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Auditory response characteristics of the piebald odorous frog and their implications

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Abstract The piebald odorous frog (*Odorrana schmackeri*), the large odorous frog (*Odorrana livida*) and the concave-eared torrent frog (*Amolops tormotus*) are sympatric species living near the same torrent streams in the vicinity of Mt. Huangshan, China. A recent study demonstrated that *A. tormotus* can use sound signals involving ultrasonic components for communication in a noisy environment, and another sympatric species, *O. livida*, can also perceive ultrasonic sound. Here we report data on the hearing range of *O. schmackeri* by studying auditory evoked potentials and single-unit data from the torus semicircularis. This frog exhibits its two most sensitive peaks at 2 kHz and 3.5–4.0 kHz with thresholds <42 dB SPL, with an upper frequency limit of hearing at 8.5 kHz with threshold of 87 dB SPL. The upper limit is much lower than those of *O. livida* and *A. tormotus*, at 22 and 34 kHz, respectively. It suggests that sympatric species may respond differently to similar environmental selection pressures sculpting auditory communication systems.

Keywords Amphibian · Frog · Hearing · Noise · *Odorrana schmackeri*

Introduction

Acoustic communication plays an important role in the lives of a wide variety of animals (Bradbury and Vehrencamp 1998; Gerhardt and Huber 2002). High-level

environmental noise, man-generated or natural, is known to affect the efficiency of acoustic communication. A variety of mechanisms have evolved to allow vocally-communicating animals to avoid the masking effects of environmental noise. These include: (a) producing call components that fall outside the dominant frequencies of the noise (Narins et al. 2004; Rabin et al. 2003; Slabbekoorn and Peet 2003), (b) increasing the intensity of their calls without changing the frequency to make their calls more audible in noisy environments (Brumm 2004; Brumm et al. 2004; Sun and Narins 2005) and (c) shifting the signaling modality from acoustic to visual (Hödl and Amézquita 2001).

There have been a number of studies focused on the changes in animal acoustic signals in noisy environments (Narins 1982; Narins et al. 1997; Brumm and Todt 2002; Slabbekoorn and Peet 2003; Brumm 2004; Brumm et al. 2004; Sun and Narins 2005; Penna et al. 2005), however, relatively little attention has been paid to potential variations in acoustic signal-receiving systems. To maximize the efficiency of acoustic communication, the receiving systems should match, to a certain extent, the conspecific acoustic signals. In frogs, the receiving systems are thought to have coevolved with the acoustic communication signals (Ryan and Wilczynski 1988; Wilczynski et al. 2001; Gerhardt and Huber 2002; Witte et al. 2005).

The piebald odorous frog (*Odorrana schmackeri*) and the large odorous frog (*O. livida*) belong to different groups of the genus *Odorrana* and are distributed over a broad geographic range across northern and eastern China (Ye and Fei 2001). *O. schmackeri* is one of the most common frog species living in Huangshan Hot Springs, Anhui Province, China; males of this species sing loudly and melodically at night. A recent study showed that *O. livida*, like *A. tormotus*, can detect ultrasonic call components (Feng et al. 2006), and the dramatic shifts of hearing into the ultrasonic range in these species may be an adaptation that minimizes masking of acoustic signals by intense low-frequency noise from local streams.

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Here we will show the auditory response characteristics of *O. schmackeri* and the notable differences in its hearing range relative to *A. tormotus* and *O. livida*, which suggest that sympatric species may have evolved differential responses to same natural environmental disturbances such as ambient noise. It should emphasize separately (a) the unusual condition of having two sensitivity peaks at high frequencies and (b) the existence of such low thresholds at higher frequencies.

