

Effects of age and size in the ears of gekkonomorph lizards: middle-ear sensitivity

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Summary

Previous studies of electrophysiological audiograms in gekkonomorph lizards revealed greater sensitivity in adults than in juveniles. We investigated whether this difference, as far as it is affected by the middle ear, is due to animal age or size. The velocity transfer function of the tympanic membrane (TM) was examined using laser interferometry in nine samples: adults of three large gekkonomorph species, adults of three small species (each related to one of the former), and juveniles of the large species, their sizes matching those of the small-species adults. Each transfer function exhibited an inverted 'V' or 'U' shape, with the velocity of TM motion peaking in the mid-frequency range and becoming poorer at lower and higher frequencies. Among samples, maximum TM velocity correlated with animal length, perhaps because of a damping change in the larger TM. The frequency at maximum velocity negatively correlated with measurements of TM area. Presumably, with a larger TM area, the best frequency shifted downward because of

increased middle-ear mass or decreased stiffness. The bandwidth of the transfer function negatively correlated with animal length, being broader in smaller animals and sharper in larger animals. This effect can be attributed to increased mass, decreased damping, or both. Among the middle-ear morphological measurements, the one most closely correlated with body length was the length of the extracolumellar anchorage at the TM. Among the physiological variables investigated, maximum velocity was negatively correlated with the frequency at which it occurred. The dependence of these transfer function variables on animal and ear size was similar, regardless of whether the comparison was among adults of species of different sizes, or among age classes within a species, so that age differences appear to be largely the result of size differences.

Key words: lizard, gecko, middle ear, transfer function, age effect, size effect.

Introduction

In recent decades the function of lizard ears has repeatedly served as a model of tetrapod ear function (Wever, 1974, 1978; Manley, 1990, 2000; Saunders et al., 2000; and references in these). A few of these investigations have shown that the ear is more sensitive in larger individual lizards than in smaller ones. As early as 1970, audiograms based on compound action potentials (CAP) in a small sample of gekkonid lizards *Underwoodisaurus milii* (Diplodactylinae) were found to be more sensitive in larger than in smaller individuals (Johnstone and Werner, 2001). Werner et al. (1998) showed that in two species of gecko, *Eublepharis macularius* (Eublepharidae) and *Oedura marmorata* (Diplodactylinae), the ears of adults were more sensitive than those of juveniles, in terms of both compound action potential (CAP) audiograms and the tympanic membrane velocity transfer function observed by laser interferometry. Werner and Igić (2002) analysed the relationship of low-frequency auditory sensitivity, assessed by audiograms based on the 'microphonic' alternating current

(AC) potentials of the inner ear, to animal size, expressed as body length. That analysis was interspecific, using 14 species or subspecies of gekkonomorph lizards belonging to four subfamilies, and also intraspecific in one of the taxa. The authors showed that auditory sensitivity, as assessed by the microphonic response, was indeed statistically correlated to body size, the larger animals being more sensitive, both interspecifically and intraspecifically. In all of these projects, however, the size factor was not properly separated from the age factor. Hypothetically these two factors could have different effects; for example, size could affect the dimensions and mass of middle-ear components and the proportions among them (Werner and Igić, 2002), while age could also be expected to affect the degree of ossification and calcification of middle-ear components.

In the present work we endeavoured to separate the effects of size and age on the functioning of the tetrapod ear, as exemplified in geckos. Geckos, lizards of the family-cluster

Gekkonomorpha, are of special interest because their vocal communication is more extensive and better known than in other lizard groups (Frankenberg and Werner, 1992; Y. L. Werner, in press).

Our experimental design was comparative. We planned to compare ear function in samples making up a triad: adults of a relatively large species, adults of a smaller species that is closely related, preferably congeneric, and juveniles of the larger species, having the size of the latter. We hoped that repetitions with comparable triads of different species would give similar results, thus demonstrating a general phenomenon. We applied to each animal four methodological approaches for assessing ear function and structure. (i) Measurement of the velocity transfer function of the tympanic membrane. (ii) Generation of audiograms from VIIIth-nerve compound action potential responses. (iii) Description and quantification of the morphology of various middle-ear structures, to gain information on the extent of mechanical and hydraulic levers. (iv) Classifying and counting the hair cells of the basilar papilla, visualised by scanning electron microscopy, to check for possible effects of their variation on auditory sensitivity.

This report deals only with the first approach, assessment of the velocity transfer function of the tympanic membrane (hereinafter termed 'transfer function'). But in addition to the analysis of the three sample-triads, we explore the interspecific correlation of the characterising variables of the transfer function (peak velocity and its frequency) with the relevant morphological size variables (measurements of the tympanic membrane, extracolumella, and whole body). Some of our data have been reported elsewhere (Werner et al., 1998, 2001; Saunders et al., 2000). The relevant parts of the middle ear are shown in Fig. 1 (see also fig. 1 in Werner and Igić, 2002).

