

## Directionality of the lizard ear

Jakob Christensen-Dalsgaard<sup>1,\*</sup> and Geoffrey A. Manley<sup>2</sup>

<sup>1</sup>*Center for Sound Communication, SDU Odense University, Campusvej 55, DK-5230 Odense M, Denmark and*

<sup>2</sup>*Lehrstuhl für Zoologie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany*

\*Author for correspondence (e-mail: JCD@biology.sdu.dk)

Accepted 26 January 2005

### Summary

Lizards have highly sensitive ears, but most lizard heads are small (1–2 cm in diameter) compared to the wavelengths of sound of frequencies to which they are most sensitive (1–4 kHz, wavelengths 34–8.5 cm). Therefore, the main cues to sound direction that mammals use – binaural time and intensity cues due to arrival-time differences and sound shadowing by the head – will be very small in lizards.

The present work shows that acoustical coupling of the two eardrums in lizards produces the largest directionality of any terrestrial vertebrate ear studied. Laser vibrometric studies of tympanic motion show pronounced directionality within a 1.8–2.4 kHz frequency band around the best frequency of hearing, caused by the interference

of ipsi- and contralateral inputs. The results correspond qualitatively to the response of a simple middle ear model, assuming coupling of the tympana through a central cavity. Furthermore, observed directional responses are markedly asymmetrical, with a steep gradient of up to 50-fold (34 dB) response differences between ipsi- and contralateral frontal angles. Therefore, the directionality is easily exploitable by simple binaural subtraction in the brain. Lizard ears are the clearest vertebrate examples of directionality generated by tympanic coupling.

Key words: hearing, auditory, eardrum, tympanum, reptile, lizard, frog, bird.

### Introduction

The middle ears of terrestrial vertebrates come in two different forms. In the pressure receiver ear found in mammals and some birds, the two middle ear cavities are (nearly) acoustically isolated, non-directional pressure receivers (Wightman and Kistler, 1993; Klump, 2000). Accordingly, binaural interaction used for the computation of sound direction is confined to the central nervous system utilizing mainly interaural differences in the sound spectra, interaural level differences (ILD) and interaural time differences (ITD). The interaural differences are caused by sound diffraction and arrival-time differences, and consequently depend strongly on head size. In all tetrapods, the middle ear cavities are connected through Eustachian tubes and the mouth cavity. In mammals, the tubes are usually closed and very narrow, and this precludes a tight acoustical coupling between the ears. In contrast, the two ears of non-mammals (lizards, frogs, turtles, crocodiles and many bird species) are connected by wide Eustachian tubes and/or interaural canals (Klump, 2000; Wever, 1978; Fay and Feng, 1987; Lewis and Coles, 1980; Christensen-Dalsgaard, 2005), sometimes of complex shape. Theoretically, such connections could enhance the directionality of the ear by allowing sound access to both sides of each tympanic membrane, cancelling or enhancing its motion, depending on the instantaneous pressure difference across the membrane. This pressure-difference receiver principle was first described

for insect ears by Autrum (1940). Thus, the driving force for tympanum vibrations, i.e. the pressure difference between its two sides, depends on the amplitude and phase of the direct and the indirect sound components, which again depend on sound direction and frequency. In such a pressure-difference receiver, binaural interaction already takes place at the tympanum, and the directionality should be strongly frequency dependent. Hence, at low frequencies the sound pressure will be nearly equal on the two sides of the tympanum. At high frequencies, the phase shift resulting from arrival-time differences exceeds a cycle and the resulting pressure difference will be a complicated, non-monotonic function of sound direction.

The directionality of the ear is also highly dependent on the interaural attenuation in the frequency range of interest (Klump, 2000). For example, in the barn owl, it has been shown that at high frequencies, the acoustical transmission of the interaural canal is highly attenuated and the ears are essentially unconnected at behaviorally important frequencies (above 5 kHz), whereas interaural transmission is efficient at lower frequencies (Moiseff and Konishi, 1981). In other birds, however, strong directional effects resulting from interaural coupling have been demonstrated. In the quail, pronounced directionality of the auditory periphery results from strong interaural coupling (less than 5 dB interaural canal

transmission attenuation at frequencies below 5 kHz; Coles et al., 1980; Hill et al., 1980). Directivity (i.e. the physical directional characteristics) patterns based on cochlear microphonic measurements were cardioid at lower frequencies and figure-of-eight shaped at high frequencies, and the patterns were changed when one eardrum was blocked. The directionality approaches 25 dB, but the resulting patterns of directionality are complicated and so strongly frequency dependent that the functional implications for sound localization, including for further neural processing, are very unclear. In frogs, where the tympana are coupled through large Eustachian tubes and the mouth cavity, the directivity patterns of eardrum vibrations are ovoidal with a maximal directional difference of 6–10 dB (Jørgensen, 1991; Jørgensen et al., 1991). Maximal sensitivity and directivity are found at around 2 kHz, depending on the size and on the species of frog (Christensen-Dalsgaard, 2005).

Lizards in general have very sensitive ears with delicate eardrums, no external ear (although a short external ear canal is present in some) and, like frogs and birds, a single auditory ossicle, the columella, that is coupled to the eardrum through a cartilaginous extracolumella (Manley, 1990). Their best frequencies of hearing range from 1 to 3 kHz, and the eardrum vibrations show band-pass characteristics (Saunders et al., 2000). The high-frequency sensitivity is influenced by the mechanics of the auditory ossicle and the extracolumella (Manley, 1990; Werner et al., 1998). At the best frequencies of hearing in lizards, the wavelengths of sound are much larger than the head dimensions, so ILD cues due to sound diffraction will be small. The Eustachian tubes are very large, however, so the eardrums are connected directly to the mouth cavity. Based on these anatomical features, it has previously been speculated that there would be considerable acoustical interaction between the eardrums, which would lead to strong directionality (Wever, 1978). The directionality would be frequency dependent and dependent on the properties of the acoustical elements, however, and given the large variation in directivity in other animals with coupled ears (frogs and birds), direct measurement is needed to demonstrate whether the acoustical coupling leads to any useful directionality. We present here such direct measurements of the directionality of eardrum vibrations in four lizard species, showing a large

eardrum directivity that in all species is dependent on acoustical interaction between the two tympana. We have found that the interaural coupling in lizards produces the largest directivity of any tetrapod studied so far and that a simple model of middle ear acoustics can account for most of the directionality.

