Otoacoustic Emission Temperature Dependence Across the Lacertilia

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Background

- OAEs are typically considered a by-product of an underlying amplification mechanism(s) at work in the ear. Presumably, such a process requires metabolic activity in order to boost detection of low-level stimuli. As such, one might expect some degree of temperature-dependence.
- In addition to metabolic effects, temperature presumably affects many aspects of auditory function and in turn OAE properties, for example: enocochlear potential, chemical/electro-dynamics (e.g., K^+ and Ca^{2+} transduction), mechanical properties (e.g., stereovillar bundle stiffness), middle ear transmission
- Although much is yet to be learned, several previous studies have examined temperature-dependent effects:
  - Neurophysiological (ANF) studies in lizards (Eatock & Manley, 1981) and mammals (e.g., Ohlemiller & Siegel, 1994)
  - Evoked potentials in lizards (e.g., Campbell, 1969; Werner, 1972)
  - OAE studies in frogs (van Dijk et al., 1989; Meenderink & van Dijk, 2006) and lizards (e.g., Manley & Köppl, 1994; Manley et al. 1996; Manley, 1997)
- Lizards serve as a good model for studying temperature-dependent effects upon hearing, as they:
  - are ectothermic (i.e., 'cold-blooded'), thus naturally experience wide body temperature variations
  - have a relatively simple anatomy (e.g., no basilar membrane traveling waves)
  - exhibit wide variations in tectorial membrane (**TM**) morphology (e.g., some lizards lack a TM altogether)
  - are robust emitters, both spontaneously (**SOAEs**) and via evoking stimuli (**eOAEs**)
- eOAEs potentially provide a robust (& non-invasive) window into emission generation mechanisms since they can readily be produced when no SOAE activity is detectable. Understanding temperature effects upon eOAEs can thereby lead to further understanding of the underlying amplification processes in the ear.

QUESTION: How does temperature affect OAEs (spontaneous & evoked) across a variety of lizard taxa?

Methods

- Results presented here are part of a broader study examining OAE properties across a wide range of lizard species with greatly differing morphological properties (see Table 1)
- All species readily emitted and numerous distinctions arose across species. However, for clarity, much of the present analysis will focus on representative species, framed as TM vs. non-TM, i.e., comparing emissions from species lacking an overlying TM versus those with some form of tectorial covering, whether a continuous ribbon as in mammals or discretized sections called sallets
- This study focused on using relatively low-level stimuli (i.e., close to threshold), where responses tend to be more linear and presumably confined to a more focused generation region
- Animals were anesthetized (save for exceptional cases) using Nembutal (~25-35 mg/kg i.p.), which was sufficient for a 2-5 hour period
- OAEs measured via an ER-10C and custom PC (see Bergevin et al. 2008 for details), the probe calibrated in-situ
- All data (except Figs.1 & 2) are from the steady-state temperature condition. Lizards were placed atop a heating pad (initially turned off) and allowed to settle to ambient room-temperature (~21-23 °C). After the COOL recordings were made, the blanket was turned on. Approximately 15-45 min. were required to reach a stable temperature (~29-32 °C), at which the WARM recordings were made.
- Temperature was monitored via a calibrated thermocouple placed either in the mouth or leg pit. Thus, recorded temperatures varied somewhat across individuals. Temperatures were also monitored cloacally at the start & end of some experiments via a quick-reading mercury thermometer, verifying the approximate change from COOL to WARM (~5-10 °C depending upon species).

Table 1. Species examined in the present study. Cited values are from Wever (1978) and Miller (1985), the latter in parentheses. Where unknown, inferences based upon similar species are included (designated via ?). Family abbreviations as follows: Ag – *Agamidae*, An – *Anguidae*, Gk – *Gekkonidae*, Gr – *Gerrhosauridae*, He – *Helodermatidae*, Ph – *Phrynosomatidae*, Po – *Polychrotidae*, Sk – *Scincidae*, Te – *Teiidae*. Families Ag, Ph and Po all fall within infraorder Iguania. The designations non–TM, sallettal, and continuous TM are meant simply to indicate the morphology of the TM over the majority of the papilla (i.e., for the bi–directional hair cells). All species except E. schneideri have a continuous TM attached to the limbic lip overlying the portion of the papilla sensitive to frequencies below 1 kHz (see Manley 2000, 2002). Note that for clarity, the TM morphologies listed here are a simplification; see Wever (1978), Miller (1992) for more detailed descriptions. Total hair cell counts in the last column are per ear. Species with data included on poster are highlighted.