Materials and methods

Animals

The choice of species depended on the opportunities to compose sample triads as explained in the Introduction. The first triad comprised adults and juveniles of *Eublepharis macularius* Blyth, 1854 and adults of the smaller species *Coleonyx mitratus* Peters, 1863 (both Eublepharidae). The *E. macularius* were bred in captivity at the Center for Reptile and Amphibian Propagation, Boerne, Texas, USA. They were received in late June 1994 and used in experiments in July 1994. The adults ($N=4$) were healthy females, 8–12 years old, of mean rostrum–anus (RA) length (Werner, 1971) 109.5 mm (range 105–119 mm) and mass 17.6 g (range 15.3–19 g). The juveniles ($N=5$) were 3–6 weeks old when studied, of mean RA 51.6 mm (50–54 mm) and mass 3.0 g (2.5–3.9 g). [Voucher specimens in the Hebrew University of Jerusalem Herpetology Collection (HUI-R) 18885–18900.] The *C. mitratus* ($N=6$) originated from Nicaragua, were purchased in June 1994 and used during July 1994. Their mean RA was 66.5 mm (58–77 mm) and mass 6.0 g (3.9–7.4 g). (Voucher specimens, HUI-R 18876–18882; 19,008.)

The second triad consisted of adults and juveniles of *Oedura marmorata* Gray, 1842 and adults of the smaller *Oedura reticulata* Bustard, 1969 (Diplodactylinae). *O. marmorata* were collected by N. Werner and Y. L. Werner from granite outcrops 6–8 km north of Mount Magnet township, Western Australia, during September–October 1993, and were used in experiments during June–July 1994. The mean RA of the adults ($N=5$) was 96.3 mm (range 85–99 mm) and mass 23.7 g (20.0–28.7 g), and for the juveniles ($N=5$) mean RA was 69.0 mm (64–74 mm), mass 8.2 g (range 5.4–11.2 g). In this species, maturation takes 5 years and is accompanied by coloration change (Cogger, 1957; Y. L. Werner, personal observation). Hence we can estimate that, when captured, the adults were at least 4.5 years old, and the juveniles were up to 2.5 years old. (Voucher specimens HUI-R 18934–18944.) *O. reticulata* ($N=5$) were collected by N. Werner and Y. L. Werner in eucalyptus groves in the Bungalbin Hills area, approximately 100 km NE of Southern Cross township, southern Western Australia, during 1–5 November 1993, and were used during July–August 1994; mean RA 60.8 mm (52–68 mm), mean mass 6.2 g (4.1–10.0 g). (Voucher specimens HUI-R 18949–18953.)

A third triad comprised adults and one juvenile of *Gehyra punctata* (Fray, 1914) and somewhat smaller adult *Gehyra variegata* (Duméril and Bibron, 1836) (Gekkonidae: Gekkoninae). The *G. punctata* were collected by N. Werner and Y. L. Werner from a granite outcrop between Gallowa and Barnong Station, west-central Western Australia, on 17–18 September 1993 and used in June 1994. Mean RA of the adults ($N=5$) was 55.9 mm (51–61 mm) and mass 5.3 g (4.0–6.9). For the single juvenile, RA was 38 mm and mass 1.3 g. (Voucher specimens HUI-R 18910–18917.) *G. variegata* ($N=6$) were collected by N. Werner and Y. L. Werner from granite outcrops 6–17 km north of Mount Magnet township, Western Australia, during September–October 1993, and used in experiments in June 1994; mean RA 43.3 mm (38–47 mm) and mass 2.6 g (1.8–4.3 g). (Voucher specimens HUI-R 18923–18927; 18929–18930.)

Biological and evolutionary background information on all species is given in Rösler (1995) and Bauer (1998), and for the *Oedura* and *Gehyra* species in Storr et al. (1990) and Cogger (1992).

Collecting in Australia and export to the USA were done under permits SF001105 from the Department of Conservation and Land Management of Western Australia, and PWS-P935483 from the Australian Nature Conservation Agency, Canberra, respectively. At the University of Pennsylvania, all geckos were maintained and tested under a protocol approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Between capture or receipt and testing (except during transportation) the animals were housed in small groups in glass terraria with shelters and access to water. They were fed mealworms, waxmoth larvae (in Australia) or crickets three times a week. The terraria were in rooms held at a constant 25°C under a 12 h:12 h light:dark cycle.

Methods

Our methods were as described earlier (Werner et al., 1998, 2001) and are summarised as follows. For anaesthesia we used a solution of 20% urethane in reptilian saline (Wever, 1978). The initial dose was 0.012 ml g^{-1} and additional doses (each 20% of the initial dose) were given to the smaller geckos, because small animals require allometrically larger doses (Sedgwick, 1986). Anaesthesia was judged by the elimination of any response to pinching. We kept seasonal variation to a minimum among samples to be compared (Köppl et al., 1990).

Tympanic membrane (TM) velocity and phase behavior were measured to a constant pure tone intensity across all test frequencies (0.15–20.0 kHz) at the TM surface (100 dB SPL). The velocity response was plotted to show only the magnitude portion of the transfer function; phase behavior was the same as previously reported (Werner et al., 1998). Since the input sound stimulus at the TM was constant, the velocity response directly indicates the transfer of sound vibrations from air to the TM. The TM was surgically exposed by minimal removal of skin and soft tissues surrounding the external ear opening. The animal was positioned on its back on a metal plate, and the head and limbs were stabilized with modeling clay, which also blocked the contralateral ear. The animal's mouth remained closed but the throat was surgically opened, as for electrode access to the round window, to enable later comparison of the results obtained from the two methods. The metal plate was secured on a heavy-duty X-Y stage resting on a large granite stone inside a sound-attenuated room. The tabletop was isolated from the stone by vibration dampers. The X-Y stage enabled the animal to be moved in these axes with micrometer precision.