### Materials and methods

The sound-induced motion of the tympanic membrane was measured in four lizard species: *Gecko gekko* L. (a gekkonid,  $N=2$ ), *Mabuya macularia* Smith (a skink,  $N=2$ ), *Leiolepis belliana* Boulenger (an agamid,  $N=2$ ), and *Ctenosaura similis* Gray (an iguanid,  $N=1$ ). In the first two species, a shallow external ear canal is present. The lizards were lightly anaesthetised (motionless, but having normal lung respiration) using a combination of initial inhalation anaesthesia (isoflurane 5%, 2–5 min inhalation in a small, closed tank) and ketamine injections (60–130  $\mu\text{g g}^{-1}$  body mass). Data from a grass frog *Rana temporaria* L. are included for comparison. The frog was lightly anesthetized by brief (<5 min) immersion in tricaine methanesulfonate (MS-222, 0.3%). After such a brief immersion the frog is awake (with normal reflexes and, infrequent, lung respiration) but sedated, and will usually remain immobile during the measurements. After sedation the animals were placed on a holder in an anechoic room. The experimental procedure was approved by the Danish Animal Experimentation Board.

Sound was emitted in turn from one of 12 loudspeakers (JBL 1G, Northridge, CA, USA) that were placed at 30° intervals around and approximately 50 cm from the animal's head. The sound was generated using Tucker-Davis (TDT, Alachua, FL, USA) system 2 hardware. The stimuli used were frequency sweeps, at levels of 80–90 dB SPL, flat from 0.2 to 8 kHz and 175 ms in duration. The signal sent to the loudspeakers was deconvoluted by the transfer function of each loudspeaker (measured using a 0.5" microphone B&K, Copenhagen, Denmark, at the center of the set-up before placing the animal) by dividing the spectrum of the sweep with the transfer function of the speaker. The sweeps were directed in turn to each of the speakers using a customised switching device. The sound at the animal's eardrum was measured with a B&K 4182

Table 1. *Physical characteristics of the four lizard species*

Species ( $N$ )	Snout–vent length (mm)	Head width (mm)	Mass (g)	IL peak amplitude ( $\text{mm s}^{-1} \text{Pa}^{-1}$ )	IL peak frequency (kHz)	Max. directional difference (dB)	Frequency at peak directionality (kHz)	Directional bandwidth (kHz)*
<i>M. macularia</i> (2)	100	13	25	4.1	3.22	32	3.03	2.05
<i>L. belliana</i> (2)	107	13	26	1.7	3.19	30	2.84	1.79
<i>C. similis</i> (1)	180	32	168	2.3	1.95	34	1.80	2.42
<i>G. gekko</i> (2)	190	22	74	4.9	1.82	34	1.36	2.15

*Mabuya macularia*, skink; *Leiolepis belliana*, agamid; *Ctenosaura similis*, iguanid; *Gekko gekko*, gekkonid.

\*The directional bandwidth is measured from the spectra as the frequency band where ipsilateral and contralateral responses differ by more than 3 dB.

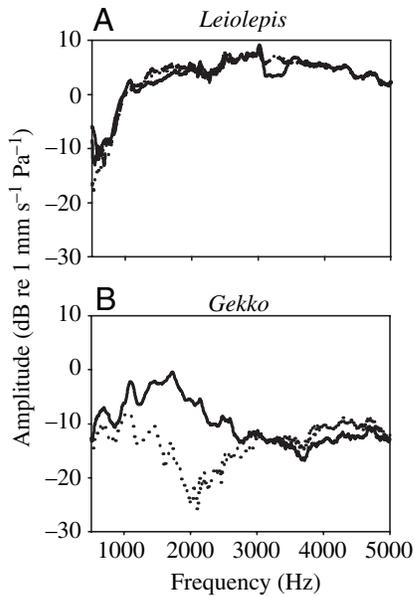
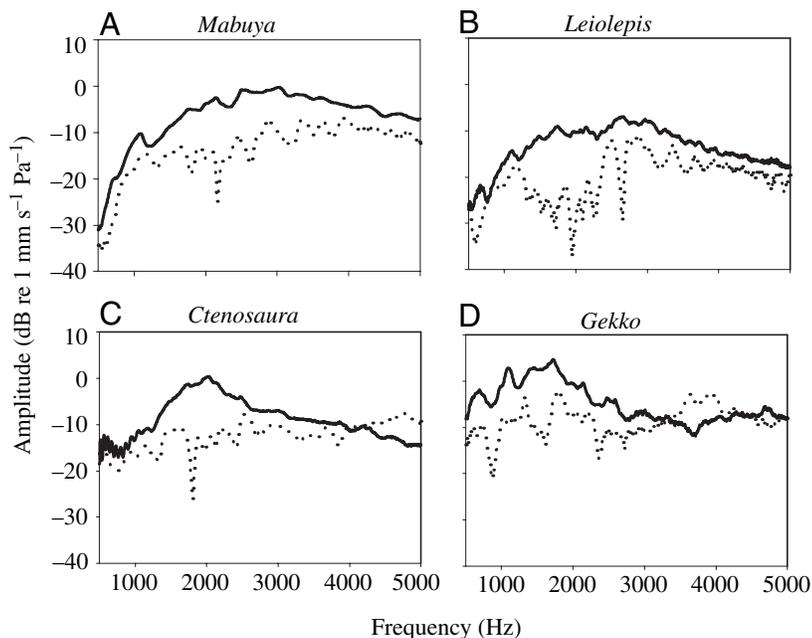


Fig. 1. Eardrum vibration velocity spectra measured at different sound intensities in (A) *Leiolepis* and (B) *Gekko* and normalized by division by the sound spectrum measured at the eardrum. Under the assumption of linearity, the normalized spectra in each figure should be identical.

probe microphone, digitised (22 kHz sample rate, 8192 samples) using the TDT AD-converter (AD2) and stored in a PC. Stimulation and recording was controlled by custom software (DragonQuest, Odense, Denmark).

Vibration of the tympanic membrane, the skin of the head and the body wall overlying the lung was measured using a Dantec laser doppler vibrometer. Tiny, highly reflecting white flakes ('Tippex') were placed on the tympanum at the tip of the columella, on the nearby skin of the head and over the ipsilateral lung. The analog laser signal was digitised using the



TDT AD-converter (AD2). Sound and laser recordings were averaged over 10 presentations. In some of the animals, for comparative measurements, the contralateral eardrum was temporarily occluded by a dome of Vaseline that did not touch the eardrum. The data were analysed using custom software.

