethodontid species are informative to our study because they are highly diverse and their inner ear morphology shows ecological trends in structural variation that may be functionally relevant for auditory sensitivity (Capshaw et al., 2019). In order to characterize the physiological variation that may correlate with morphological variation in this group, we included species representing a subset of the ecological diversity found in family Plethodontidae including two facultative cave-dwelling species (*E. lucifuga* and *G. porphyriticus*), two terrestrial, direct developing species (*P. cinereus* and *P. glutinosus*) and two semi-aquatic species (*D. fuscus* sp. and *E. cirrigera*). All animal handling and experimental procedures were performed in accordance with the University of Maryland Institutional Animal care and Use Committee, permitted under protocol numbers R-16-59 and R-SEP-19-41.

### Acoustic stimuli

We used a pure tone simultaneous masking technique (Brandt et al., 2018) to assess auditory sensitivity to a range of frequencies in

salamanders. We recorded ABRs in response to a broadband click stimulus in the presence and absence of a masking tone (Fig. 1); hereafter referred to as a mABR (*sensu* Brandt et al., 2018). Auditory sensitivity to the masking tone was calculated as the difference between the masked and unmasked response. This method ensures low-frequency specificity by using longer duration tones that include sufficient cycles of the sinusoid, compared with transient toneburst signals that introduce spectral artifacts ('frequency splatter') at low nominal frequencies (typically below 1 kHz) when the signals only contain very few cycles.

The click stimulus was generated using one half cycle of a 2000 Hz sinusoid to create a broadband stimulus with sufficient power across a frequency range of 10–1000 Hz to evoke a measurable click response. Clicks were presented at a level that elicited a neural response at 90% of the maximal amplitude from each individual to ensure a clear, measurable evoked potential. Click stimuli were overlaid with a pure tone masker presented at increasing levels until the click response was masked. We used pure tone maskers of 50–1200 Hz for testing sound pressure sensitivity, and 20–1200 Hz for testing vibration sensitivity. Masked and unmasked click responses were averaged over 800 presentations, and every second stimulus presentation was phase inverted to reduce artifacts.

#### Data collection and analysis

Salamanders were anesthetized with 20% w/v benzocaine applied to the ventral body surface. Evoked potentials were recorded using three Teflon-coated silver wire electrodes inserted subcutaneously: the recording electrode was placed dorsal to the left ear, approximating the location of the eighth cranial nerve, the inverting electrode was placed on the midline of the head dorsal to the brainstem, and the ground electrode was inserted into the tail. We adjusted electrode placement to optimize the signal-to-noise ratio for detection of the neural response.

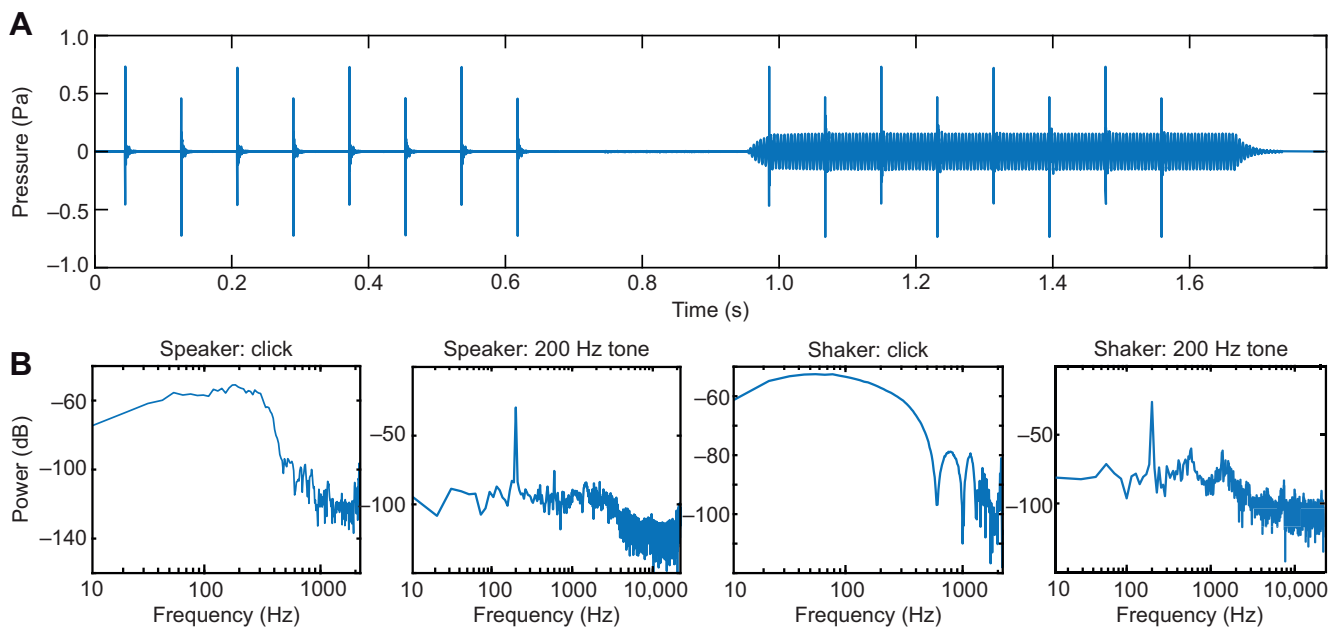
mABRs were recorded within a custom-built anechoic chamber lined with 500 mm acoustic foam wedges and shielded by a Faraday

cage. We generated stimuli and recorded neural responses using QuickABR, custom software developed by Christian Brandt (University of Southern Denmark, Odense, Denmark). Evoked potentials were amplified with a RA4PA Medusa pre-amplifier and recorded by a RM2 digital signal processor (Tucker Davis Technologies, Alachua, FL, USA) at a sampling rate of 24.4 kHz. Recordings were filtered using a 200 Hz high-pass and 2000 Hz low-pass Butterworth filter.

Seismic vibration stimuli were generated by a miniature vibration exciter (Bruel & Kjaer 4810 mini-shaker) connected to the RM2 via a conditioning amplifier (Bruel & Kjaer 1704). The anesthetized salamander was placed on a platform connected to the mini-shaker and oriented to apply sinusoidal vibrations along three orthogonal planes of the animal: the mediolateral (*x*), anteroposterior (*y*) and dorsoventral (*z*) body axes. The mini-shaker was calibrated using a piezoelectric accelerometer (Bruel & Kjaer 4381), which was itself calibrated using a calibration exciter (Bruel & Kjaer 4294) with an output of  $10 \text{ m s}^{-2}$  at 159.15 Hz.

Sound pressure stimuli were generated by a low-frequency loudspeaker (Electro-Voice TL606A, flat frequency response from 50 to 400 Hz) placed 1.5 m from the anesthetized salamander and driven by an RM2 mobile processor. The speaker was calibrated using a 0.5-inch condenser microphone (GRAS 40AP) with a constant current power supply (GRAS power module 12AK) placed at the approximate position of the salamander head. The microphone was calibrated using an acoustic calibrator (GRAS type 4230) with an output of 94 dB SPL at 1000 Hz. Because acoustic detection of substrate-borne vibration has been implicated as a primary mechanism for terrestrial auditory sensitivity in salamanders (Zeyl and Johnston, 2016), we measured the vibrations induced in the substrate by the speaker output at the position of the salamander using the piezoelectric accelerometer (Bruel & Kjaer 4381) connected to a charge to Deltatron converter (Bruel & Kjaer 2467-A).

Auditory sensitivity thresholds were determined as the lowest level at which the pure tone had a masking effect on the neural click



**Fig. 1. The acoustic stimuli used for masked auditory brainstem response recordings.** (A) Waveform representing a single stimulation cycle including an unmasked series of clicks of alternating polarity, followed by a series of clicks overlaid with a simultaneous 200 Hz pure tone masker. (B) Power spectra for the click stimulus and 200 Hz tone produced by the speaker (left-hand panels) and the vibration exciter (right-hand panels).



response, observed as a reduction in the amplitude of the click response in the presence of the masker. Thresholds were verified by visual inspection of evoked potential traces using MATLAB (version R2016b, MathWorks). Vibration thresholds represent the vector norm of evoked responses to sinusoidal stimuli traveling in all three planes, along the  $x$ -,  $y$ - and  $z$ -axes. Baseline sensitivity thresholds for sound pressure and seismic vibration were plotted against frequency to generate audiograms and vibrograms for all species. We compared auditory sensitivity across species using linear mixed models that incorporated sensitivity thresholds as the response variable, species, frequency, and the species by frequency interaction as fixed effects, and individual as a random effect. We tested the contributions of the air-filled lungs and of habitat-correlated morphological variation of the inner ear to observed differences in auditory sensitivity using linear mixed models that included the presence or absence of the lungs and species habitat type as fixed effects, and species and individual as nested random effects. We used Type III tests of fixed effects to evaluate significant differences among factors, followed by *post hoc* pairwise comparisons with a Bonferroni correction for multiple comparisons. Statistical analyses were performed in R (<https://www.r-project.org/>) using the package nlme (v 3.1-131; <https://CRAN.R-project.org/package=nlme>), and *post hoc* comparisons were performed using the package lsmeans (v 2.26-3; <https://cran.r-project.org/web/packages/lsmeans/index.html>).