		Anatomical parameters		
Species (common name)	Family	TM type (> 1 kHz)	Papilla Length [mm]	# of hair cells
Agama agama (rainbow agama)	Ag	none	0.4	240 (220)
Anolis carolinensis (green anole)	Po	none	0.45 (0.5)	160 (182)
Aspidoscelis tigris (whiptail lizard)	Te	continuous	0.65	370 (465)
Callisaurus draconoides (zebra–tail lizard)	Ph	none	(0.2)	65 (73)
Elgaria multicarinata (southern alligator lizard)	An	none	0.4	160
Eublepharis macularius (leopard gecko)	Gk	sallets & continuous	1.25	970
Eumeces schneideri (Schneider's skink)	Sk	sallets	?	500?
Gekko gecko (tokay gecko)	Gk	sallets & continuous	1.8	1620 (2100)
Gerrhosaurus flavigularis (yellow–throated plated lizard)	Gr	sallets	0.8?	530
Heloderma suspectum (Gila monster)	He	continuous	0.5–1?	300?
Pogona vitticeps (bearded dragon)	Ag	?	?	?
Sceloporus magister (desert spiny lizard)	Ph	none	0.35 (0.35)	80 (90)
Tupinambis teguixin (black & white tegu)	Te	continuous	1.4	1400
Urosaurus ornatus (ornate tree lizard)	Ph	none	0.29?	55
Uta stansburiana (common side–blotched lizard)	Ph	none	0.22? (0.2)	52 (55)

I - Temperature Effects on Spontaneous OAEs

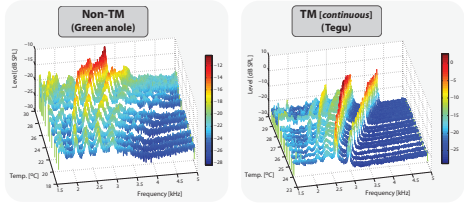
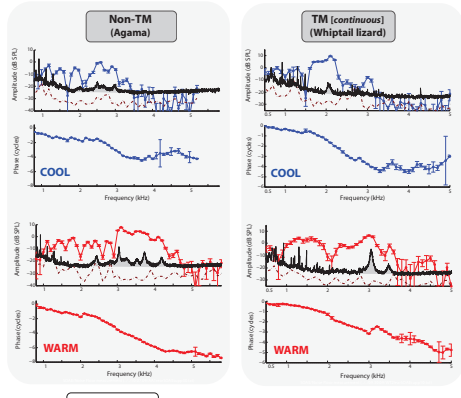


Figure 1 - SOAE temperature-dependence in two different individuals (*Anolis carolinensis* on left, *Tupinambis teguixin* on right). Lizards were warmed up from room temperature via a heating pad over the course of ~25-35 min.

Figure 2 - SOAE temperature-dependence in an individual *Aspidoscelis tigris*. A total of ~20 min. passed between the first and third recordings. Note the merger of the two peaks between 3.5-4.5 kHz (similar to Manley et al., 1996).

- Lizard SOAEs spectrally consist either of a plateau and/or distinct peaks (typically a super-position of both); peaks were more apparent and sharper in species with some form of TM
- Consistent with previous reports (Manley & Köppl, 1994; Manley et al. 1996; Manley, 1997), significant upward SOAE frequency shifts were seen with increasing temperature, though no consistent magnitude change was readily apparent
- While not always so dramatic (e.g., the *Phrynosomatidae* family, whose papilla have <100 hair cells), the qualitative behavior shown in Fig.1 was routinely observed in all species
- While differences were apparent between TM and non-TM species, temperature effects were broadly similar despite significant variations in TM morphology
- Data indicate that different generators likely interact in a complex, temperature-dependent fashion (e.g., peak merging in Fig.2), with TM coupling likely playing a significant role (Manley, 1997)

II - Covariation Between SOAEs and SFOAEs



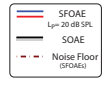


Figure 3 - Temperature-dependence co-variation between SOAEs and SFOAEs (Lp= 20 dB SPL). Data shown for both an individual *Anolis carolinensis* (left) and *Aspidoscelis tigris* (right). Top row shows the room-temperature case (blue) while the bottom row is the warm condition (red). Noise floor indicated by dashed line (brown). SOAEs were measured both before and after the SFOAE sweep and found to be stable. Additional grey shading is added for clarity to highlight approximate regions of SOAE activity. Errorbars show standard error of the mean over 35 time-averaged waveforms.