#### Data analysis

The laser spectra were corrected for small directional variations in the sound spectrum due to sound diffraction by subtracting the spectrum recorded by the probe microphone from the laser spectrum. Since the speakers were equalized and centred at the start of the experiment, sound diffraction could be measured by comparing the probe spectrum recorded with ipsilateral and contralateral stimulation. Eardrum directivity was displayed either as conventional line plots or cylinder surface plots. Cylinder surface plots are interpolated contour plots of amplitude with direction ( $x$ , 12 directions) and frequency ( $y$ , 500 frequency bands) as independent variables. Each horizontal line corresponds to a polar plot, and each vertical line corresponds to an amplitude spectrum of eardrum motion stimulated by sound from a certain direction. All plots were generated by SigmaPlot, version 8.0. The directional bandwidth was measured from the spectra as the frequency band where the response to ipsilateral and contralateral stimulation differed by more than 3 dB.

To study the possible neural processing of such input signals to the ears, we used the interaural vibration amplitude difference (IVAD) function (Jørgensen et al., 1991) to predict the output of a simplistic model neuron that is excited by the ipsilateral ear and inhibited by the contralateral ear (an EI neuron; Goldberg and Brown, 1969). The function computes the vibration amplitude difference (in dB) between the input from the ipsi- and contralateral ear. The directionalities of the two ears are assumed to be mirror reflections along the frontal-caudal axis.

The model data presented were based on a lumped-element electrical analog of a lizard middle ear (see diagram in fig. 7 in Fletcher, 1992). The model has two sound inputs ( $P_1$  and  $P_2$ ) that differ in phase by:

$$\Delta\phi = (\omega/c)d\sin\theta, \quad (1)$$

where  $\omega$  is the angular frequency,  $c$  the velocity of sound,  $d$  the interaural distance and  $\theta$  the sound incidence angle relative to the body axis. The parameters used were based on measurements from *Mabuya*: interaural distance 13 mm, cavity volume

Fig. 2. Eardrum vibration velocity spectra (amplitude, dB) for the four species studied: *Mabuya* (A), *Leiolepis* (B), *Ctenosaura* (C) and *Gekko* (D). The spectra are normalized by division by the sound spectra measured at the eardrum. Thick line: ipsilateral stimulation, broken line: contralateral stimulation. Values are in dB re  $1 \text{ mm s}^{-1} \text{ Pa}^{-1}$ , i.e. 0 dB corresponds to a vibration velocity of  $1 \text{ mm s}^{-1}$  at 1 Pa (94 dB SPL).

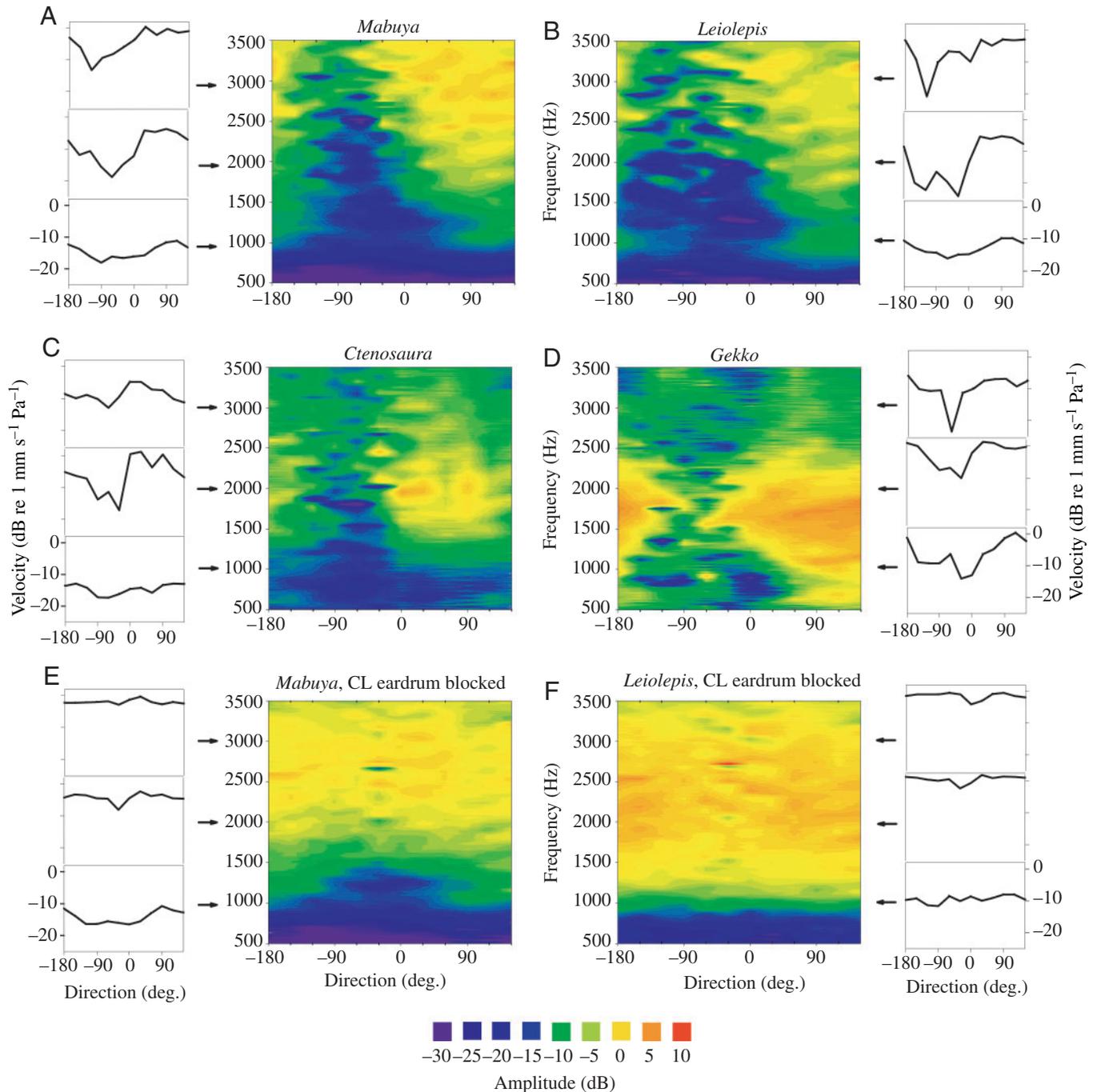


Fig. 3. Cylinder surface plots (for details, see Materials and methods) of eardrum directionality in the four lizard species *Mabuya* (A), *Leiolepis* (B), *Ctenosaura* (C) and *Gekko* (D). The normalized velocities (colour scale, in dB re 1 mm s<sup>-1</sup> Pa<sup>-1</sup>) are plotted as a function of direction ( $x$ -axis, contralateral angles on the left and ipsilateral angles on the right) and frequency ( $y$ -axis). The right and left row of figures show line plots of velocity ( $y$ -axis, dB re 1 mm s<sup>-1</sup> Pa<sup>-1</sup>) as a function of direction ( $x$ -axis) at three frequencies (1000, 2000 and 3000 Hz, arrows), corresponding to three horizontal lines in the cylinder surface plot. (E,F) Eardrum directionality after occluding one eardrum by a dome of Vaseline. E, *Mabuya*; F, *Leiolepis*.