Materials and methods

Animal preparation

Piebald odorous frogs (*O. schmackeri*, $n=5$, weight: 4.5–5.3 g) were collected in Huangshan Hot Springs (Anhui Province, China). Frogs were anesthetized with MS222 (tricaine methanesulfonate, 0.5%) for about 5–10 min, then placed on crushed ice to minimize bleeding during surgery. The skin on the dorsal surface of the head was then incised, and a small hole was made in the skull above the right optic tectum. After recovery from anesthesia, the frog was immobilized with an intramuscular injection of *d*-tubocurarine chloride (10 mg/kg body weight) and was wrapped in moist gauze to facilitate cutaneous respiration. During the recording session, the frog was placed dorsal side up on a vibration-isolated table inside a sound-proof, anechoic, electromagnetically-shielded room ($2.3 \times 1.5 \times 2.15 \text{ m}^3$). The temperature was maintained at 19–21°C by a central air conditioner. During recording, supplemental doses of *d*-tubocurarine chloride were given to maintain immobilization.

Sound presentation and recording procedures

Acoustic stimuli were generated using Tucker-Davis technologies system (TDT system 3). Stimulus frequency and intensity, and automated collection of auditory responses were controlled by a computer (Acer). Tone bursts (50-ms duration, 5-ms rise and fall times, 1 stimulus/s) were presented through a loudspeaker (YD78, China; impedance 6 Ω , frequency response: ± 5 dB between 500 and 10 kHz) located directly facing and 15 cm from the frog's contralateral eardrum. Sound pressure levels (SPLs) of acoustic stimuli were determined prior to a physiological experiment with a microphone (Brüel & Kjaer 4135, Denmark) and a measuring amplifier (B&K 2610), and were expressed in dB SPL, 0 dB re 20 μPa rms.

Auditory evoked potentials (AEPs) and single-unit spikes were recorded extracellularly with glass microelectrodes from the central or rostral portion of the TS at a depth between 700 and 1,400 μm . The microelectrodes (tip diameters: 1–2 μm , impedances: 1–10 M Ω) were filled with a 3.0 M solution of sodium acetate. Microelectrodes were positioned over the dorsal surface of the optic

tectum and inserted using a remotely-controlled microdrive (SM-21, Narishige, Japan). Neural responses of the TS neurons to tone bursts were amplified by a pre-amplifier (DAM-80, World Precision Instruments) and displayed on an oscilloscope (YB4320G, Tektronix). In addition, the responses recorded from single-units were band-pass filtered between 300 and 3 kHz; a lower band-pass filter (20–200 Hz) was used for AEPs. The potentials or spikes were extracted using BrainWare software (TDT) and stored on the computer for off-line analysis. AEPs were averaged over 20 trials. Tone bursts were also presented 20 times to construct a peristimulus time histogram (PSTH) for the single-unit recordings.

When AEPs were robust, 19 different sound frequencies (from 1 to 10 kHz in increments of 500 Hz) were tested at a constant intensity in the range from 42 to 87 dB SPL (with a step of 5–10 dB SPL). These measurements were repeated along the dorsoventral axis of the TS every 100 μm -increasing in depth. For single-unit recordings, the characteristic frequency (CF) and sensitive frequency range were determined for a well-isolated unit by manually varying the acoustic stimulus frequency and intensity. Iso-intensity spike count-frequency curves for single units were then automatically obtained (BrainWare).

Results

Auditory evoked potentials and single-unit spikes from the frog's TS were recorded extracellularly in response to tone bursts in the frequency range from 1 to 10 kHz and in the intensity range from 42 to 87 dB SPL.

Auditory evoked potentials from TS

The AEPs recorded from the frog's TS were quite consistent in shape although the amplitudes and latencies of the AEPs showed significant variation as a function of stimulus frequency and intensity. An AEP consisted of an initial sharp, large negative peak (N_1) followed by a large positive peak (P_1) and a small, slow negative wave (N_2) with a long latency (Fig. 1). For each stimulus, N_1 reached its maximum amplitude when the recording electrode was located at a depth of about 1,000–1,200 μm beneath the tectal surface. The amplitude difference between N_1 and P_1 was used as an index of auditory sensitivity of the TS. As shown in Fig. 1, the most sensitive responses of the frog's TS were observed within the frequency range from 1 to 4 kHz at an intensity of 87 dB SPL.