Free-field acoustic stimulation was provided by a 14 cm midrange speaker (model 405-8H, Altec Lansing Inc., Milford, PA, USA) suspended approximately 12 cm above the animal's head. Pure tone stimuli from the speaker were generated with a computer-controlled frequency synthesiser (model System One, Audio Precision Inc., Beaverton, OR, USA). The synthesiser was programmed to step logarithmically through 141 frequencies between 0.15 and 20.0 kHz, sequentially. The frequency range was extended beyond the 10.0 kHz limit that is conventional when testing lizards, because some gecko vocalisations contain ultrasound, even up to 60 kHz (Brown, 1985; Frankenberg and Werner, 1992). A probe tube (50 mm × 0.5 mm i.d.) leading to a 12.5 mm condenser microphone was positioned with its tip at the perimeter of the TM. The probe tube was calibrated in free field against a 3.13 mm condenser microphone (Brüel and Kjaer, model 4138, Viby, Denmark). During calibration of sound at the TM surface, the synthesiser stepped through all test frequencies, while maintaining the voltage across the loudspeaker constant. The output signal from the probe-tube microphone was led to the analyser section of the synthesiser. The analyser section measured the voltage of each test tone, as detected by the microphone. Low-frequency noise was eliminated by a moving one-third octave filter centred about each test tone. This filter

was a built-in function of the analyser. A look-up table was created (under software control) with test frequency and voltage in adjacent columns. The software then calculated corrections for each frequency so that the voltage delivered to the speaker by the generator portion of the synthesiser would produce a constant SPL of 100 dB at the surface of the TM. All measurements in dB were referenced against $20 \mu\text{Pa}$.

Once the sound level at the TM surface was calibrated, the interferometer laser beam was focused on the TM. The optical head of the laser interferometer was mounted on an x-y-z micromanipulator 20 cm from the TM. Either the animal or the laser head could be moved in order to align the laser beam with the desired point on the TM.

Glass microbeads, 15–30 μm diameter (5–10 ng), were placed at two, or sometimes three, locations on the external surface of the TM (Bigelow et al., 1996). These microbead positions, chosen for repeatability and representing locations to show the mechanical leverage and the input to the ossicular chain, were (A) opposite the junction of the extracolumellar shaft with the extracolumellar processes radiating on the inside of the TM, (B) at the tips of pars inferior of the extracolumella (TPI) and, sometimes (C) on the free TM, approximately midway between the TPI and the anterior rim of the TM (Fig. 1). The tip of a single human body hair was used to

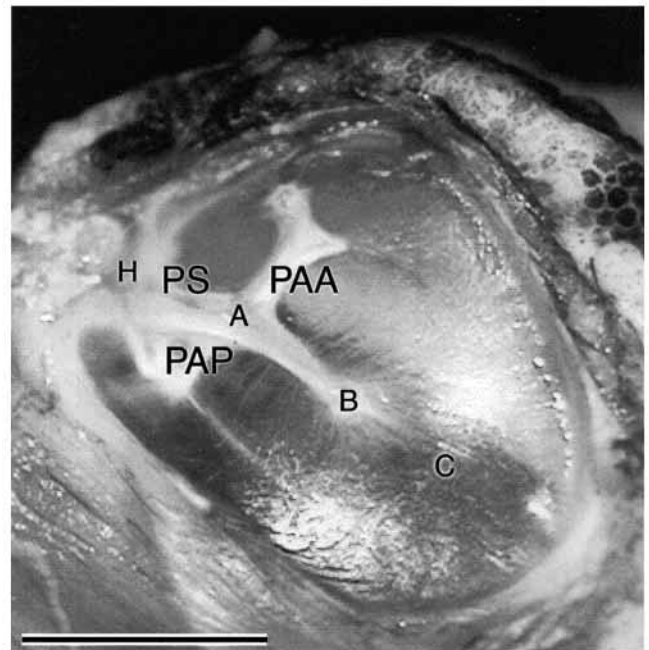


Fig. 1. Right-side ear of freshly dead adult *Eublepharis macularius* (Nikon Multiphot Macrophotography system). The tympanic membrane (TM) has been completely exposed surgically. The four processes of the extracolumella spreading on the inside of the TM are clearly seen through. A, location of the microbead opposite the columella-extracolumella shaft (hidden from view); B, location of the microbead at the tip of the pars inferior of the extracolumella; C, location of the microbead on the free sector of the TM; H, hinge of the extracolumellar mechanical lever; PAA, anterior process; PAP, posterior process; PS, superior process. Scale bar, 3 mm.

deposit the microbead on the TM surface. The beads adhered to the TM, presumably because of the cerumen on the TM surface (Cohen et al., 1992). The glass beads increased the reflectance of the laser beam by >300% compared with the reflectance of the TM surface itself. The alignment of the laser beam with the bead was adjusted for maximum signal strength. These procedures significantly improved the signal-to-noise ratio of the velocity signal received by the interferometer. The velocity transfer function of the TM was measured at each bead location.

The synthesiser then stepped through the series of test frequencies, each presented at 100 dB SPL. The output signal from the interferometer was connected to the analyser section of the synthesiser and, as each tone was presented, a voltage signal for the velocity of the TM was stored in the computer memory and transferred to a disk file.

Each experiment was performed on a group of 4–6 animals (with the exception of the single *Gehyra punctata* juvenile). At each frequency the mean velocity response and standard error of the mean (S.E.M.) were calculated for the group. The resulting TM-response plots underwent a smoothing procedure, which calculated a running average over a moving window of six test frequencies. This smoothing procedure moderated local perturbations due to minor anomalies of calibration or unique responses of the TMs.