$V=0.7$  cm<sup>3</sup>, tympanum area 20 mm<sup>2</sup> and tympanum thickness 8  $\mu$ m. Estimated parameters were tympanum resonance frequency 2800 Hz, mass of tympanum (loaded by middle ear) 0.5 mg, tympanum quality factor  $Q$  1.2.

From these parameters, the tympanic impedance  $Z_t$  and the

cavity impedance  $Z_V$  can be calculated (see Fletcher, 1992 for details) as:

$$Z_t = R_t + i\omega L_t + 1/i\omega C_t \quad (2)$$

and

$$Z_V = 1 / [i\omega(V/\rho c^2)], \quad (3)$$

where  $R_t=7.8\times 10^{-6}\text{ kg m}^{-4}\text{ s}^{-1}$ ,  $L_t=1783\text{ kg m}^{-4}$  and  $C_t=6.06\times 10^{-12}\text{ kg m}^{-4}\text{ s}^2$ .

These values deviate by up to a factor 30 from the impedances calculated for the frog tympanum by Aertsen et al. (1986). A part of the discrepancy is probably due to the different structure of the lizard tympanum (smaller mass), but the calculations in Aertsen et al. (1986) were also based on the acoustical measurements, whereas the parameters used here are estimated from the structural characteristics of the ear.

## Results

We investigated the directionality of eardrum vibrations in four lizard species from four different families: the skink *Mabuya macularia*, the agamid *Leiolepis belliana*, the iguanid *Ctenosaura similis* and the gekkonid *Gekko gecko* (Tokay gecko). Physical characteristics of the different species are shown in Table 1; note especially the differences in the size and head width.

### Linearity

Calculations of transfer functions between sound and eardrum vibrations presuppose that the eardrum vibrations are linear at the amplitude ranges used. We therefore investigated the linearity of the eardrum vibrations. As an example, Fig. 1A shows the response of the agamid lizard *Leiolepis* stimulated at three levels. When the sound spectrum is subtracted, the spectra have nearly identical shapes, and the levels are also similar, indicating a linear response. This was also characteristic of the data from *Mabuya* and *Ctenosaura*. In contrast, the response of *Gekko* is very different. Here, the vibration amplitude (when corrected for sound level) decreases at high stimulus levels, and the vibration spectrum also has a different shape at low and high stimulus levels (Fig. 1B), indicative of a non-linear response at high stimulus levels. Consequently, for *Gekko* only data from low-level stimulation are used.

### Directionality

Fig. 2 shows eardrum vibration amplitude spectra for the four species. In all animals, the vibration spectrum has band-pass characteristics with peak response to ipsilateral stimulation around 3 kHz in the smaller species (*Mabuya*, Fig. 2A; *Leiolepis*, Fig. 2B) and 1.8 kHz in the larger species (*Ctenosaura*, Fig. 2C; *Gekko*, Fig. 2D). Peak vibration amplitudes are up to  $4.9\text{ mm s}^{-1}$  at 94 dB SPL (1 Pa). Data are summarized in Table 1. There are large and consistent differences (up to 28 dB) between the responses to ipsi- and contralateral stimulation. The response to contralateral stimulation shows a reduction in vibration amplitude compared to the response to ipsilateral stimulation, and one or more pronounced dips in the frequency spectrum. Generally, at all frequencies responses from all contralateral angles are lower than or approximately equal to ipsilateral responses. The directional bandwidth (for definition, see Materials and

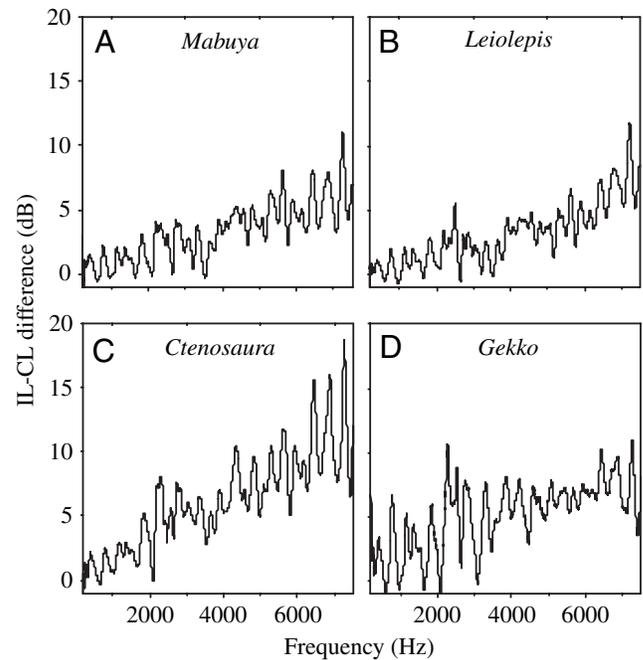


Fig. 4. Diffraction measured as ipsilateral–contralateral sound pressure difference measured by a probe microphone at the eardrum in *Mabuya* (A), *Leiolepis* (B), *Ctenosaura* (C) and *Gekko* (D).

methods) was measured from the spectra and ranges from 1.79 kHz to 2.42 kHz (see Table 1).

In Fig. 3 the directional responses of the eardrum are displayed as cylinder surface plots (for details, see Materials and methods) to facilitate comparison between the species. The plots are similar in the four species and show large ipsi–contralateral differences. The response is markedly asymmetrical with a steep gradient across the midline ( $0^\circ$ ) and, due to this asymmetry, the difference is maximal between  $60^\circ$  ipsi- and contralateral angles (up to 34 dB). When the contralateral eardrum was occluded by Vaseline, the directivity changed to an omnidirectional pattern (Fig. 3E,F). The maximal response amplitude of the ipsilateral eardrum is, however, comparable before and after occlusion.