- Correlation observed between SOAEs and SFOAEs: larger SFOAE magnitudes in regions where SOAE activity is present (though the converse not necessarily true)
- SFOAEs shift upwards in frequency with increasing temperature in a fashion similar to SOAEs, suggesting commonality in generator between two OAE types
- Little overall temperature effect upon SFOAE phase (further addressed in Fig.6)
- While not specifically explored, presumably each species has a unique 'optimal' temperature that corresponds to maximum sensitivity and relates to actual temperatures experienced in the native environment (Campbell, 1969; Werner, 1972)

III - Temperature Effects on Evoked OAEs

SFOAE
Lp= 20 dB SPL

COOL
WARM

DPOAE
(2f1-f2)
L_1=L_2=45 dB SPL
f2/f1=1.22

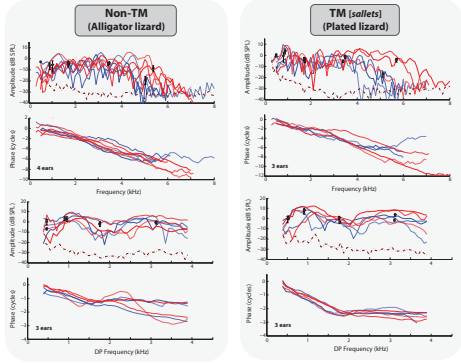


Figure 4 - Variations in both SFOAE and DPOAE (2f1-f2) magnitude and phase for *Elgaria multicarinata* (left) and *Gerrhosaurus flavigularis* (right). Individual points included indicate mean values (and standard error) for magnitudes averaged across octave-wide bins. Different line thicknesses/shading are unique to a particular ear across the cool and warm conditions. Each ear comes from a unique individual. Some phase curves were shifted vertically for clarity.

- Each lizard ear has a unique set of peaks & valleys
- Low-mid frequency (~0.5-4 kHz) magnitudes little affected by temperature
- Emissions extend to higher frequencies in the warm condition, consistent with other measures (e.g., ANF, evoked potentials)
- Consistent with other figures, larger effect with temperature in TM species [e.g., Manley, 1997]
- SFOAE results broadly consistent with model [Bergevin & Shera, 2010] where underlying (slightly irregular) mechanical oscillators shift their center frequency with temperature
- Plated lizard shows clear shift in DPOAE phase behavior about 1.5-2 kHz (e.g., place-fixed to wave-fixed mechanism shift?) independent of temperature; similar to apical/basal shift as seen in mammals?

IV - Temperature Effects on Tuning Estimates

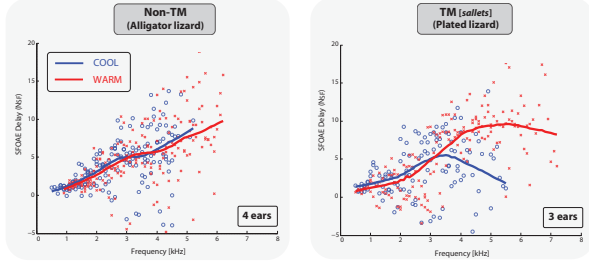


Figure 5 - Temperature-dependence of N_{SF}, the phase-gradient delay associated with the SFOAE (i.e., slopes of phase curves from Fig.4) expressed in periods. Data shown for both *Elgaria multicarinata* (left) and *Gerrhosaurus flavigularis* (right) [same SFOAE data as shown in Fig.4]. Trend line is a locally-weighted regression curve. Only N-values whose corresponding magnitude was at least 10 dB above the noise floor are included.

- Theoretical model indicates N_{SF} proportional to sharpness of auditory tuning [Bergevin & Shera, 2010]
- Little overall effect upon N_{SF} due to temperature (except to extend outwards to higher frequencies)
- Consistent with ANF studies (e.g., Eatock & Manley, 1981; Ohlemiller & Siegel, 1994), indicates tuning not strongly affected by temperature
- Lowering the body temperautre (i.e., below room-temperature) could provide further insight into changes in metabolic activity underlying emission generators (e.g., Meenderink & van Dijk, 2006)

CONCLUSIONS

- eOAE temperature-dependence is qualitatively similar to that observed for SOAEs, ANFs, & evoked potentials
- Results further support that SFOAEs provide an objective/non-invasive measure of auditory tuning due to consistency with ANF studies showing temperature-invariance of tuning
- Larger temperature effect upon OAEs apparent in species with a continuous TM
- A temperature-dependent frequency range is apparent for lizard hearing (consistent w/ previous studies)

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