Temperature affects reptilian auditory sensitivity such that optimal audiograms are obtained at the ecologically preferred body temperature of the species (Campbell, 1969; Werner, 1976). Although a minor contributing effect on the middle ear has been indicated (Werner, 1983), the main effect is at the level of the inner ear (Eatock and Manley, 1981). Our experiments were conducted at stable room temperature, 20–22°C, well within the normal efficient

activity range of the subjects, as all these species are nocturnal foragers.

Results

Size effects among species

We first explored size effects among species by checking the Pearson correlations within all possible pairs among seven relevant physiological and morphological variables. The use of this test was justified since none of the data deviated significantly from normal. Correlations were tested for a population of nine samples, comprising the three triads described above. The physiological variables were the peak velocity (mm s^{-1}), the frequency of the peak velocity (kHz), and the bandwidth, defined as the frequency range, in octaves, between the low- and high-frequency intercepts at half-maximum velocity (the range, in octaves, of frequencies that attained at least half the peak velocity) of the averaged TM transfer functions, all measured both at the TPI and opposite the columella. The morphological variables (Fig. 1), from unpublished data, were the TM area (mm^2), the anchorage length of the extracolumella on the TM (pars inferior + pars superior, mm), length of the pars inferior (mm), and RA length (mm). These sample means are shown in Table 1. The correlations among the seven variables are presented in Table 2. All four measurements of size (of the body and of middle-ear structures) were strongly and significantly correlated with each other, $r > 0.9$, $P < 0.05$; the strongest correlation was that between the lengths of the extracolumellar anchorage and the pars inferior alone ($r = 0.999$, $P < 0.01$).

However, the four morphological measures differed a little in their effects on the velocity transfer function variables. Maximum velocity was most strongly correlated with RA

Table 1. *Sample averages for three physiological and four morphological variables*

Sample	Species code	Transfer function			Morphological measures			
		Maximum velocity (mm s^{-1})	Peak frequency (kHz)	Peak width (octave)	TM area (mm^2)	Extracolumella anchorage (mm)	Pars inferior (mm)	RA (mm)
Eublepharid triad								
<i>Eublepharis macularius</i>	2	4.3	2.5	0.53	27	3.26	2.61	109.5
<i>E. macularius</i> juveniles	3	2.9	2.9	1.89	8.07	1.79	1.47	51.6
<i>Coleonyx mitratus</i>	1	3.2	2.4	1.86	8.45	2.11	1.64	66.5
Diplodactylid triad								
<i>Oedura marmorata</i>	7	4.1	2.3	0.78	15.2	2.71	2.17	96.3
<i>O. marmorata</i> juveniles	8	2.3	3	1.65	5.59	2.13	1.66	67.4
<i>Oedura reticulata</i>	9	3.3	3.1	1.82	5.64	1.64	1.3	60.8
Gekkonine triad								
<i>Gehyra punctata</i>	4	2.3	5	0.84	6.37	1.35	1.06	57.2
<i>G. punctata</i> juvenile	5	2.3	4.5	2.85	3.31	1.04	0.79	38
<i>Gehyra variegata</i>	6	2.04	4.1	1.88	3.76	1.23	0.98	43.2

TM, tympanic membrane; RA, rostrum–anus length.

The species codes relate to Figs 2–4.

The physiological variables of the TM transfer function were measured at the tip of the pars inferior of the extracolumella.

Table 2. Correlations (r) among three TM transfer function variables (measured at the tip of the pars inferior of the extracolumella) and four morphological size measurements, from data summarised in Table 1

	RA length	Pars inferior length	Extracolumella anchorage length	TM area	Bandwidth (octave)	Peak frequency
Maximum velocity	0.880**	0.870**	0.864**	0.858**	-0.596	-0.762*
Peak frequency	-0.682*	-0.806**	-0.805**	-0.569	0.297	
Band width	-0.813**	-0.717*	-0.709*	-0.736*		
TM area	0.918**	0.913**	0.907**			
Extracolumella anchorage	0.966**	0.999**				
Pars inferior length	0.964**					

TM, tympanic membrane; RA, rostrum–anus length.

$N=9$ in each group.

*Correlation is significant at $P<0.05$ level (two-tailed t -test).

**Correlation is significant at $P<0.01$ level (two-tailed t -test).

(Fig. 2). The frequency at maximum velocity was most strongly negatively correlated with the extracolumellar anchorage length and the length of pars inferior (Fig. 3). The octave bandwidth of the velocity function was, again, most strongly negatively correlated with RA (Fig. 4). Maximum velocity strongly and significantly negatively correlated with its frequency. However, the correlation of maximum velocity and of peak frequency with octave bandwidth was not significant.