The diffraction of sound around the body of the lizard produces possible additional directional cues. Fig. 4 shows diffraction data from the four species. Diffraction measured as ipsilateral–contralateral sound pressure difference increases with frequency. A 2 dB difference is found at approximately 2 kHz in the smaller lizards (*Leiolepis*, *Mabuya*) and at approximately 1 kHz in the larger lizards (*Gekko*, *Ctenosaura*).

A common type of binaural interaction in neurons of the auditory brain is that the input from one ear is excitatory and the input from the other ear inhibitory (EI neuron; Goldberg and Brown, 1969). As a simple model, the resulting directivity of an EI neuron can be found by subtracting the directivity of one ear by its mirror reflection along the midline of the animal. The result of this operation is the interaural vibration amplitude difference (IVAD) plot shown for the four species in Fig. 5. Generally, the asymmetrical directivity is sharpened

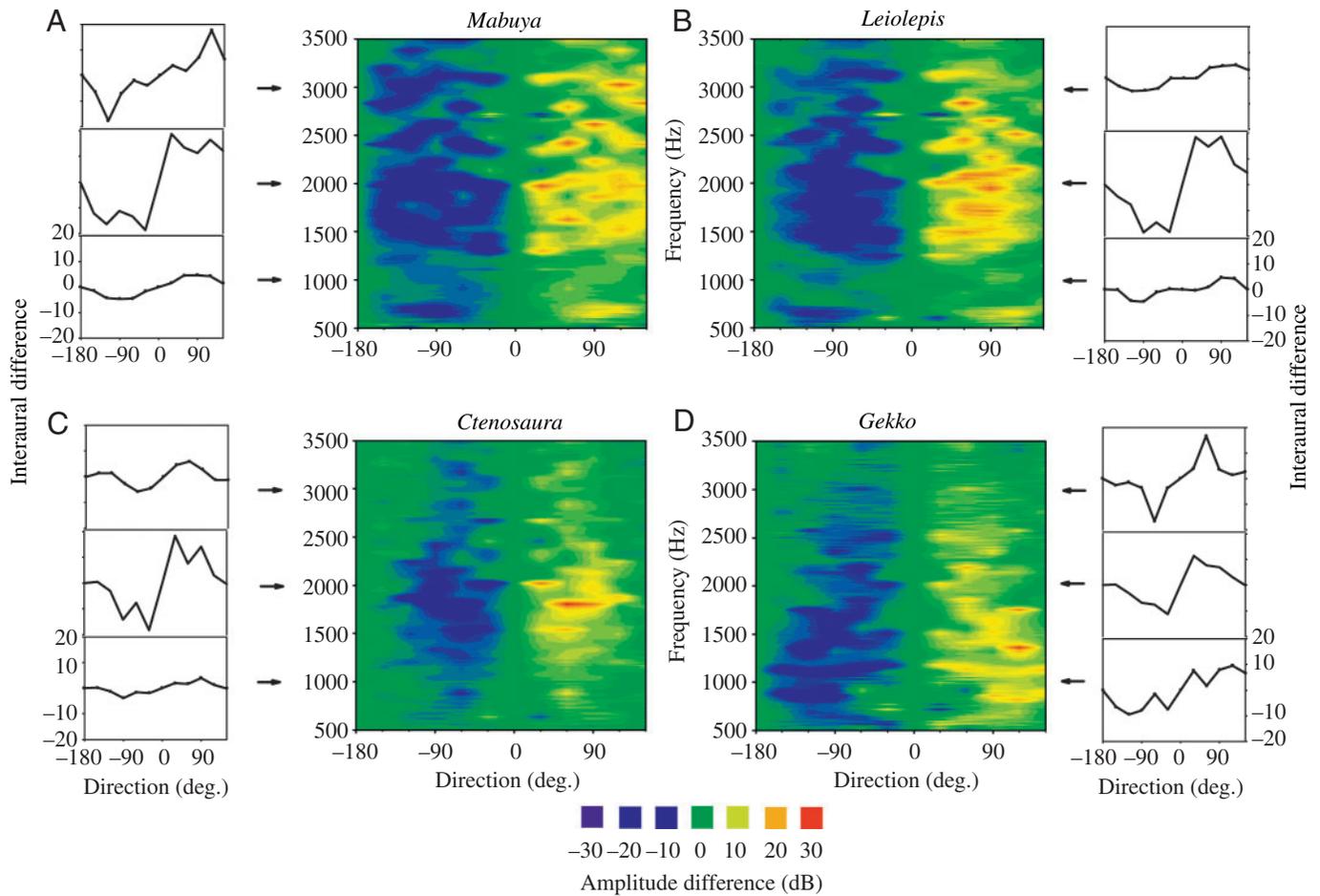


Fig. 5. Interaural difference plot modeling the output of a binaural difference (EI) neuron in the four lizard species *Mabuya* (A), *Leiolepis* (B), *Ctenosaura* (C) and *Gekko* (D). The eardrum vibration data set is subtracted from its reflection along the body axis. Other details as in Fig. 3, except that the colour scale now is relative interaural differences in dB. The line plots in the left and right column show relative interaural differences (y-axis) as a function of direction (x-axis) at three frequencies (1000, 2000 and 3000 Hz, arrows). Note that the interaural differences include the effect of sound diffraction by the body of the lizard.