We evaluated the effects of the air-filled mouth cavity on aerial sound detection by recording mABRs with the salamander's mouth opened and partially filled with water-soaked cotton gauze. The amount of gauze was adjusted for head size to ensure that the jaw was not hyperextended during experiments. In this way, we removed the resonant capacity of the buccopharyngeal cavity without damaging the jaw or disrupting cranial kinesis. We used linear mixed models in the R package nlme to compare sensitivity thresholds across experimental conditions relative to baseline mABR data (recorded prior to mouth-open mABR experimentation), incorporating experimental condition (mouth open/closed) and presence or absence of lungs as fixed effects, and individual as a random effect.

We assessed bone conduction of airborne sound energy by measuring the amplitude of head vibrations induced by free-field sound pressure stimulation using laser Doppler vibrometry. Laser vibrometry experiments were conducted in an IAC Acoustics anechoic room covered with 500 mm foam rubber acoustic wedges. Vibrations were measured in the horizontal plane using an OFV-5000 laser Doppler vibrometer with an OFV-505 sensor (Polytec, Waldbronn, Germany). Measuring sound-induced vibrations in the horizontal plane is consistent with the planes of orientation of the low-frequency vibration-sensitive saccular macula and amphibian papilla that are oriented in the frontal–vertical and horizontal planes, respectively. Sound pressure stimuli were 100 ms frequency sweeps (0.1–10 kHz at 81 dB SPL) generated by a JBL 1G loudspeaker placed 1 m from the animal, ipsilateral to the left ear. Laser Doppler measurements were recorded with the salamanders prone on a heavy steel platform to reduce the amplitude of vibrations induced in the platform by the sound stimuli. Sound pressure levels at the location of the salamander head were measured using a 0.5-inch probe microphone (BK 4182) 5 mm from the head. We also measured sound-induced vibrations in the platform 4–5 mm below the position of the salamander head as a control. Sound stimuli were generated and data were recorded and digitized using Tucker-Davis Technologies system 2 hardware and custom software (DragonQuest, Odense, Denmark). Sound and laser data were

recorded with a 22.64 kHz sampling rate and averaged over ten presentations. We used DragonQuest to create transfer functions of the amplitude of tissue displacement produced by sound pressure impinging on the left lateral surface of the salamander head. We compared transfer functions for sound and head vibrations with auditory sensitivity data in MATLAB, and assessed the significance of differences in these data using a non-parametric Wilcoxon signed rank test.

## RESULTS

Auditory evoked potentials recorded in salamanders show two to three prominent peaks that were elicited approximately 4–6 ms after the acoustic stimulus reached the salamander. Fig. 2 shows typical ABR waveforms recorded from an adult salamander in response to an unmasked click stimulus (Fig. 2A) and to the click stimulus overlaid with a 100 Hz pure tone masker (Fig. 2B).

### Seismic vibration sensitivity

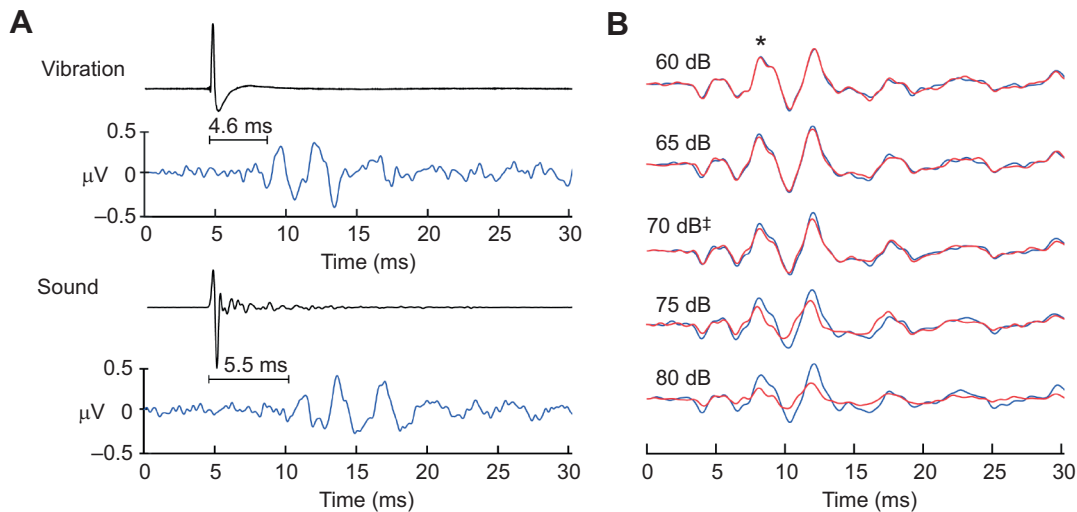
The average mABR-derived vibrograms for the eight salamander species tested demonstrate a characteristic U-shape with a frequency bandwidth from 20 to 1200 Hz (Fig. 3A). Intraspecific vibration sensitivity curves are relatively flat from 20 to 80 Hz, achieving peak sensitivity between 80 and 200 Hz and declining rapidly at frequencies above 250 Hz. We were unable to elicit an auditory response to vibration stimuli greater than 1200 Hz. Because all thresholds were at least 14 dB above ambient vibration noise levels, quantified as the vector sum of vibrational noise in all three planes, vibration sensitivity curves were unlikely to have been influenced by octave noise during ABR recording.

Variation in seismic sensitivity was both species and frequency dependent (species:  $F_{7,859}=115.08$ ,  $P<2.2\times 10^{-16}$ ; species $\times$ frequency:  $F_{77,859}=5.47$ ,  $P<2.2\times 10^{-16}$ ), with greatest differences in sensitivity occurring for frequencies below 400 Hz. There were significant differences in vibration detection thresholds for frequencies below 400 Hz when comparing facultative cave species with all other species ( $F_{33,826}=9.56$ ,  $P<2.2\times 10^{-16}$ ). The facultative cave species *E. lucifuga* and *G. porphyriticus* had nearly equivalent best sensitivities at 150 Hz of  $-59.2$  and  $-58.5$  dB re.  $1\text{ m s}^{-2}$ , respectively, approximately 10 dB lower than the next best species' peak sensitivity (*P. cinereus*:  $-49.5$  dB re.  $1\text{ m s}^{-2}$  at 150 Hz).

### Sound pressure sensitivity

Fig. 3B compares mean threshold sensitivity to airborne sound pressure for the eight species tested. All audiograms show peak sensitivities occurring at approximately 100 Hz and 200–250 Hz, and a rapid decline in sensitivity to frequencies above 250 Hz. Similar to seismic sensitivity, variation in sound pressure sensitivity was species and frequency dependent (species:  $F_{7,782}=46.29$ ,  $P<2.2\times 10^{-16}$ ; species $\times$ frequency:  $F_{77,782}=9.45$ ,  $P<2.2\times 10^{-16}$ ), with greatest differences in sensitivity occurring for frequencies below 400 Hz. There were significant differences in sensitivity thresholds for tones below 1000 Hz when comparing facultative cave species with all other species ( $F_{33,719}=12.65$ ,  $P<2.2\times 10^{-16}$ ). *Eurycea lucifuga* demonstrated the lowest aerial sound detection thresholds with a mean sensitivity of 56.9 dB re. 20  $\mu\text{Pa}$  at 100 Hz. All thresholds were at least 16 dB above octave noise levels in the anechoic chamber; however, ambient noise notably increased for frequencies below 200 Hz relative to higher frequencies and it is therefore possible that sensitivity curves are somewhat masked for low frequencies.

The presence of air-filled lungs did not enhance the frequency range of sensitivity to airborne sound pressure in salamanders, nor



**Fig. 2. Representative auditory brainstem response waveforms.** (A) Click response measured using seismic vibration (top) and sound pressure stimuli (bottom). All waveforms represent the average of 800 stimulus presentations. (B) mABR waveforms recorded from *Eurycea cirrigera* in response to seismic vibration stimulation using a simultaneous 100 Hz pure tone masker. Masked (red) and unmasked (blue) click response waveforms were overlaid and scaled to a stimulus onset at 0 ms; threshold (indicated with a ‡) was determined as the lowest level that the tone had a masking effect on the first prominent peak of the click response that was clearly discriminable from noise (indicated with an asterisk).

did it confer more favorable detection thresholds. In contrast, we observed that lungless plethodontid species were more sensitive to sound pressure stimuli below 250 Hz ( $F_{10,719}=5.15$ ,  $P=2.8\times 10^{-7}$ ). This pattern appears to be driven by the sensitivity of the facultative cave-dwelling species *E. lucifuga* and *G. porphyriticus*, which have best thresholds 20 to 25 dB lower than the best thresholds of ambystomatid species that possess lungs (Fig. 3A). Additionally, there were no significant differences in sensitivity thresholds obtained via mABR with the mouth open or closed ( $F_{1,585}=4.63$ ,  $P=0.992$ ), indicating that the contribution of the air-filled mouth cavity to airborne sound detection is negligible in salamanders. This pattern remained consistent when comparing the effects of the mouth cavity on airborne sound detection among lungless species to species with lungs ( $F_{1,585}=0.27$ ,  $P=0.587$ ).