Figure 2 illustrates the frequency properties of AEPs from the TS of *O. schmackeri* at two selected intensities, 87 and 47 dB SPL ($n=7$). At the intensity of 87 dB SPL, AEP amplitudes were largest between 3–4 kHz, then decreased at higher frequencies. AEP latency was minimal at 1.5 kHz, and increased little between 2–6 kHz. However, at the intensity of 47 dB SPL, the AEP disappeared at lower (≤ 1.5 kHz) and higher (≥ 5.5 kHz)

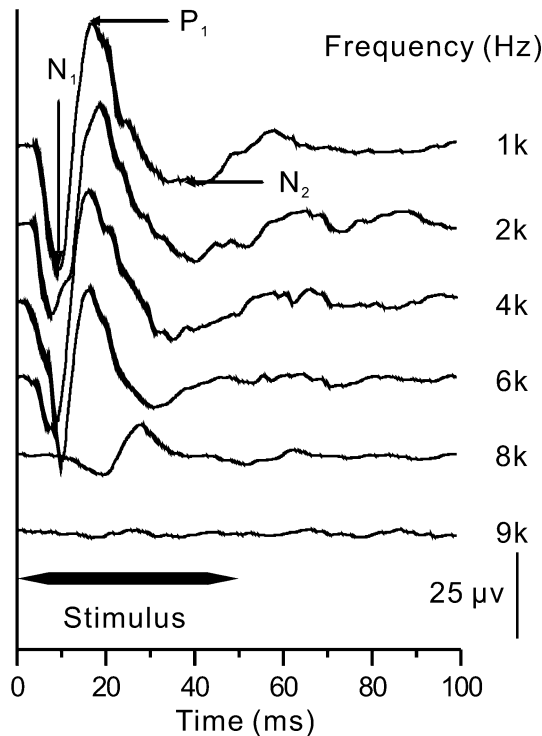


Fig. 1 Auditory evoked potentials (AEPs) recorded from the torus semicircularis (TS) of the frog *Odorrana schmackeri* in response to tone bursts (shown as horizontal bars 50-ms duration, 5-ms rise and fall times, intensity: 87 dB SPL) of different frequencies (shown in Hertz). Rate of stimulus presentation, 1 click/s. AEPs were averaged over 20 stimuli. Calibration bar = 25 μ V

frequencies. Two of AEP amplitude peaks were found at 2 and 3.5–4 kHz, but there were no significant differences between AEP amplitudes in response to 2 and 4 kHz ($t=1.22$, $P>0.05$, $n=14$), or between 3.5 and 4 kHz ($t=0.715$, $P>0.05$, $n=14$). The minimum latency was found at 4 kHz, but there were also no significant differences between 3.5 and 4 kHz ($t=-0.115$, $P>0.05$, $n=12$), or between 2 and 4 kHz ($t=1.16$, $P>0.05$, $n=12$).

The relationships between both N_1 - P_1 -peak amplitudes and N_1 latencies of AEPs versus stimulus frequency at various intensities are shown in Fig. 3a, b. Although AEPs were robust at frequencies from 1 to 8.5 kHz at 87 dB SPL, the maximum peak-to-peak amplitude of the AEP was found at 1 kHz and 3–4 kHz, and the minimum of the peak latency at 2 kHz in response to tone bursts at an intensity of 87 dB SPL. AEP amplitude decreased and latency increased at frequencies >6 kHz.

The AEP amplitude decreased and latency increased with decreasing stimulus intensity at all frequencies tested. There is an inverse correlation between AEP amplitudes and latencies at different intensities for a fixed stimulus frequency. AEP amplitudes increased significantly when the stimulus intensity changed from 42 to 67 dB SPL, and reached saturation at 87 dB SPL for lower (<1.5 kHz) and mid (2–6 kHz) frequencies, as