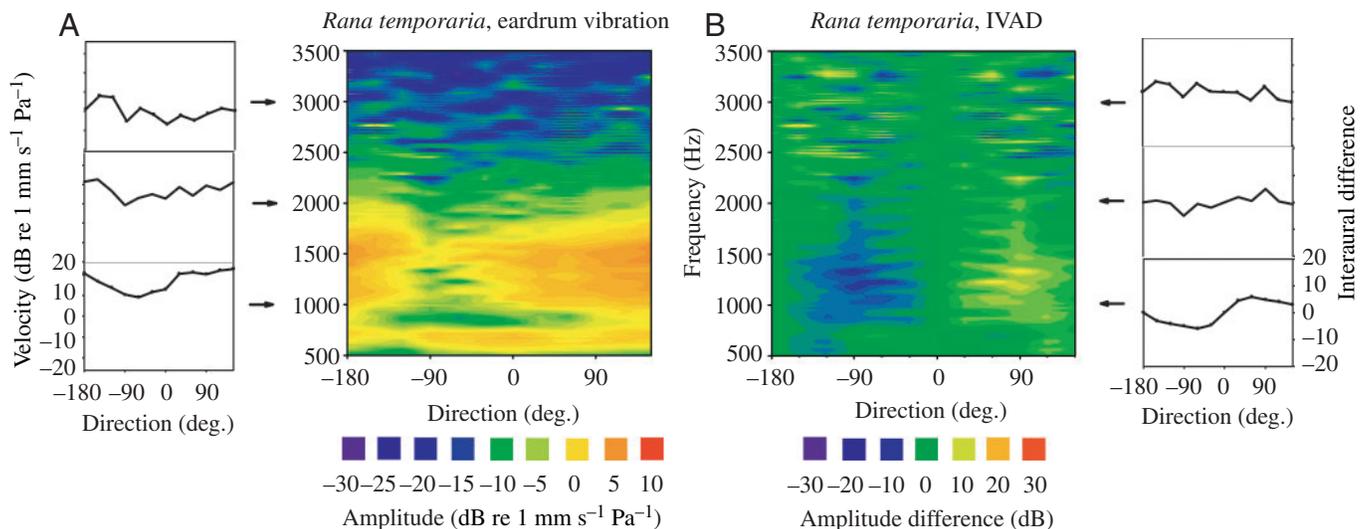


Fig. 6. Cylinder surface plots of eardrum directionality (A) and interaural difference (B) of a grass frog, *Rana temporaria*. Other details in A as in Fig. 3A-D; in B as in Fig. 5A-D.

(differences across the frontal–caudal axis are doubled) by the subtraction, increasing the steepness of the gradient across the frontal midline to more than 50 dB by this simple operation.

It is instructive to compare the directivity of the lizards with the directivity of another animal with acoustically coupled ears. Fig. 6A shows results from measurements of eardrum vibrations in a grass frog *Rana temporaria*. The frog tympanum also has a band-pass characteristic, with a more limited frequency response than the lizards. Also, the spectrum clearly shows two peaks around 670 Hz and 1570 Hz. The directionality is largest between the two peaks. The directional bandwidth (as defined in Materials and methods) is 550 Hz, and the maximal interaural difference (IVAD plot, Fig. 6B) is 14 dB.

The occlusion experiments (Fig. 3E,F) indicate that the directionality is generated by acoustical coupling of the two eardrums. The eardrums are effectively connected by a common air space, since the Eustachian tubes connecting the middle ear and mouth cavities in lizards are very wide. To investigate the effects of acoustical coupling, we used a simple acoustical model of the ear (Fig. 7). The model (Fletcher and Thwaites, 1974; Fletcher, 1992) is based on an electrical analog of the ear (Fig. 7B), and the parameters used are estimated from measurements (for the parameter values, see Materials and methods). The model generates a directional response in the frequency range of 1–2.5 kHz (Fig. 7D), with reduced vibration spectrum for contralateral stimulation and a pronounced dip in the contralateral frequency response (Fig. 7C). Note, however, that this simple two-input model will necessarily produce a response that is symmetrical around the interaural axis.

### Discussion

We have shown that the ear of lizards is highly directional and that the directionality is dependent on interaural coupling of the tympana. The tympana of all species show a band-pass characteristic with maximal response around 1–3 kHz, where the peak vibration velocity amplitudes range from 1.7 to 4.9 mm s<sup>-1</sup> Pa<sup>-1</sup> (Table 1), comparable to earlier measurements from gekkonid lizards (2–4 mm s<sup>-1</sup> at 100 dB SPL; Manley, 1992; Saunders et al., 2000; Werner et al., 2002). The non-linear decrease in vibration amplitude at high sound levels in *Gekko* (Fig. 1B) is probably due to a middle-ear muscle found in geckos that may reduce the columellar vibrations, analogous to the stapedial reflex of mammals (Wever, 1978). The larger species (*Ctenosaura*, *Gekko*) have maximal directionality at lower frequencies than the smaller species. The pressure-

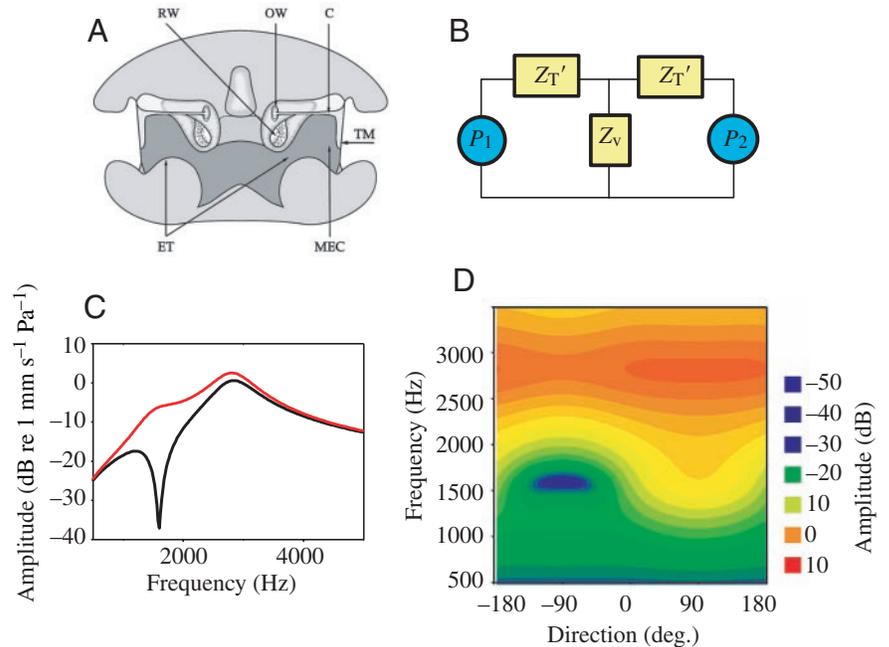


Fig. 7. A simple model of lizard ear directionality (Fletcher 1992). (A) Diagram of a transverse section of a lizard head (*Sceloporus*; redrawn and altered from Wever, 1978). TM, tympanic membrane; C, columella; ET, Eustachian tube; MEC, middle ear cavity; RW, round window; OW, oval window. (B) The electrical analog circuit ( $Z_V$  impedance of mouth cavity,  $Z_T$  impedance of tympana,  $P_1$  and  $P_2$  sound inputs; details of parameters in Materials and methods). (C) Vibration velocity spectra for ipsilateral (red curve) and contralateral (black curve) sound directions (compare Fig. 2). (D) Cylinder surface plot of the model response (compare Fig. 3).