In order to determine if the observed auditory responses to airborne sound stimuli could be attributed to seismic sensitivity, we measured the amplitude of substrate vibrations induced by threshold-level sound pressure stimuli at the position of the salamander and compared these with species-specific vibration detection thresholds (Fig. 3C). We calculated relative substrate vibration by subtracting the vibrations generated in the floor of the anechoic chamber by sound stimuli emitted by the speaker from the mean vibration detection thresholds for each species and for each frequency. The total amplitude of substrate vibrations generated by threshold-level sound pressure stimuli, calculated as the vector norm of the amplitudes measured in the  $x$ -,  $y$ - and  $z$ -axes, were generally greatest at low frequencies between 50 and 150 Hz. The amplitudes of sound-induced substrate vibration remained below seismic sensitivity thresholds for all frequencies tested, indicating that acoustic stimuli emitted by the speaker during ABR recording experimentation did not produce detectable vibrations in the substrate.

### Sound-induced head vibrations

We measured vibrations generated in the salamander head by a frequency sweep sound pressure stimulus using a laser Doppler vibrometer and calculated transfer functions (in dB re.  $1\text{ mm s}^{-1}\text{ Pa}^{-1}$ ) by comparing the vibration velocity recorded by the vibrometer with the sound pressure level of the stimulus at the location of the

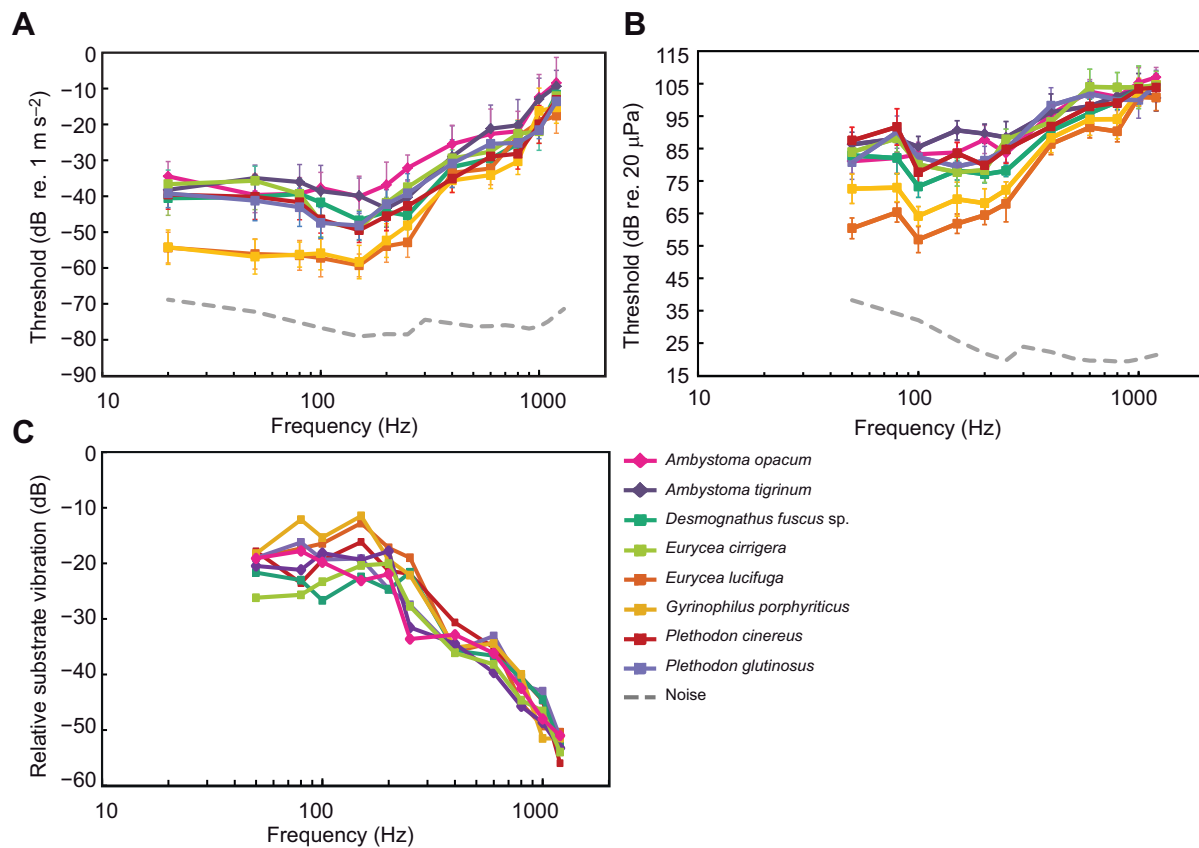
salamander. The average transfer functions between the sound stimulus and measured head vibrations for all species (Fig. 4) indicate that airborne sound pressure is sufficient to induce a greater amplitude of vibration velocity of the salamander head compared with the steel platform for all frequencies below 600 Hz. From 10 to 600 Hz, sound pressure stimuli generated vibrations in the salamander that were 6–20 dB greater than those generated in the steel recording platform, 5 mm below the salamander head. Vibration velocity amplitudes decreased with increasing stimulus frequency, with maximum displacement occurring between 10 and 50 Hz and minimum displacement between 800 and 1000 Hz for all species tested.

From these transfer functions, we calculated the equivalent head vibrations induced by threshold-level sound pressure and compared these with mean vibration detection thresholds for each species. The amplitudes of head vibrations induced by airborne sound pressure generally approximate vibration sensitivity curves for all species tested (Fig. 5). Low-frequency sound pressure below 150 Hz induced head vibrations above seismic sensitivity thresholds for all species, ranging from a 2 dB difference at 50 Hz in *E. cirrigera* to a 20 dB difference at 80 Hz in *P. cinereus*. Head vibrations induced by sounds below 100 Hz were significantly above threshold for all species except for *A. tigrinum* were 4–10 dB below threshold, although these deviations from threshold were not significant ( $P>0.05$  for all comparisons).

### DISCUSSION

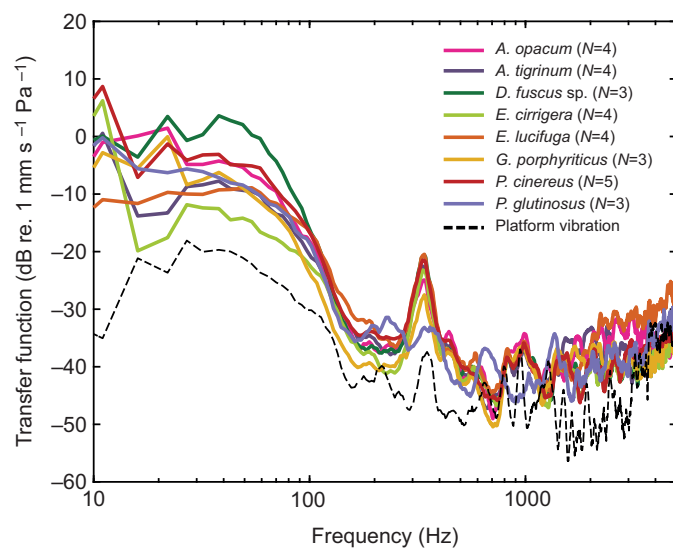
In this study, we used auditory brainstem response recording to demonstrate that salamanders are sensitive to sound pressure in air, with two peaks in sensitivity generally occurring at 100 Hz and from 200 to 250 Hz (Fig. 3B). We also found that salamanders are highly sensitive to seismic vibration, with best sensitivity to low frequencies between 80 and 200 Hz (Fig. 3A), and that the sound sensitivity can be largely explained by sensitivity to sound-induced vibrations of the head (Fig. 5).

The amphibian inner ear contains up to three acoustically sensitive end organs: the amphibian papilla, the basilar papilla, and



**Fig. 3. Audiograms of eight salamander species.** Sensitivity thresholds to (A) seismic vibration and (B) sound pressure stimuli. Thresholds signify the lowest masker level to reduce the click response. Values are means  $\pm$  s.e.m. Octave noise levels are indicated by a dashed line. (C) Amplitude of vibrations generated in the substrate by mean threshold-level sound pressure stimuli relative to mean vibration sensitivity thresholds for each species, demonstrating that sound-induced substrate vibration amplitudes are sub-threshold for all frequencies tested.