Next we applied stepwise regression analysis in order to view each of the three physiological variables as a function of the four size measurements (Draper and Smith, 1998). The results matched the above in principle but indicated stricter

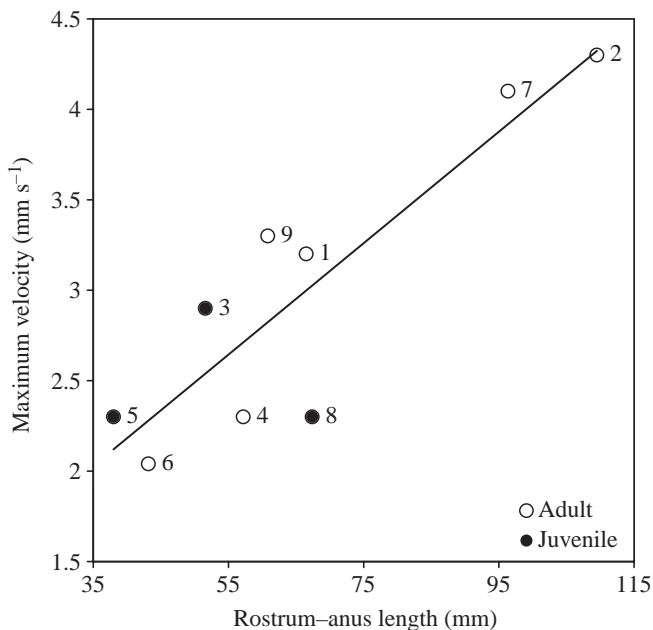


Fig. 2. Correlation ($r=0.88$) of the maximum velocity of tympanic membrane vibration (mm s^{-1}), with animal rostrum–anus (RA) length, among nine samples of gekkonomorph lizards. Sample codes are explained in Table 1. The line describes the linear regression, maximum velocity = $0.95 + 3.081 \times 10^{-2} \text{RA}$.

restriction of the factors affecting the physiology. Statistically, maximum velocity was determined by RA alone ($r_{\text{adj}}^2=0.743$, $P<0.01$). The frequency at maximum velocity was determined only by pars inferior length ($r_{\text{adj}}^2=0.599$, $P<0.01$). The octave bandwidth was determined by RA alone ($r_{\text{adj}}^2=0.613$, $P<0.01$).

Finally, we asked whether juveniles differ from adults in the relationship of the measured properties of the TM transfer function to the morphological variation. We performed linear regressions, using only the size measurements that significantly affected the physiological variables. The two age categories

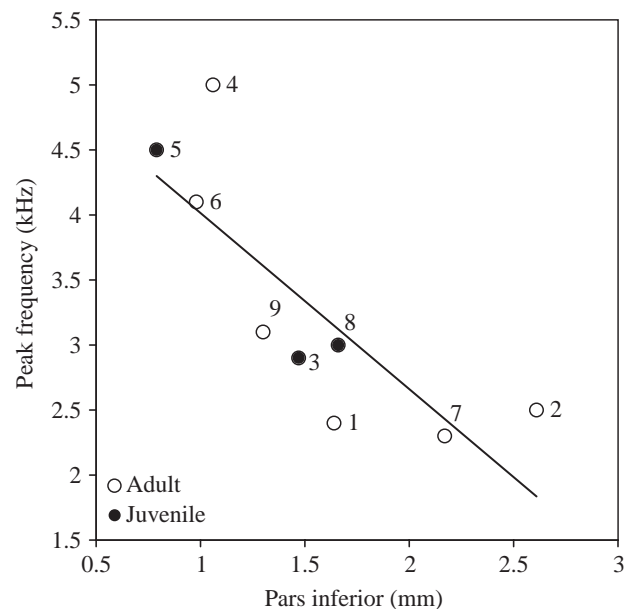


Fig. 3. Correlation ($r=0.806$) of the frequency at which tympanic membrane vibration reaches peak velocity (kHz), with pars inferior length among nine samples of gekkonomorph lizards. Sample codes are explained in Table 1. The line describes the linear regression, peak frequency = $5.369 - 1.354 \times \text{pars inferior length}$. The correlation of the peak frequency with extracolumella anchorage length (combined lengths of pars superior and pars inferior) is $r=0.805$ and this regression equation is peak frequency = $5.41 - 1.095 \times \text{anchorage length}$.

were included as dummy variables (Draper and Smith, 1998). To correct for size differences between adults and juveniles, the measurements were taken on a relative scale; the maximum measurements for each age category were used as an anchor

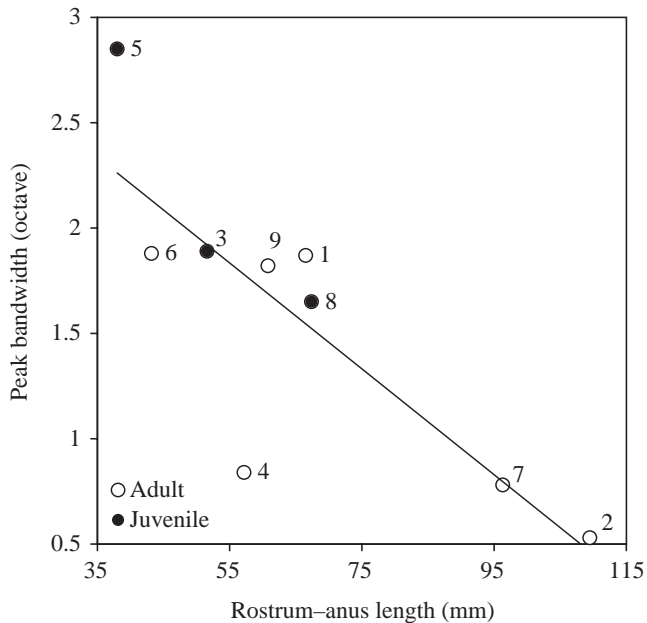


Fig. 4. Correlation ($r=0.813$) of the peak bandwidth of the tympanic membrane transfer function (octave range of frequencies achieving at least half the velocity of the peak), with animal rostrum-anus (RA) length, among nine samples of gekkonomorph lizards. Sample codes are explained in Table 1. The line describes the linear regression, peak width= $3.219-2.51\times 10^{-2}RA$.

point and given the value of one. All other size measurements were scaled relative to them. An Arcsine transformation was used on the relative size values in order to adjust their distribution to a normal one ($y=\arcsin\sqrt{p}$, where p is the relative size value) (Sokal and Rohlf, 1997). The transformations performed on the data did not allow for analysis of the intercept. No significant differences were found in all analyses of juvenile and adult responses.