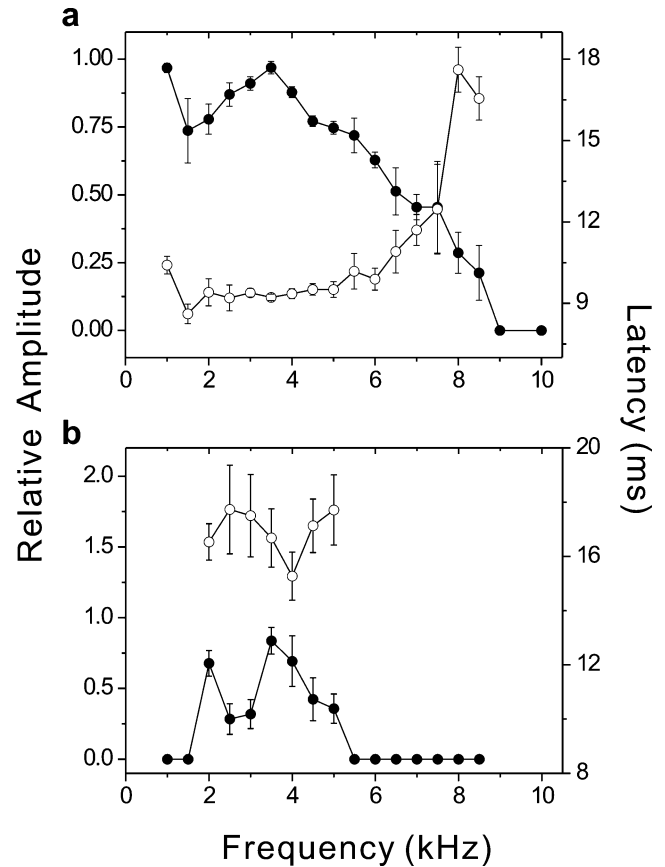


Fig. 2 Amplitude and latency of AEPs from the TS of *O. schmackeri* plotted as a function of stimulus frequency in response to constant-intensity tone bursts: **a** 87 dB SPL and **b** 47 dB SPL ($n=7$). At each position, amplitudes are normalized to the maximum AEP. Solid circles are the mean and standard deviation of the amplitudes; open circles are the mean and standard deviation of the latencies

shown in Fig. 3a. A gradual increase in latency was observed with decreasing intensity at each frequency, but increased rapidly at higher (>6 kHz) and lower frequencies. At the lowest intensity tested of 42 dB SPL, responses were observed only in response to frequencies from 2–4 kHz.

Auditory responses of single TS units

The CF and minimum threshold of well-isolated single TS units ($n=6$) ranged from 1 to 3.8 kHz and from 52 dB SPL to 80 dB SPL, respectively. Most units (five out of six) showed broad frequency tuning and robust spike firing from 1 to 6 kHz; the highest frequency limit was 7.0 kHz at 87 dB SPL. Figure 4 shows the spike rate versus frequency functions and PSTHs of two TS neurons in response to acoustic stimuli at a constant intensity of 87 dB SPL. Both units responded with the onset discharge pattern (Fig. 4 b, d) and their best frequencies were 2 and 1 kHz, respectively, as shown in Fig. 4 a, c, and another peak at 5 kHz. However, no spikes were recorded at frequencies ≥ 7 kHz (Fig. 4a) or 8 kHz (Fig. 4c).