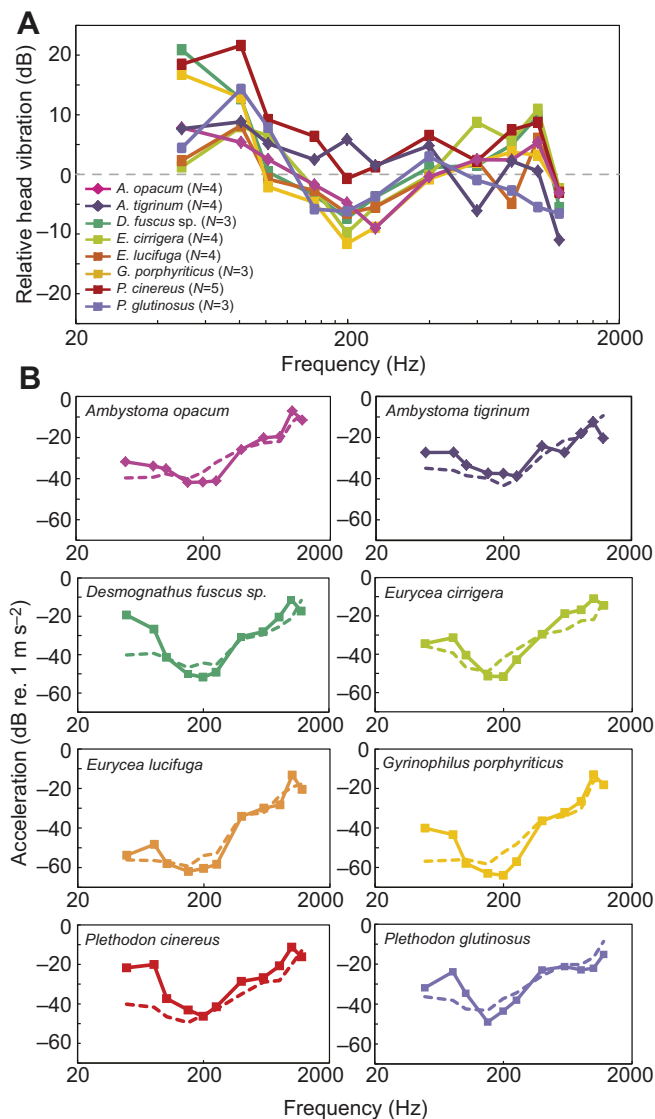
the saccular macula (reviewed by Smotherman and Narins, 2000). In anuran amphibians for which physiological studies have been conducted, the amphibian papilla and basilar papilla demonstrate



**Fig. 4. Average amplitude of sound-induced vibrations in the lateral surface of the salamander head.** Transfer functions were measured in response to a frequency-modulated sweep (0.1–10 kHz) broadcast ipsilaterally to the recording ear.

distinct frequency responses with best sensitivities to mid-frequencies from 100 to 1250 Hz and to high frequencies from 2000 to 4500 Hz, respectively (Capranica and Moffat, 1974, 1975; Feng et al., 1975; Frishkopf and Geisler, 1966; Liff, 1969; Moffat and Capranica, 1974; Narins and Capranica, 1976; Ronken, 1991; Sachs, 1964; Smotherman and Narins, 2000; Wilczynski et al., 1984). The basilar papilla is reduced in salamanders (or absent, as is the case for all plethodontid species), and so the amphibian papilla is typically considered to be of greater functional significance (Mullinger and Smith, 1969; Smith, 1968). In our results, we found a peak in sound pressure sensitivity from 200 to 250 Hz that falls within the frequency response bandwidth of the amphibian papilla. High sensitivity at lower frequencies (e.g. the peak in sound pressure sensitivity observed at 100 Hz) is most likely imparted by the saccule, which is sensitive to low-frequency sound and vibration from 20 to 150 Hz in most amphibians in which it has been studied (Christensen-Dalsgaard and Jørgensen, 1988; Christensen-Dalsgaard and Narins, 1993; Koyama et al., 1982; Lewis, 1988; Moffat and Capranica, 1976; Ross and Smith, 1980; Yu et al., 1991).

The average detection thresholds and range of sensitivity for airborne sound pressure and seismic vibration reported here are similar to those from other recent studies of salamander auditory function. Christensen et al. (2015a) measured similar sound pressure sensitivity curves in adult tiger salamanders (*A. tigrinum*) and metamorphosed axolotls (*Ambystoma mexicanum*) with peak sensitivities ranging from 78 to 83 dB re. 20  $\mu$ Pa at 80 and 320 Hz



**Fig. 5. Relationship between sound-induced head vibrations and seismic sensitivity.** (A) Head vibrations induced by threshold-level sound pressure relative to seismic vibration sensitivity for eight salamander species. The dashed line denotes equivalent values for head vibration and threshold seismic sensitivity; values above and below this line indicate super- and sub-threshold head vibrations, respectively. (B) Sound-induced head vibrations (continuous lines) compared with seismic vibration sensitivity (dashed lines) for each species.

that are well matched to the aerial sound sensitivity curves from *A. tigrinum* in the present study. A comparative study by Zeyl and Johnston (2016) incorporated a similarly diverse sampling of species, and reported lower sound pressure detection thresholds at best frequency among *Eurycea* species relative to other species tested, which corroborates our findings here; however, Zeyl and Johnston found peak sensitivity for *Eurycea* species to occur at higher frequencies (400–500 Hz) compared with those observed in the present study. Differences in sensitivity across studies may be due to the different methods used for recording evoked potentials. Achieving accurate neurophysiological recordings in response to low-frequency sound is difficult due to the prevalence of low-frequency environmental noise and the difficulty of generating a pure tone for long wavelength stimuli. Here we used a masked ABR recording paradigm that incorporated a longer duration acoustic

stimuli which may produce divergent results compared with ABR recordings using transient stimuli, particularly for low frequencies where short duration stimuli may introduce spectral artifacts that influence assessments of auditory response to pure tones.

Zeyl and Johnston (2016) also noted that the increased terrestriality of *P. glutinosus* did not correlate with enhanced sensitivity to airborne sound, as their *Plethodon* audiogram closely matched those of the semi-aquatic *Eurycea* species and pedomorphic *Ambystoma talpoideum*. We observe similar results: aerial sound pressure sensitivity curves are consistent across species that inhabit ecologically diverse microhabitats, including among terrestrial *Plethodon* species, semi-aquatic *Eurycea* and *Desmognathus* species, and fossorial *Ambystoma* species. However, we observed that facultative cave-dwelling species *E. lucifuga* and *G. porphyriticus* are significantly more sensitive to low-frequency sound and vibration compared with other species. A previous assessment of morphometric variation in the salamander ear found evidence for hypertrophy of the pars inferior of cave-dwelling species relative to closely related epigeal species (Capshaw et al., 2019). The results of our physiological study reveal variation in salamander auditory sensitivity, indicating that the hypertrophic pars inferior may support an expansion of the saccule to accommodate greater numbers of sensory hair cells or an increase in the size of the otoconial mass overlying the saccular macula that could enhance responsiveness to lower frequencies (De Vries, 1950; Lychakov and Rebane, 2000). A comparative histological investigation of the salamander auditory end organs is necessary to confirm a microstructural basis for the observed correlation between the morphology of the inner ear and physiological performance of the auditory system.

#### Comparisons of auditory performance among atympanic vertebrates

In a terrestrial environment, atympanic salamanders can detect acoustic energy as both vibrations in the substrate and as sound pressure-induced vibrations of the head and body. This mechanism is similar to that used by snakes to detect aerial sound (Christensen et al., 2012), and vibration sensitivity curves in salamanders are comparable to those measured in snakes (e.g. *Crotalus viridis*: best sensitivity of approximately  $-62$  dB re.  $1 \text{ m s}^{-2}$  at 200 Hz; Hartline, 1971; *Python regius*: best sensitivity of  $-54$  dB re.  $1 \text{ m s}^{-2}$  at frequencies of 80 and 120 Hz; Christensen et al., 2012) (see Fig. 6). Salamanders are less sensitive to seismic vibrations relative to eared and earless toads (family Bufonidae) that respond to 300 Hz vibrational stimuli down to approximately  $-65$  dB re.  $1 \text{ m s}^{-2}$  (Womack et al., 2017); however, data are not available for bufonid sensitivity to vibrations below 200 Hz, and so we are unable to make direct comparisons for these frequencies. Seismic sensitivity measured in several frog species is generally much greater than that observed in salamanders, with best thresholds ranging from 0.01 to  $0.28 \text{ cm s}^{-2}$  ( $-80$  to  $-51$  dB re.  $1 \text{ m s}^{-2}$ ) recorded from low-frequency fibers originating from the amphibian papilla in the grass frog, *Rana temporaria* (Christensen-Dalsgaard and Jørgensen, 1996), to  $0.01$ – $1.0 \text{ cm s}^{-2}$  ( $-80$  to  $-40$  dB re.  $1 \text{ m s}^{-2}$ ) recorded from the amphibian papillar and saccular fibers of the northern leopard frog, *Lithobates (Rana) pipiens*, and the white-lipped frog, *Leptodactylus albilabris*, in response to 10–80 Hz vibrations (Christensen-Dalsgaard and Narins, 1993). In general, terrestrial atympanic vertebrates demonstrate better sensitivity to sound pressure relative to the lungfish (*Protopterus annectens*; Christensen et al., 2015b), which has aquatically adapted otolithic ears. In all terrestrial species, the addition of a papillar end organ



sensitive to acoustic energy confers lower detection thresholds and a greater frequency response range for airborne sound.