Size and age effects within species-triads

The results from the three triads of species are graphically summarised in Figs 5–7, in each of which the averaged TM transfer functions of the three samples comprising the triad are superimposed. Because the point of measurement (glass-bead location) affected the sensitivity of the transfer function, i.e. the velocity attained, but not its shape (fig. 6 in Werner et al., 1998; fig. 2.18 in Saunders et al., 2000), we present for each triad the results from one location only (location A, opposite the columella–extracolumella shaft). From Figs 5–7 it can be seen that in each of the triads the general shape of the transfer functions is relatively similar for the two functions representing the juveniles of the larger species and the similarly sized adults of the smaller species. Both together differ from the more sharply tuned transfer function of the adults of the larger species. We now consider these visual observations in Figs 5–7 more quantitatively.

Rather than complicate Figs 5–7 with the S.E.M. values on each curve at each test frequency, we present a separate example of the amount of variation. Fig. 5A shows a transfer function averaged from the raw data, without the smoothing procedure, with S.E.M. values calculated from the raw data. The

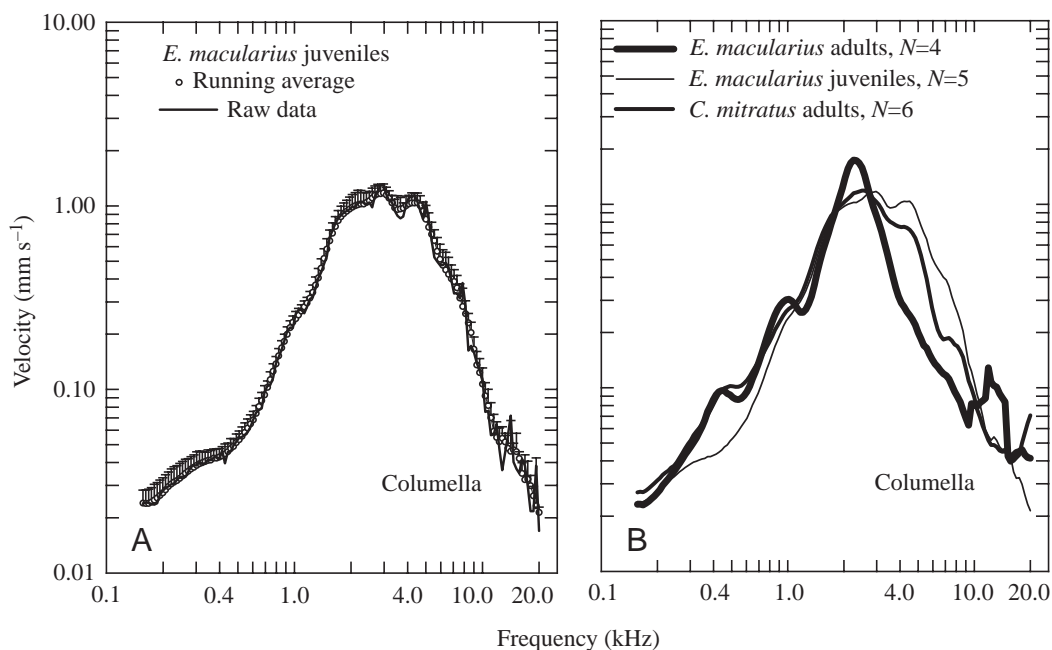


Fig. 5. (A) The averaged raw (unsmoothed) transfer function for juvenile *Eublepharis macularius*. Values are means + 1 S.E.M. This variability was typical of all the curves reported here. (B) Averaged peak-to-peak velocity functions of the tympanic membrane, measured at the junction of the columella, for the eublepharid triad.

variability shown in Fig. 5A is typical of that seen in all the transfer functions that we report here (except for one curve based on a single animal) and elsewhere (figs 3–6 in Werner et al., 1998; fig. 2.18 in Saunders et al., 2000; fig. 2 in Werner et al., 2001).

The nine transfer functions measured opposite the columella (Figs 5–7) or at the TPI (Table 1) may be compared in quantitative terms by determining for each the greatest velocity, the frequency at the point of peak velocity, and the octave bandwidth. Table 1 shows that (at the TPI) in the eublepharid triad the TM attains a much higher velocity in the large adults than in the juveniles and small adults, which resemble each other. Similarly, the octave bandwidth of the velocity function in the large adults is less than half those of the juveniles and small adults, which resemble each other. However, the peak frequency of the small adults resembles that of the large adults (compare with Fig. 5). In the diplodactylid triad the small adults resembled the juveniles and differed from the large adults both in the best frequency and in octave bandwidth. In TM velocity the small adults were intermediate between the two other groups (compare with Fig. 6). Finally, in the gekkonine triad the peak frequency and the octave bandwidth are similar in the small adults and the juveniles, and together different from the large adults. The peak velocity of the juvenile is intermediate between the two adult groups (compare with Fig. 7).