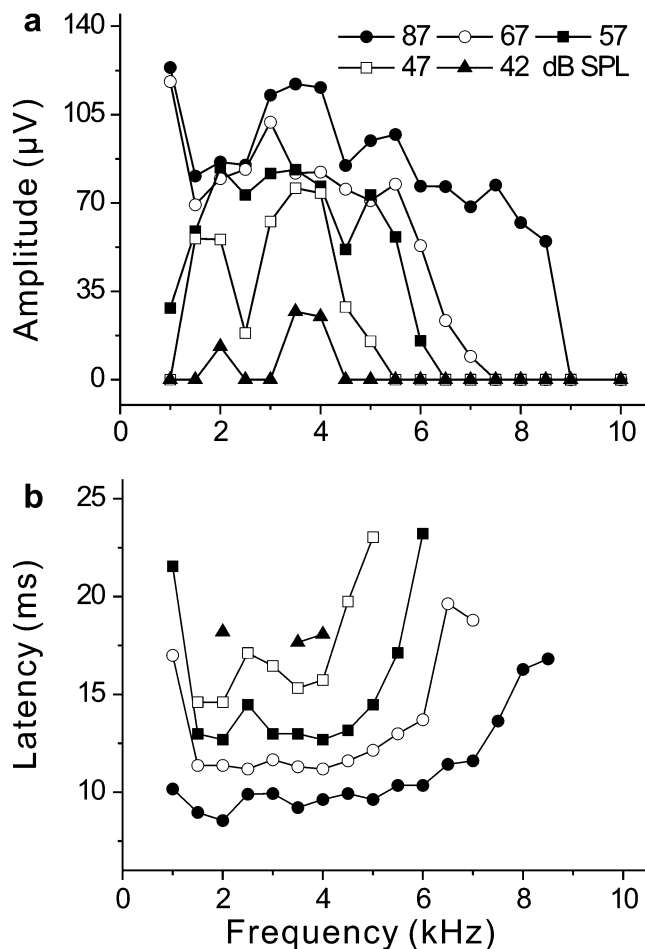


Fig. 3 Amplitude and latency versus frequency of the AEPs recorded from the TS of one *O. schmackeri* in response to tone burst stimuli. The amplitude differences between N_1 -peak to P_1 -peak (a) and N_1 -peak latencies (b) were measured at various intensities (symbols are the same as in a and b). Stimulus frequency varied from 1 to 10 kHz in steps of 0.5 kHz and intensity ranged from 87 to 42 dB SPL.

Discussion

The frogs *O. schmackeri*, *O. livida* and *A. tormotus* are sympatric species living in the vicinity of a fast-flowing mountain stream. Males of both *O. schmackeri* and *O. livida* prefer calling from rocks in the stream, singing very loudly and melodically at night. *O. schmackeri* prefers an open and moist environment quite close to the stream, while *O. livida* moves about near the brushwood (Fei 1999). Calling males of *A. tormotus* were collected in brushwood away from the stream. Ambient factors (foliage, humidity, topography, etc.) can produce strong selective pressure on acoustic communication signals by affecting the transmission and reception of acoustic signals (Wiley and Richards 1978). Animals living in a given habitat have evolved acoustic communication systems with features that are often “designed” to counteract

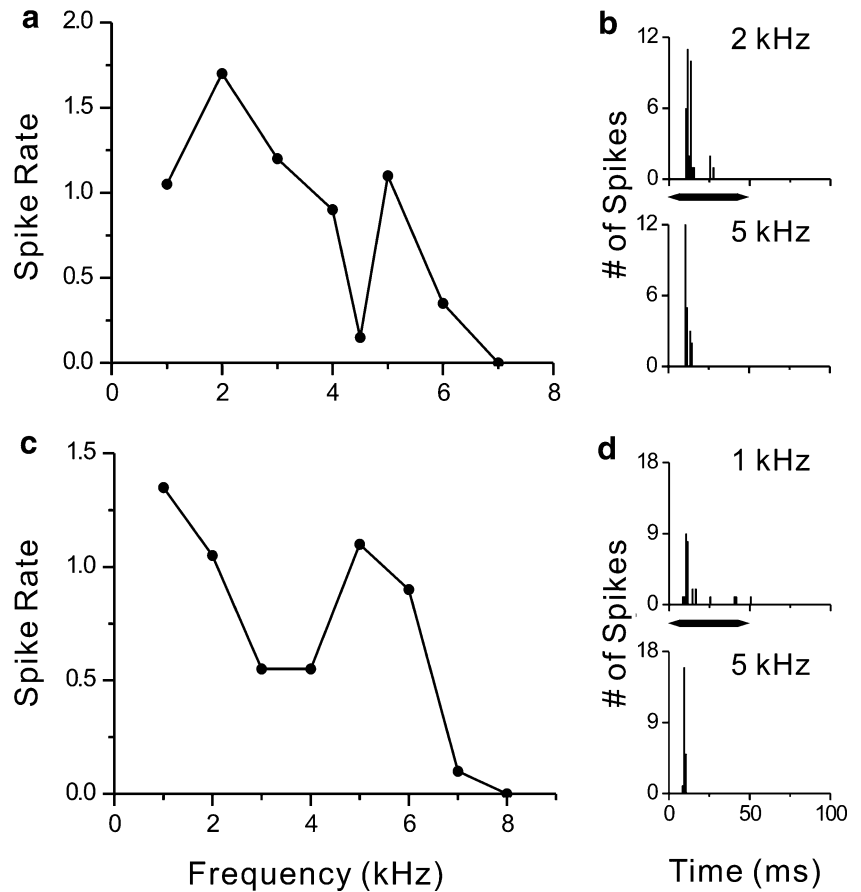
these effects such as attenuation, degradation, reflection, etc., thus making communication more effective. For example, frog calls suffer greater attenuation and degradation in dense forest than open habitats; and frog populations in forest habitats generally are tuned to higher frequencies than populations in open habitats (Ryan et al. 1990; Ryan and Wilczynski 1991). However, some investigators have found that there was no correspondence between frog call structure and environment (Penna and Solis 1998; Kime NM et al. 2000; Penna et al. 2006).