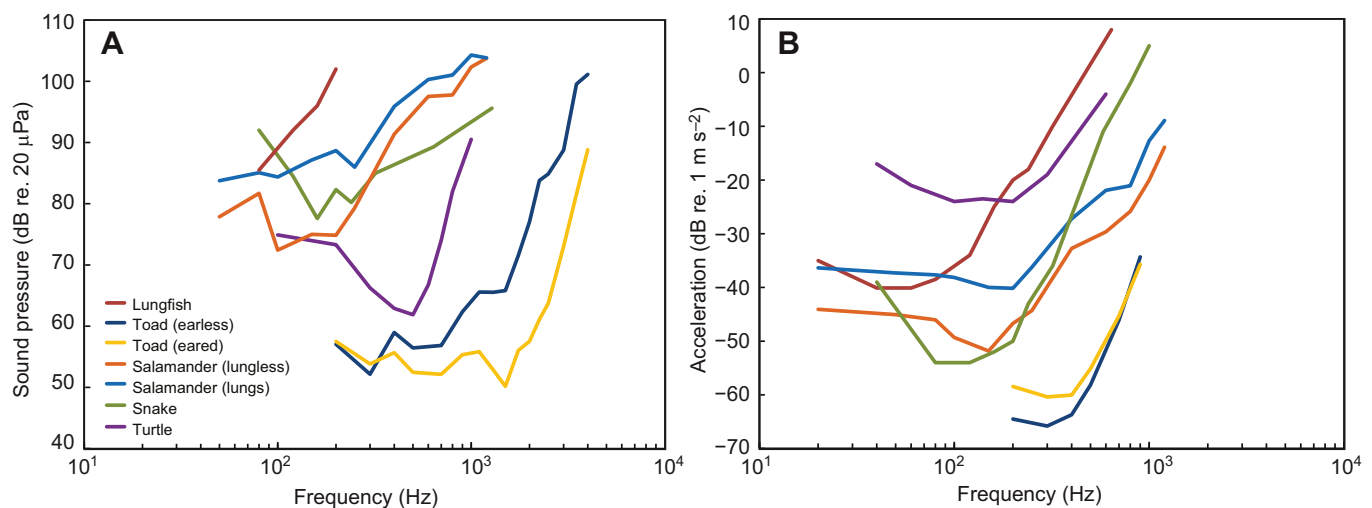
### Pathways for airborne sound transmission to an atympanic ear

The main objective of our study was to evaluate the extratympanic pathways that enable auditory sensitivity to airborne sound pressure in atympanic salamander species. To understand the mechanistic basis for hearing in the absence of an impedance-matching tympanic ear, we evaluated several proposed pathways for the extratympanic transmission of sound energy from the environment to the inner ear in salamanders including seismic sensitivity, cavity resonance and bone conduction of sound. In a terrestrial environment, sound energy is transmitted in the air as a pressure wave and through the substrate as vibrations; therefore it is likely that seismic vibration detection greatly influences auditory sensitivity in salamanders, especially at threshold levels for sound pressure detection. We observed that, although auditory sensitivity in salamanders is restricted to high-amplitude, low-frequency sounds, threshold-level sound pressure stimuli used during ABR experimentation generated vibrations in the substrate of the anechoic chamber that were sub-threshold for seismic detectors in the ear. Therefore, the sensitivity of the salamander auditory system to airborne sound pressure is not dependent on the ability to detect substrate vibrations, although seismic sensitivity imparted by the saccule probably contributes substantially to salamander auditory function under naturalistic conditions where substrate vibrations are not experimentally minimized.

In anuran amphibians with and without tympanic middle ears, sound detection has been linked to the resonance of the air-filled lungs (Ehret et al., 1990; Ehret et al., 1994; Hetherington, 1992, 2001; Hetherington and Lindquist, 1999; Jørgensen, 1991; Jørgensen et al., 1991; Lindquist et al., 1998; Narins et al., 1988) and mouth cavity (Boistel et al., 2013; Chung et al., 1981). In many of these studies, however, sound transmission from the lungs provided acoustic input to the ear through modulation of the inner surface of the tympanum, and therefore the lung–ear route in these

species may not be considered truly extratympanic (Ehret et al., 1990, 1994; Jørgensen, 1991; Jørgensen et al., 1991; Narins et al., 1988). The pathway for sound transmission from the lungs to the inner ear has not been experimentally determined in atympanic amphibians, although several have been proposed. For example, sound-induced vibrations in the lungs may travel through the mouth cavity to the inner ear via the round window (Hetherington and Lindquist, 1999), through endolymphatic sacs that extend into the vertebral canal (Narins et al., 1988), or through the perilymphatic system that protrudes into the cranial cavity (Hetherington, 2001). Here, we compared auditory function of lungless plethodontid species with ambystomatid species that have lungs and found that the presence of air-filled lungs did not confer an extended frequency range of sensitivity or lower detection thresholds to sound pressure or seismic vibration. On average, lungless plethodontid species were more sensitive to both sound pressure and seismic vibration, particularly for frequencies below 400 Hz; however, this pattern was largely driven by the facultative cave-dwelling species incorporated into our study. In general, the sensitivity curves of species with and without lungs were well matched. The minimal role of the air-filled lungs for aerial sound detection in our sample is in agreement with the conclusions presented by Zeyl and Johnston (2017) using salamanders and by Womack et al. (2018) in harlequin frogs (genus *Atelopus*) with reduced or absent tympanic middle ears. Additionally, experimental removal of the resonant capacity of the air-filled mouth cavity had a negligible influence on sound pressure sensitivity thresholds for species with and without lungs.

The enclosed volumes of the lungs and the mouth cavity are quite small in amphibians and are predicted to resonate at high frequencies. For example, the total enclosed volumes of the mouth cavity and Eustachian tubes measured in two ranid frog species impart intrinsic resonance frequencies of approximately 1.8–2.0 kHz that match the peak amplitude of vibration of the tympanic membrane at 1.7 kHz (Chung et al., 1981) and the high-frequency sensitivity peak of the auditory response in these species (Pettigrew et al., 1981). The resonance of the mouth cavity therefore contributes to the frequency response of the anuran



**Fig. 6. Comparative auditory sensitivity to sound pressure and seismic vibration.** (A) Audiograms representing sound pressure sensitivity; (B) vibrograms representing seismic vibration sensitivity. Data represent mean detection thresholds in response to acoustic stimuli in ambystomatid (with lungs) and plethodontid (lungless) salamander species from the present study, compared with thresholds measured in African lungfish (*Protopterus annectens*; Christensen et al., 2015b), eared and earless toads (multiple species within the family Bufonidae; Womack et al., 2017), royal python (*Python regius*; Christensen et al., 2012) and red-eared slider (*Trachemys scripta elegans*; Christensen-Dalsgaard et al., 2012). Variation in sensitivity may occur due to methodological differences and threshold criteria across studies.



auditory system, which is characterized by several structures that support high-frequency hearing including a flexible tympanum and a basilar papilla specialized for detection of high-frequency sound. In contrast, the basilar papilla is reduced or entirely absent in salamanders and so an equivalent ensoufflement of the lungs and/or mouth cavity would induce high-frequency resonance above the physiological limits of, and therefore undetectable by, the salamander auditory system (see also Goutte et al., 2017). Accordingly, we observed no significant physiological differences in auditory function when comparing aerial sound detection thresholds of species with and without lungs, and before and after the occlusion of the mouth cavity. We conclude that cavity resonance does not contribute significantly to the ability to detect airborne sound pressure in salamanders.