Discussion

Comments on the methodology

A number of potential concerns about the methodology used warrant comment. The upside-down posture during

experimentation is quite natural for scansorial (climbing) geckos, comprising four of the six species used. Only *Eublepharis* and *Coleonyx* are cursorial (ground-dwelling), and their results were not different from those of the scansorial gecko.

The surgical window in the throat was shown by Werner et al. (2001), using *Eublepharis* as a model, to have little effect on the results except at the lowest (<0.5 kHz) and highest (>8.0 kHz) frequencies. The effect above 8.0 kHz is discussed below.

Although the contralateral ear was blocked with plasticine, the open sound system could stimulate the ipsilateral TM from both sides because of the fenestrated throat. However, the acoustic pathway through the throat was complicated and we have no evidence that it would allow a stimulus of sufficient intensity to effectively stimulate the inner surface of the TM. Moreover, if there was an effect, it was probably uniform across all the subjects being compared. Any effects of the varying relationship of sound wavelength to head size can be discounted because the transfer function shape correlated with animal size within triads rather than among triads; the adult *Gehyra punctata* had a transfer function shape like the adults of *Eublepharis macularius* and *Oedura marmorata* but a body size like their juveniles or their respective small adults (Table 1). The size effect thus seems to occur independently in parallel within the genera or subfamilies (see below).

Comments on the results

The TM transfer functions in Figs 5 and 6 reveal irregularities in the highest frequencies, although the plots represent group means. These 'jumps' in the velocity–frequency functions may well be due to the

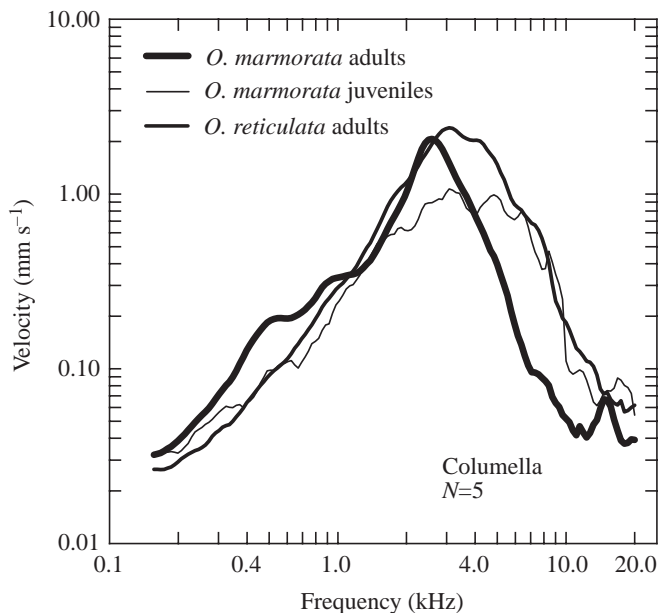


Fig. 6. Averaged peak-to-peak velocity functions of the tympanic membrane, measured at the junction of the columella, for the diplodactylid triad.

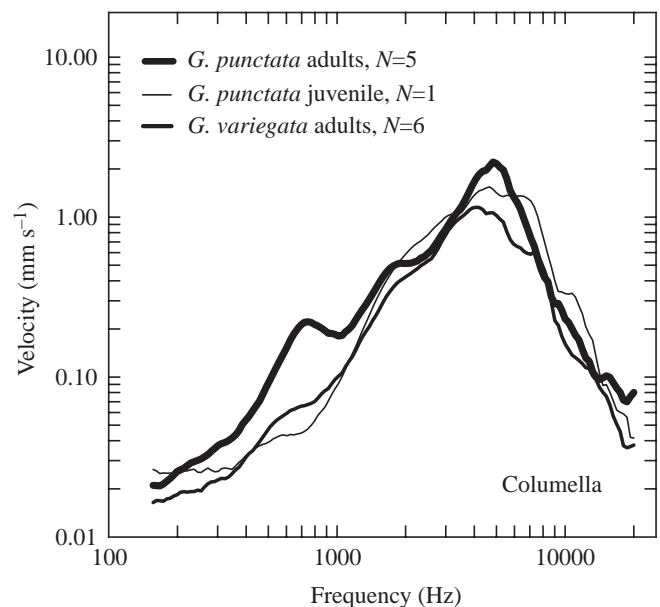


Fig. 7. Averaged peak-to-peak velocity functions of the tympanic membrane, measured at the junction of the columella, for the gekkonine triad.

fenestration of the throat (Werner et al., 2001). Hence an investigation of the high-frequency capacity of gecko ears, interesting because of the enigmatic high-frequency components in some of their vocalizations (Brown, 1985; Frankenberg and Werner, 1992), should preferably employ methods that exclude such fenestration. But our data do carry a lesson. From our data, as shown in Figs 5 and 6, in the smaller animals the jumps occur at higher frequencies. In Fig. 7, representing even smaller species, only for the group of large adults does the clear onset of a jump occur at the upper extremity of the frequency range; in the curves of the juvenile and small adults there are only marginal indications of a jump. This dependence of the frequency at which the disturbance occurs on animal size (which could be expected) means that when testing even larger lizards the fenestration could also cause disturbance at lower frequencies. Additional, minor peaks in the transfer functions (e.g. in Fig. 5, notably at 0.4 and 1 kHz) cannot yet be interpreted and may deserve attention in future research.