gradient directionality – up to 34 dB difference between ipsi- and contralateral sound directions – is higher in the lizards than in any other vertebrates. A comparison of the lizard and frog data (Figs 3 and 6) shows that the sensitivities are comparable, but that the directionality of the frog ear is smaller than that of the lizard ear and confined to a narrower frequency band. Also, the directivity in lizards is asymmetrical, leading to a large gradient along the frontal directions. In contrast, the directional difference generated by sound diffraction is small, at most amounting to 2–3 dB in the frequency range where the ear is most sensitive. The directionality is dependent on contralateral input (Fig. 3E,F), and many features of the observed directionality can be simulated in a simple, two-input model (Fig. 7), such as the dip in the contralateral spectrum and the high directionality in a 1.5 kHz frequency band below the peak frequency. Although the important front–back asymmetry cannot be generated by two symmetrical inputs, there are possible sources of a third input, perhaps the nares. Previous modelling studies of the frog ear, based on carefully measured parameters, could model the response of the eardrum in a closed coupler (Palmer and Pinder, 1984; Aertsen et al., 1986). However, the models were not able to produce a good fit to experimental free-field data as shown in Fig. 6 (and in Jørgensen, 1991), either predicting largest directionality at very low frequencies and small directionality in the frequency range

of maximal sensitivity (Aertsen et al., 1986) or a figure-of-eight characteristic at low frequencies (Palmer and Pinder, 1984). The likely reason is that the acoustics of the frog auditory system are very complicated compared to those of the lizard. For example, it is suggested that the entire head region is transparent to sound (Aertsen et al., 1986) and it has been shown that another major sound input is through the lungs, an area that covers most of the dorsum (Jørgensen et al., 1991; Narins et al., 1990; Christensen-Dalsgaard, 2005). These inputs probably cannot be modelled by simple lumped elements.

The consequence of the strong frontal asymmetry of the lizard ear is that binaural comparison will intensify the directionality. As shown in Fig. 5, when the directional response of one ear is subtracted from its mirror image, the directionality is greatly enhanced, generating a very steep gradient along the frontal angles in the frequency range from 1.5 to 3 kHz. This operation can be seen as a simplified model of the output of a central binaural neuron that is excited by inputs from one ear and inhibited by input from the other ear (that is, an EI neuron; Goldberg and Brown, 1969). The steep gradient is especially useful, since a very simple behavioral rule, i.e. turning towards the side of excitatory input to the binaural neuron, would direct the lizard's head to the sound source with considerable precision. Since the neural input will also reflect sound diffraction effects around the animal, the data in Fig. 5 are not corrected for sound diffraction. We only show amplitude data in the plot, but the concomitant phase changes in the tympanic responses would result in an increased time difference between ipsi- and contralateral sound directions (up to 180° ipsilateral lead, i.e. 250 µs at 2 kHz), which could be important for inhibitory interactions that sharpen the neural directionality.

The difference between the lizards and other tetrapods that have acoustically connected eardrums (i.e. birds and frogs) is that the interaural attenuation is probably much smaller in lizards than in birds (Klump, 2000) and that, as stated above, the directionality of the frog ear (Fig. 6) is complicated by the fact that sound also enters the middle ear cavity *via* the floor of the (closed) mouth and *via* the lungs (Jørgensen, 1991; Jørgensen et al., 1991; Narins et al., 1988; Christensen-Dalsgaard, 2005). The mouth floor in lizards is probably relatively impermeable to sound and, while sound is also received *via* the lungs in the lizards (Hetherington, 2001), in the species investigated here the effects were very small and confined to a narrow frequency range around 1 kHz (data not shown). Thus, lizards are the clearest tetrapod example of pressure-difference receiver ears.

The neural processing of directional information in lizards is almost unknown (Manley, 1981; Szpir et al., 1990), but it is nevertheless likely that EI neurons are present in the auditory brain, since such binaural neurons are found in both the mammalian (Goldberg and Brown, 1969; Irvine, 1992) and anuran (Feng and Capranica, 1976) CNS. The only available data on directional sensitivity of neurons in the auditory pathway of lizards (single-cell recordings from torus semicircularis in the midbrain of *Gekko gekko*; Manley, 1981)

show pronounced differences between ipsi- and contralateral stimulation that probably reflect both acoustical interaction between the ears and neural interaction in the auditory pathway of the brain. Similarly, behavioral data on sound localization in lizards are very scarce, limited to one study showing that Mediterranean house geckos *Hemidactylus tursicus* intercept calling crickets and approach loudspeakers transmitting cricket calls (Sakaluk and Bellwood, 1984). The paucity of data reflects the fact that it is very difficult to condition lizards to respond to sound (Manley, 2000), and therefore the use of the acute directional hearing in lizards is presently unknown. The present data do, however, strongly suggest that acute directional hearing is likely to be a much more generally useful and advantageous feature of lizard ears.

One interesting additional feature of the data may reveal the reason why, in all auditory papillae of lizards, the hair cells are divided into two areas. There is one area of hair cells that is evolutionarily stable, almost certainly plesiomorphic (primitive) and in which the cells respond best to frequencies below roughly 1 kHz. There is in addition one or, in some groups, two, areas of hair cells that respond to frequencies above 1 kHz. This is true of all groups, in spite of the independent evolution of the particular configurations shown by the different lizard families and the high anatomical variability of the higher-frequency hair-cell areas (Manley, 2002). With the present data, interaural differences were negligible at frequencies below 1 kHz, but were large at frequencies processed in the variable high-frequency hair-cell areas. This suggests that the family-specific patterns of the higher-frequency hair-cell areas could have been due to the presence in the auditory nerve of information relevant to sound localization.

It should be noted, however, that pressure gradient receivers, often regarded as specializations of small animals for directional hearing, may more likely reflect the plesiomorphic state of the auditory system in the terrestrial vertebrates, since an ancestral ear having tympana formed from skin covering skull fenestrations that opened into the mouth cavity would essentially show the response of the model in Fig. 7. Any enclosure of the middle ear and tympanum in a middle ear cavity leading to a pressure-sensitive (e.g. mammalian-type) ear would be a derived condition, maybe caused by an increase in the size of the brain and by the benefits of isolating the middle ear from the respiratory and food-intake pathways.