Finally, we demonstrated that sound pressure is able to generate vibrations in the salamander head that are of greater amplitude than the vibrations induced in the underlying substrate. The acoustical size of an animal, determined as the product of the wave number  $k$  ( $2\pi$  divided by the wavelength of the incident sound) and body size  $a$  (radius of the head), may be used to predict whether an animal is transparent to sound ( $ka \ll 1$ ) or if it reflects sound ( $ka \gg 1$ ). Small animals such as salamanders have a  $ka$  ranging from 0.009 to 0.027 at frequencies from 100 to 300 Hz, and so low-frequency airborne sound can effectively produce a translational displacement of the animal's body. If the sensory organs of the inner ear are capable of detecting these displacements, sound-induced translation may represent a key mechanism by which aerial sound pressure can stimulate an atympanic auditory system. We measured sound-induced translation in salamanders and observed that the amplitude of the vibrations generated in the salamander head approximates the seismic sensitivity curves that we obtained using evoked potential recordings, and therefore are detectable to the salamander auditory system (Fig. 5). We conclude that auditory sensitivity in atympanic salamander species is a matter of sensitivity to sound-induced vibrations, and terrestrial extratympanic hearing can be accomplished via bone conduction of sound from the environment to the inner ear.

### Extratympanic sound transduction in the inner ear of salamanders

In this study we show that sensitivity to airborne sound in salamanders lacking an impedance-matching tympanic ear is not dependent on detection of substrate-borne vibrations and is unaffected by the resonance of air-filled body cavities. Rather, threshold-level sound pressure is sufficient to induce a translational movement in the animal that is detectable by the auditory system as sound-induced vibration of the head (i.e. bone conduction). Once sound-induced vibrations are generated in the head, they may be transmitted to the inner ear via relative movement of the stapes (ossicular inertia), deformation of the bony otic capsules, and/or fluid inertia within the inner ear that enables mechanical transduction of acoustic energy into a neural signal by sensory hair cells (reviewed by Stenfelt, 2013).

Auditory sensitivity in atympanic species such as salamanders is generally restricted to frequencies below 1 kHz, and extensive study has shown that at such low frequencies the skull constitutes a rigid body that is translated in place as a single unit, with the middle ear ossicles vibrating in phase with the surrounding cranial bones (Stenfelt, 2011, 2016; Stenfelt et al., 2002). Therefore, ossicular inertia is unlikely to be a relevant mechanism for hearing in salamanders. We note that our previous morphological study of the plethodontid otic region did not show any significant differences in the size of the middle ear ossicle across diverse species: its mass and

volume was low even among fossorial and cave species that demonstrate other signatures of hypertrophy in the ear (e.g. an enlarged pars inferior) (Capshaw et al., 2019). Similarly, effective transduction of acoustic energy via sound-induced compression and expansion of the otic capsules is limited to wavelengths less than 10 times the size of the otic capsule (Stenfelt, 2011, 2016; Stenfelt and Goode, 2005). The salamander otic capsule can be approximated as a sphere with a diameter ranging from 1 to 2 mm (based on measurements described in Capshaw et al., 2019), and so the lowest effective frequency for the distortional component of bone conduction is roughly 17 kHz – far beyond the physiological limits of the salamander auditory system.

Although ossicular inertia and deformation of the otic capsules are unlikely to contribute to low-frequency extratympanic hearing in salamanders, acoustic energy traveling as vibrations in the surrounding bone and tissue in the salamander's head can induce inertial movement of the fluid enclosed within the otic capsule. Fluid inertia has also been suggested as the main transduction mechanism for bone-conducted sound in humans (Stenfelt, 2015). Generation of a fluid pressure wave within the ear is dependent on the presence of compliant fenestrations of the otic capsule to permit fluid pressure relief within the ear. Most terrestrial species possess two such fenestrations: the oval window that serves as an inlet for acoustical energy and the round window that serves as a fluid pressure outlet. The round window is absent in salamanders and fluid pressure relief occurs at the perilymphatic foramen on the medial wall of the otic capsule, allowing energy to dissipate into the brain cavity (Smith, 1968; Wever, 1978b). Otolithic organs like the saccular macula are readily stimulated by fluid inertia that displaces the overlying otoconial mass, shearing it against the sensory hair cells. In contrast, the amphibian papilla is not an otolith organ but is overlain with a gelatinous tectorium, yet fluid inertia within the ear due to extratympanic transmission of free-field sound may still be capable of stimulating the hair cells of the amphibian papilla. Smith (1968) observed that the location of the amphibian papilla adjacent to the perilymphatic foramen in the salamander inner ear permits high-amplitude fluid displacements over the hair cells of the amphibian papilla in response to vibrational stimulation and probably also to sound-induced head vibrations.

### Evolutionary implications of extratympanic hearing

The origin of modern amphibians (Lissamphibia) remains an area of active research characterized by several competing hypotheses, including (1) the temnospondyl hypothesis, which proposes that Lissamphibia is a monophyletic group derived from temnospondyl ancestors, (2) the lepospondyl hypothesis, which proposes that Lissamphibia is a monophyletic group descended from lepospondyls, and (3) the polyphyletic hypothesis, in which batrachians (frogs and salamanders) descend from temnospondyl ancestors while caecilians are derived from lepospondyl ancestors (reviewed by Anderson, 2008; Marjanović and Laurin, 2009; Sigurdson and Green, 2011). The resolution of these hypotheses would influence our interpretation of the evolution of the amphibian ear, namely whether the atympanic condition observed in salamanders represents a plesiomorphic condition more likely under the lepospondyl hypothesis, or a derived condition more likely under the temnospondyl hypothesis whereby the tympanic ear, present in the temnospondyl ancestor, was secondarily lost. Regardless of whether the atympanic ear in salamanders may be considered secondarily reduced, we observe that the salamander auditory system is capable of pressure hearing via sound-induced vibrations in the head, despite lacking any specializations for

airborne sound detection. Terrestrial extratympanic hearing in salamanders is therefore dependent on the presence of auditory end organs capable of receiving and transducing bone-conducted acoustic energy.

Although the presence and degree of elaboration of amphibian auditory end organs varies among species, the evolutionary history of the vertebrate ear indicates that acoustic sensors were probably present prior to the development of peripheral structures to permit transduction of aerial sound pressure (e.g. the tympanic middle ear). The earliest tetrapod ear is presumed to have possessed several specialized sensory organs prior to the transition from water to land, including two otolithic organs, the saccular and lagenar maculae, that functioned as near-field particle motion sensors (reviewed by Fritsch, 1999). The saccular macula of this ancestral tetrapod ear would have been capable of detecting low-frequency seismic energy, and this could have conferred a rudimentary ability to detect acoustic energy assuming that the inner ear was capable of receiving that energy, as is the case in recent lungfish (Christensen et al., 2015b). Here we demonstrate that salamanders detect airborne sound via simple translation of the animal by sound pressure energy that should induce fluid inertial movement in the inner ear. The detection of sound-induced head vibrations by sensory organs in the inner ear requires no specialized superficial tissues and probably represents the simplest form of auditory transduction, applicable in any animal with  $ka \ll 1$  in the absence of any functional middle ear structures. We conclude that extratympanic hearing in salamanders may reflect a generalized terrestrial hearing mechanism enabling species to detect low-frequency airborne sound in the absence of an impedance-matching ear, and that this could have served as an early strategy for aerial sound detection in ancestral tetrapods.

#### Acknowledgements

We thank Hilary Bierman for lending her expertise during experimental planning and set-up, Matthew Niemiller for his invaluable assistance in collecting salamanders, and Dennis Higgs for his support with preliminary data collection during our pilot studies. We thank Alexander Navarro for graciously providing us with *Plethodon cinereus* at the completion of his studies. All other plethodontid salamander species were collected with permission from the Tennessee Wildlife Resources Agency (permit numbers 1386, 1388, 1605 and 3838).

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: G.C., D.S., J.C.-D., C.E.C.; Methodology: G.C., D.S., J.C.-D., C.E.C.; Software: J.C.-D.; Validation: G.C., J.C.-D.; Formal analysis: G.C., J.C.-D.; Investigation: G.C., D.S., J.C.-D.; Resources: D.S., J.C.-D., C.E.C.; Writing - original draft: G.C.; Writing - review & editing: G.C., D.S., J.C.-D., C.E.C.; Visualization: G.C.; Supervision: D.S., C.E.C.; Project administration: D.S., C.E.C.; Funding acquisition: G.C., D.S., J.C.-D., C.E.C.

#### Funding

This work was supported by the National Institute on Deafness and Other Communication Disorders (NIDCD/NIH) training grant T32 DC-000046 to the University of Maryland's Center for Comparative and Evolutionary Biology of Hearing, and NIDCD/NIH grant DC-000436 (to C.E.C.), research grants from The Explorer's Club Washington Group (to G.C.) and the American Society of Ichthyologists and Herpetologists (Gaige Award to G.C.), and grants from the Carlsberg Foundation (2009-01-0292 and 2012-01-0662 to J.C.-D.). Deposited in PMC for release after 12 months.

#### References

Anderson, J. S. (2008). Focal review: the origin(s) of modern amphibians. *Evol. Biol.* **35**, 231-247. doi:10.1007/s11692-008-9044-5

Bárány, E. (1938). A contribution to the physiology of bone conduction. *Acta Otolaryngol. Suppl.* **26**, 1-233.