Our analysis of interspecific correlations showed that statistically both the peak velocity of the TM (in mm s^{-1}) and the octave bandwidth of the transfer functions were determined by body size. The frequency at which velocity peaked was determined by the pars inferior length. The difference between these two determining factors is small: among the three variables describing ear size, it is the extracolumellar anchorage length that shows the strongest correlation with RA ($r=0.966$) but the correlation of the pars inferior was similar ($r=0.964$; Table 2). Body length (RA) may be described as representing the three middle-ear size descriptors as follows. We performed a multiple regression of RA over the three ear-size measurements, using the stepwise method. The function used only the anchorage length as an explaining variable. The whole model was significant ($r_{\text{adj}}^2=0.925$; $P<0.001$), but when testing the significance of the regression, only the slope was significantly different from zero ($P<0.001$). Since when ear size approaches zero, initial body size has no meaning, we excluded the intercept from the equation. Thus the best body size description as function of ear size is: $\text{RA}=31.499 \times \text{anchorage length}$.

In the above considerations, we note that the three juvenile samples did not differ significantly from the adults. Since the samples were few, we can only conclude that we could show no difference.

As outlined in the Introduction, we had hoped that any differences among the samples comprising a triad would be similar in all three triads, enabling an unambiguous conclusion to be made about whether age effects are distinct from size effects, and if so, in what way. This hope has been fulfilled only to a small extent. Graphically, in each of the three triads the general shape of the transfer functions is relatively similar for the two functions representing the juveniles of the larger species and the similarly sized adults of the smaller species. Both have a relatively broad velocity peak, and together differ from the more sharply tuned transfer function of the adults of the larger species. In quantitative terms, we may consider the

bandwidth that achieved at least half the peak velocity. For the adults of the large species in all three triads, mean bandwidth = 0.66 octaves (range 0.53–0.78 octaves). In contrast, for the juveniles of the same three species, mean bandwidth = 2.13 octaves (1.65–2.85 octaves), and for the adults of the smaller species of the three triads, mean bandwidth = 1.60 octaves (0.84–1.88 octaves).

We applied the Tukey's multiple-comparisons test to analyze whether the three categories – juveniles, small adults and big adults – differ in their mean octave ranges (Sokal and Rohlf, 1997). The juveniles differed from the large-adults group ($P<0.05$), but neither of these two groups differed significantly from the small-adults group.

In principle, our finding that the larger animals (individuals or species) have more sensitive TM responses parallels the findings of Rosowski et al. (1984, 1988) in the alligator lizard and the findings of Huang (1999) in the cat family.

We may interpret the size-related variation observed here in the TM transfer function, in light of the physics of mammalian middle-ear function as elucidated by Relkin (1988) and Rosowski (1994; pp. 217). In our data obtained for geckos, in accordance with the mammalian model, increases in body size and ear size are accompanied by at least three changes in middle-ear function.

First, peak TM velocity increases, perhaps due to decreased damping in TMs with a larger surface area, accompanied by the increased hydraulic ratio due to the columellar footplate not growing as much as the TM (Werner et al., 1998). Rosowski (1994) raised the possibility that a disproportional enlargement of the TM in mammals could lead to 'overmatching', which would result in a reduction in middle-ear sound transmission efficiency. Nevertheless, the situation in these two animal classes differs. In mammals there is a general trend for interspecific uniformity in the hydraulic ratio (Rosowski, 1994), while in reptiles (or at least geckos) the norm is interspecific variation, with the larger species having a higher ratio (Werner and Igić, 2002).

Second, the downward frequency shift in the transfer function velocity peak might arise from increased mass, or perhaps also decreased stiffness, in the conducting structures of the middle-ear system of the larger animals. At the moment, we lack any data to support the latter possibility.

Third, the shape of the transfer function changes. As body size increases, the function becomes more sharply tuned (i.e. the octave bandwidth is reduced), as predicted for a system dominated by the effects of mass (fig. 10 in Relkin, 1988). A decrease in the damping factor of the conducting apparatus could also produce a more sharply tuned function. Again, data in support of this mechanism remain to be identified.

These suggestions on the roles of damping, mass and stiffness should not be lumped as an oversimplified holistic explanation of the effects of size. In view of the complexity of the middle ear, they only indicate the types of mechanisms that may contribute to the size-related differences among the TM transfer functions.

Although the characteristics of the TM transfer function did

not differ statistically between the juveniles of the large species and the adults of the small species, scrutiny of Figs 5–7 indicates a possible qualitative difference. In each of the three triads, the very top of the function appears to be broadest in the juveniles. Because these functions are somewhat irregular in the relevant range, this phenomenon is not readily quantified but it warrants further investigation.

The relevance of these observations to the stimulation of the inner ear will be further discussed elsewhere when reporting on the audiograms derived from the same animals, based on VIIIth-nerve compound action potentials.

Conclusions

In gekkonomorph lizards, the transfer function of the tympanic membrane velocity, as seen under constant-sound stimulation, varies with animal size as follows. (i) The maximum velocity response is most strongly correlated with animal (rostrum–anus) length. (ii) The frequency at which maximum velocity occurs is most strongly negatively correlated with the pars inferior length. (iii) The octave bandwidth is most strongly negatively correlated with animal length.

Among the morphological measurements of the middle ear, the one with which body length is most closely correlated is the extracolumellar anchorage length.

Among the physiological variables, the maximum velocity is strongly and significantly negatively correlated with the frequency at which it occurs.

The dependence of the physiological variables of the tympanic membrane velocity transfer function on animal and ear size, is similar whether the comparison is among age classes within a species, or among adults of species of different size. Thus the age effects are largely due to size effects but the possibility of a separate age effect needs further research.

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