We thank Gunilla Ørbech for assistance with the experiments, Finn Østergaard Andersen for assistance with the anesthetic procedure and Darcy B. Kelley, Catherine Carr, Christine Köppl, Annemarie Surlykke and two anonymous referees for comments on earlier versions of the manuscript. Supported by the Danish National Research Foundation.

## References

- Aertsen, A. M. H. J., Vlaming, M. S. M. G., Eggermont, J. J. and Johannesma, P. I. M. (1986). Directional hearing in the grassfrog (*Rana temporaria* L.). II. Acoustics and modelling of the auditory periphery. *Hear. Res.* **21**, 17-40.

- Autrum, H.-J.** (1940). Über Lautäusserungen und Schallwahrnehmung bei Arthropoden. II. Das Richtungshören von *Locusta* und Versuch einer Hörtheorie für Tympanalorgane vom Locustidentyp. *Z. vergl. Physiol.* **28**, 326-352.
- Christensen-Dalsgaard, J.** (2005). Directional hearing of non-mammalian tetrapods. In *Sound Source Localization* (ed. A. N. Popper and R. R. Fay). New York: Springer-Verlag, in press.
- Coles, R. B., Lewis, D. B., Hill, K. G., Hutchings, M. E. and Gower, D. M.** (1980). Directional hearing in the Japanese quail (*Coturnix coturnix japonica*). II. Cochlear physiology. *J. Exp. Biol.* **86**, 153-170.
- Fay, R. R. and Feng, A. S.** (1987). Mechanisms for directional hearing among nonmammalian vertebrates. In *Directional Hearing* (ed. W. A. Yost and G. Gourevitch), pp. 179-213. New York: Springer-Verlag.
- Feng, A. S. and Capranica, R. R.** (1976). Sound localization in anurans. I. Evidence of binaural interaction in the dorsal medullary nucleus of the bullfrog (*Rana catesbeiana*). *J. Neurophysiol.* **39**, 871-881.
- Fletcher, N. H.** (1992). *Acoustic Systems in Biology*. Oxford: Oxford University Press.
- Fletcher, N. H. and Thwaites, S.** (1979). Physical models for the analysis of acoustical systems in biology. *Quart. Rev. Biophys.* **12**, 25-65.
- Goldberg, J. M. and Brown, P. B.** (1969). Response of binaural neurons of dog superior olivary complex: an anatomical and electrophysiological study. *J. Neurophysiol.* **32**, 613-636.
- 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* **187**, 499-507.
- Hill, K. G., Lewis, D. B., Hutchings, M. E. and Coles, R. B.** (1980). Directional hearing in the Japanese quail (*Coturnix coturnix japonica*). I. Acoustical properties of the auditory system. *J. Exp. Biol.* **86**, 135-151.
- Irvine, D. R. F.** (1992). Auditory brainstem processing. In *The Mammalian Auditory Pathway: Neurophysiology* (ed. A. N. Popper and R. R. Fay), pp. 153-231. New York: Springer-Verlag.
- Jørgensen, M. B.** (1991). Comparative studies of the biophysics of directional hearing in anurans. *J. Comp. Physiol. A* **169**, 591-598.
- 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.
- Klump, G. M.** (2000). Sound localization in birds. In *Comparative Hearing: Birds and Reptiles* (ed. R. J. Dooling, R. R. Fay and A. N. Popper), pp. 249-307. New York: Springer-Verlag.
- Lewis, B. and Coles, R.** (1980). Sound localization in birds. *Trends Neurosci.* **3**, 102-105.
- Manley, G. A.** (1972). The middle ear of the tokay gecko. *J. Comp. Physiol.* **81**, 239-250.
- Manley, G. A.** (1981). A review of the auditory physiology of the reptiles. In *Progress in Sensory Physiology* (ed. H.-J. Autrum, D. Ottoson, E. Perl and R. F. Schmidt), pp. 49-134. Berlin: Springer-Verlag.
- Manley, G. A.** (1990). *Peripheral Hearing Mechanisms in Reptiles and Birds*. Berlin: Springer-Verlag.
- Manley, G. A.** (2000). The hearing organs of lizards. In *Comparative Hearing: Birds and Reptiles* (ed. R. J. Dooling, R. R. Fay and A. N. Popper), pp. 139-196. New York: Springer-Verlag.
- Manley, G. A.** (2002). Evolution of structure and function of the hearing organ of lizards. *J. Neurobiol.* **53**, 202-211.
- Moiseff, A. and Konishi, M.** (1981). The owl's interaural pathway is not involved in sound localization. *J. Comp. Physiol. A* **144**, 299-304.
- 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.
- Palmer, A. R. and Pinder, A. C.** (1984). The directionality of the frog ear described by a mechanical model. *J. Theor. Biol.* **110**, 205-215.
- Sakaluk, S. K. and Bellwood, J. J.** (1984). Gecko phonotaxis to cricket calling song: A case of satellite predation. *Anim. Behav.* **32**, 659-662.
- Saunders, J. C., Duncan, R. K., Doan, D. E. and Werner, Y. L.** (2000). The middle ear of reptiles and birds. In *Comparative Hearing: Birds and Reptiles* (ed. R. J. Dooling, R. R. Fay and A. N. Popper), pp. 13-69. New York: Springer-Verlag.
- Szpir, M. R., Sento, S. and Ryugo, D. K.** (1990). Central projections of cochlear nerve fibers in the alligator lizard. *J. Comp. Neurol.* **295**, 530-547.
- Werner, Y. L., Montgomery, L. G., Safford, S. D., Igic, P. G. and Saunders, J. C.** (1998). How body size affects middle-ear structure and function and auditory sensitivity in gekkonoid lizards. *J. Exp. Biol.* **201**, 487-502.
- Werner, Y. L., Igic, P. G., Seifan, M. and Saunders, J. C.** (2002). Effects of age and size in the ears of gekkonomorph lizards: middle-ear sensitivity. *J. Exp. Biol.* **205**, 3215-3223.
- Wever, E. G.** (1978). *The Reptile Ear. Its Structure and Function*. Princeton: Princeton University Press.
- Wightman, F. L. and Kistler, D. J.** (1993). Sound localization. In *Human Psychophysics* (ed. W. A. Yost, A. N. Popper and R. R. Fay), pp. 155-192. New York: Springer-Verlag.