Boistel, R., Aubin, T., Cloetens, P., Peyrin, F., Scotti, T., Herzog, P., Gerlach, J., Pollet, N. and Aubry, J.-F. (2013). How minute sooglossid frogs hear without a

middle ear. *Proc. Natl. Acad. Sci. USA* **110**, 15360-15364. doi:10.1073/pnas.1302218110

Brandt, C., Brande-Lavridsen, N. and Christensen-Dalsgaard, J. (2018). The masked ABR (mABR): a new measurement method for the auditory brainstem response. *J. Assoc. Res. Otolaryngol.* **19**, 753-761. doi:10.1007/s10162-018-00696-x

Capranica, R. R. and Moffat, A. J. M. (1974). Frequency sensitivity of auditory fibers in the eighth nerve of the spadefoot toad *Scaphiopus couchi*. *J. Acoust. Soc. Am.* **55**, S85. doi:10.1121/1.1920023

Capranica, R. R. and Moffat, A. J. M. (1975). Selectivity of the peripheral auditory system of spadefoot toads (*Scaphiopus couchi*) for sounds of biological significance. *J. Comp. Physiol. A* **100**, 231-249. doi:10.1007/BF00614533

Capshaw, G., Soares, D. and Carr, C. E. (2019). Bony labyrinth morphometry reveals hidden diversity in lungless salamanders (Family Plethodontidae): structural correlates of ecology, development, and vision in the inner ear. *Evolution (N. Y.)* **73**, 2135-2150. doi:10.1111/evo.13837

Christensen, C. B., Christensen-Dalsgaard, J. and Madsen, P. T. (2015b). Hearing of the African lungfish (*Protopterus annectens*) suggests underwater pressure detection and rudimentary aerial hearing in early tetrapods. *J. Exp. Biol.* **218**, 381-387. doi:10.1242/jeb.116012

Christensen, C. B., Christensen-Dalsgaard, J., Brandt, C. and Madsen, P. T. (2012). Hearing with an atympanic ear: good vibration and poor sound-pressure detection in the royal python, *Python regius*. *J. Exp. Biol.* **215**, 331-342. doi:10.1242/jeb.062539

Christensen, C. B., Lauridsen, H., Christensen-Dalsgaard, J., Pedersen, M. and Madsen, P. T. (2015a). Better than fish on land? Hearing across metamorphosis in salamanders. *Proc. R. Soc. B Biol. Sci.* **282**, 20141943. doi:10.1098/rspb.2014.1943

Christensen-Dalsgaard, J. and Carr, C. E. (2008). Evolution of a sensory novelty: tympanic ears and the associated neural processing. *Brain Res. Bull.* **75**, 365-370. doi:10.1016/j.brainresbull.2007.10.044

Christensen-Dalsgaard, J. and Jørgensen, M. B. (1988). The response characteristics of vibration-sensitive saccular fibers in the grassfrog, *Rana temporaria*. *J. Comp. Physiol. A* **162**, 633-638. doi:10.1007/BF01342638

Christensen-Dalsgaard, J. and Jørgensen, M. B. (1996). Sound and vibration sensitivity of VIIIth nerve fibers in the grassfrog, *Rana temporaria*. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **179**, 437-445. doi:10.1007/BF00192311

Christensen-Dalsgaard, J. and Narins, P. M. (1993). Sound and vibration sensitivity of VIIIth nerve fibers in the frogs *Leptodactylus albilabris* and *Rana pipiens pipiens*. *J. Comp. Physiol. A* **172**, 653-662. doi:10.1007/BF00195391

Christensen-Dalsgaard, J., Brandt, C., Willis, K. L., Christensen, C. B., Ketten, D. R., Edds-Walton, P., Fay, R. R., Madsen, P. T. and Carr, C. E. (2012). Specialization for underwater hearing by the tympanic middle ear of the turtle, *Trachemys scripta elegans*. *Proc. R. Soc. B Biol. Sci.* **279**, 2816-2824. doi:10.1098/rspb.2012.0290

Chung, S.-H., Pettigrew, A. and Anson, M. (1978). Dynamics of the amphibian middle ear. *Nature* **272**, 142-147. doi:10.1038/272142a0

Chung, S. H., Pettigrew, A. G. and Anson, M. (1981). Hearing in the frog: dynamics of the middle ear. *Proc. R. Soc. Lond. B* **212**, 459-485. doi:10.1098/rspb.1981.0048

Clack, J. A. (1997). The evolution of tetrapod ears and the fossil record. *Brain. Behav. Evol.* **50**, 198-212. doi:10.1159/000113334

Clack, J. A. (2002). Patterns and processes in the early evolution of the tetrapod ear. *J. Neurobiol.* **53**, 251-264. doi:10.1002/neu.10129

De Vries, H. (1950). The mechanics of the labyrinth otoliths. *Acta Otolaryngol.* **38**, 262-273. doi:10.3109/00016485009118384

Ehret, G., Keilwerth, E. and Kamada, T. (1994). The lung-ear drum pathway in three treefrog and four dendrobatid frog species: some properties of sound transmission. *J. Exp. Biol.* **195**, 329-343.

Ehret, G., Tautz, J., Schmitz, B. and Narins, P. M. (1990). Hearing through the lungs: lung-ear drum transmission of sound in the frog *Eleutherodactylus coqui*. *Naturwissenschaften* **77**, 192-194. doi:10.1007/BF01131168

Feng, A. S., Narins, P. M. and Capranica, R. R. (1975). Three populations of primary auditory fibers in the bullfrog (*Rana catesbeiana*): their peripheral origins and frequency sensitivities. *J. Comp. Physiol. A* **100**, 221-229. doi:10.1007/BF00614532

Frishkopf, L. S. and Geisler, C. D. (1966). Peripheral origin of auditory responses recorded from the eighth nerve of the bullfrog. *J. Acoust. Soc. Am.* **40**, 469-472. doi:10.1121/1.1910097

Fritsch, B. (1999). Hearing in two worlds: theoretical and actual adaptive changes of the aquatic and terrestrial ear for sound reception. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 15-42. New York: Springer-Verlag New York Inc.

Goutte, S., Mason, M. J., Christensen-Dalsgaard, J., Montealegre-Z, F., Chivers, B. D., Sarria-S, F. A., Antoniazzi, M. M., Jared, C., Almeida Sato, L. and Felipe Toledo, L. (2017). Evidence of auditory insensitivity to vocalization frequencies in two frogs. *Sci. Rep.* **7**, 12121. doi:10.1038/s41598-017-12145-5

Hartline, P. H. (1971). Physiological basis for detection of sound and vibration in snakes. *J. Exp. Biol.* **54**, 349-371.

- Hetherington, T. E.** (1985). Role of the opercularis muscle in seismic sensitivity in the bullfrog *Rana catesbeiana*. *J. Exp. Zool.* **235**, 27–34. doi:10.1002/jez.1402350105
- Hetherington, T. E.** (1988). Biomechanics of vibration reception in the bullfrog, *Rana catesbeiana*. *J. Comp. Physiol. A* **163**, 43–52. doi:10.1007/BF00611995
- Hetherington, T. E.** (1992). The effects of body size on functional properties of middle ear systems of anuran amphibians. *Brain. Behav. Evol.* **39**, 133–142. doi:10.1159/000114111
- Hetherington, T. E.** (2001). Laser vibrometric studies of sound-induced motion of the body walls and lungs of salamanders and lizards: implications for lung-based hearing. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **187**, 499–507. doi:10.1007/s003590100220
- Hetherington, T. E. and Lindquist, E. D.** (1999). Lung-based hearing in an 'earless' anuran amphibian. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **184**, 395–401. doi:10.1007/s003590050338
- Hetherington, T. E., Jaslow, A. P. and Lombard, R. E.** (1986). Comparative morphology of the amphibian opercularis system: I. General design features and functional interpretation. *J. Morphol.* **190**, 43–61. doi:10.1002/jmor.1051900105
- Jørgensen, M. B.** (1991). Comparative studies of the biophysics of directional hearing in anurans. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **169**, 591–598. doi:10.1007/BF00193548
- Jørgensen, M. B., Schmitz, B. and Christensen-Dalsgaard, J.** (1991). Biophysics of directional hearing in the frog *Eleutherodactylus coqui*. *J. Comp. Physiol. A* **168**, 223–232. doi:10.1007/BF00218414
- Kingsbury, B. F. and Reed, H. D.** (1908). The columella auris in Amphibia. *Anat. Rec.* **2**, 81–91. doi:10.1002/ar.1090020302
- Kitazawa, T., Takechi, M., Hirasawa, T., Adachi, N., Narboux-Nême, N., Kume, H., Maeda, K., Hirai, T., Miyagawa-Tomita, S., Kurihara, Y. et al.** (2015). Developmental genetic bases behind the independent origin of the tympanic membrane in mammals and diapsids. *Nat. Commun.* **6**, 6853. doi:10.1038/ncomms7853
- Koyama, H., Lewis, E. R., Leverenz, E. L. Baird, R. A.** (1982). Acute seismic sensitivity in the bullfrog ear. *Brain Res.* **250**, 168–172. doi:10.1016/0006-8993(82)90964-7
- Lewis, E. R.** (1988). Tuning in the bullfrog ear. *Biophys. J.* **53**, 441–447. doi:10.1016/S0006-3495(88)83120-5
- Liff, H.** (1969). Responses from single auditory units in the eighth nerve of the leopard frog. *J. Acoust. Soc. Am.* **45**, 512–513. doi:10.1121/1.1911405
- Lindquist, E. D., Hetherington, T. E. and Volman, S. F.** (1998). Biomechanical and neurophysiological studies on audition in eared and earless harlequin frogs (*Atelopus*). *J. Comp. Physiol. A Neuroethol. Sensory Neural Behav. Physiol.* **183**, 265–271. doi:10.1007/s003590050254
- Lombard, R. E. and Straughan, I. R.** (1974). Functional aspects of anuran middle ear structures. *J. Exp. Biol.* **61**, 71–93.
- Lychakov, D. V. and Rebane, Y. T.** (2000). Otolith regularities. *Hear. Res.* **143**, 83–102. doi:10.1016/S0378-5955(00)00026-5
- Marjanović, D. and Laurin, M.** (2009). The origin(s) of modern amphibians: a commentary. *Evol. Biol.* **36**, 336–338. doi:10.1007/s11692-009-9065-8
- Mason, M. J.** (2007). Pathways for sound transmission to the inner ear in amphibians. In *Hearing and Sound Communication in Amphibians* (ed. P. M. Narins, A. S. Feng, R. R. Fay and A. N. Popper), pp. 147–183. New York: Springer Science+Business Media, LLC.
- Moffat, A. J. M. and Capranica, R. R.** (1974). Sensory processing in the peripheral auditory system of treefrogs (*Hyla*). *J. Acoust. Soc. Am.* **55**, 480. doi:10.1121/1.3438013
- Moffat, A. J. M. and Capranica, R. R.** (1976). Auditory sensitivity of the sacculle in the American toad (*Bufo americanus*). *J. Comp. Physiol. A* **105**, 1–8. doi:10.1007/BF01380048
- Monath, T.** (1965). The opercular apparatus of salamanders. *J. Morphol.* **116**, 149–170. doi:10.1002/jmor.1051160202
- Mullinger, A. M. and Smith, J. J. B.** (1969). Some aspects of the gross and fine structure of the amphibian papilla in the labyrinth of the newt, *Triturus cristatus*. *Tissue Cell* **1**, 403–416. doi:10.1016/S0040-8166(69)80013-3
- Narins, P. and Capranica, R.** (1976). Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui*. *Science* **192**, 378–380. doi:10.1126/science.1257772
- Narins, P. M., Ehret, G. and Tautz, J.** (1988). Accessory pathway for sound transfer in a neotropical frog. *Proc. Natl. Acad. Sci. USA* **85**, 1508–1512. doi:10.1073/pnas.85.5.1508
- Pettigrew, A. G., Anson, M. and Chung, S. H.** (1981). Hearing in the frog: a neurophysiological study of the auditory response in the midbrain. *Proc. R. Soc. Lond. Biol. Sci.* **212**, 433–457. doi:10.1098/rspb.1981.0047
- Ronken, D. A.** (1991). Spike discharge properties that are related to the characteristic frequency of single units in the frog auditory nerve. *J. Acoust. Soc. Am.* **90**, 2428–2440. doi:10.1121/1.402047
- Ross, R. J. and Smith, J. J. B.** (1980). Detection of substrate vibrations by salamanders: frequency sensitivity of the ear. *Comp. Biochem. Physiol. A Physiol.* **65A**, 167–172. doi:10.1016/0300-9629(80)90218-2
- Sachs, M. B.** (1964). Responses to acoustic stimuli from single units in the eighth nerve of the green frog. *J. Acoust. Soc. Am.* **36**, 1956–1958. doi:10.1121/1.1919303
- Sigurdson, T. and Green, D. M.** (2011). The origin of modern amphibians: a re-evaluation. *Zool. J. Linn. Soc.* **162**, 457–469. doi:10.1111/j.1096-3642.2010.00683.x
- Smith, J. J. B.** (1968). Hearing in terrestrial urodeles: a vibration-sensitive mechanism in the ear. *J. Exp. Biol.* **48**, 191–205.
- Smotherman, M. S. and Narins, P. M.** (2000). Hair cells, hearing and hopping: a field guide to hair cell physiology in the frog. *J. Exp. Biol.* **203**, 2237–2246.
- Stenfelt, S.** (2011). Acoustic and physiologic aspects of bone conduction hearing. *Adv. Otorhinolaryngol.* **71**, 10–21. doi:10.1159/000323574
- Stenfelt, S.** (2013). Bone conduction and the middle ear. In *The Middle Ear: Science, Otolaryngology, and Technology* (ed. S. Puria, R. R. Fay and A. N. Popper), pp. 135–169. New York: Springer Science+Business Media, LLC.
- Stenfelt, S.** (2015). Inner ear contribution to bone conduction hearing in the human. *Hear. Res.* **329**, 41–51. doi:10.1016/j.heares.2014.12.003
- Stenfelt, S.** (2016). Model predictions for bone conduction perception in the human. *Hear. Res.* **340**, 135–143. doi:10.1016/j.heares.2015.10.014
- Stenfelt, S. and Goode, R. L.** (2005). Bone-conducted sound: physiological and clinical aspects. *Otol. Neurotol.* **26**, 1245–1261. doi:10.1097/01.mao.0000187236.10842.d5
- Stenfelt, S., Hato, N. and Goode, R. L.** (2002). Factors contributing to bone conduction: the middle ear. *J. Acoust. Soc. Am.* **111**, 947–959. doi:10.1121/1.1432977
- Tonndorf, J.** (1962). Compressional bone conduction in cochlear models. *J. Acoust. Soc. Am.* **34**, 1127–1131. doi:10.1121/1.1918259
- Tonndorf, J.** (1966). Bone conduction. Studies in experimental animals. *Acta Otolaryngol. Suppl.* **213**, 1–132.
- Tonndorf, J.** (1972). Bone conduction. In *Foundations of Modern Auditory Theory, Vol. II* (ed. J. V. Tobias), pp. 195–237. New York: Academic Press.
- von Békésy, G.** (1948). Vibration of the head in a sound field and its role in hearing by bone conduction. *J. Acoust. Soc. Am.* **20**, 749–760. doi:10.1121/1.1906433
- Wever, E. G.** (1978a). *The Reptile Ear*. Princeton, NJ: Princeton University Press.
- Wever, E. G.** (1978b). Sound transmission in the salamander ear. *Proc. Natl. Acad. Sci. U. S. A.* **75**, 529–530. doi:10.1073/pnas.75.1.529
- Wever, E. G.** (1985). *The Amphibian Ear*. Princeton, NJ: Princeton University Press.
- Wilczynski, W., Zakon, H. H. and Brenowitz, E. A.** (1984). Acoustic communication in spring peepers. Call characteristics and neurophysiological aspects. *J. Comp. Physiol. A* **155**, 577–584. doi:10.1007/BF00610843
- Womack, M. C., Christensen-Dalsgaard, J., Coloma, L. A. and Hoke, K. L.** (2018). Sensitive high-frequency hearing in earless and partially eared harlequin frogs (*Atelopus*). *J. Exp. Biol.* **221**, jeb169664. doi:10.1242/jeb.169664
- Womack, M. C., Christensen-Dalsgaard, J., Coloma, L. A., Chaparro, J. C. and Hoke, K. L.** (2017). Earless toads sense low frequencies but miss the high notes. *Proc. R. Soc. B Biol. Sci.* **284**, 20171670. doi:10.1098/rspb.2017.1670
- Yu, X., Lewis, E. R. and Feld, D.** (1991). Seismic and auditory tuning curves from bullfrog sacculus and amphibian papular axons. *J. Comp. Physiol. A* **169**, 241–248. doi:10.1007/BF00215871
- Zeyl, J. N. and Johnston, C. E.** (2016). Comparative and developmental patterns of amphibious auditory function in salamanders. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **202**, 879–894. doi:10.1007/s00359-016-1128-6
- Zeyl, J. N. and Johnston, C. E.** (2017). Do body wall vibrations over the lungs aid aerial hearing in salamanders? An investigation into extratympanic hearing mechanisms. *Herpetologica* **73**, 283–290. doi:10.1655/Herpetologica-D-16-00076.1