Based on AEP data, *O. schmackeri* does not respond to sounds higher than 8.5 kHz. All single units recorded also support this notion. It is generally accepted that the dominant frequency of the advertisement calls of frogs roughly matches their auditory sensitivity peak. Unlike the frog *A. tormotus* that emits calls with much higher frequency components, *O. schmackeri* has a sensitive frequency range with peaks at 2 kHz and 3.5–4 kHz, just above the main energy distribution of noise in the study area (Narins et al. 2004). Living in the same intense noisy environment, *A. tormotus* has extended much of their call frequencies above the ambient noise to make advertisement calls more easily detected (Feng et al. 2006); however, *O. schmackeri* appears to choose another strategy: combining a discrete displacement of its hearing sensitivity with an increase in the loudness of their calls to overcome noise interference. Thus, different sympatric species of frogs, living in the same microhabitat, can choose different tactics to avoid the impaired effects produced by high levels of ambient noise (Sun and Narins 2005).

The frog auditory system contains two separate auditory sensory organs, the amphibian papilla (AP) and the basilar papilla (BP), with each tuned to a different frequency range (Lewis and Narins 1999). Most frog species exhibit two frequency sensitivity peaks, the lower of which corresponds to the output from the AP and the higher to the BP (Frishkopf 1966), respectively. The lower peak is usually found at around 100–200 Hz; the upper peak is more variable and species-specific, and ranges from hundreds of hertz to a few kilohertz (Pettigrew et al. 1978; Loftus-Hills and Johnstone 1970; Loftus-Hills 1973; Zakon and Wilczynski 1988).

The auditory system of the frog *O. schmackeri* exhibits its two sensitive frequency regions with peaks at 2 kHz and 3.5–4 kHz, respectively. The frequency of the upper peak is similar to that of other frog species, for example, *Crinia parinsignifera* and *C. signifera* (Loftus-Hills and Johnstone 1970); the lower peak, presumably receiving inputs from the AP, occurs at a frequency (2 kHz) much higher than that of other frog species. In low-frequency noise habitats, frogs with high-frequency auditory filters are more effective than those with low-frequency filters at mitigating interference from environmental noise (Witte et al. 2005). The background noise in which *O. schmackeri* calls is low-frequency dominated with a peak around 0.1 kHz (Narins et al. 2004). In order to enhance the communication by decreasing the sensitivity to low-frequency ambient noise, *O. schmackeri* is likely to shift the lower peak to frequency as high as 2 kHz.

Fig. 4 Spike rate and PSTHs of two single units recorded from the TS of *O. schmackeri*. The unit (shown in **a, b**) exhibits best responses to tone bursts at 2 kHz, with another peak at 5 kHz; the unit (shown in **c, d**) responded best to tones at 1 kHz, next at 5 kHz. Stimulus intensity: 87 dB SPL; bin width: 1 ms



In frogs, minimum thresholds of the upper peaks are considerably 10–20 dB higher than those of the lower peaks (see review by Smotherman and Narins 2003). Although thresholds of upper peaks are variable among species, there is a gradual increase in thresholds of upper peaks with increasing frequency (Loftus-Hills 1973). For instance, when the upper peaks are up to 3 kHz, the thresholds are higher than 60 dB SPL (Loftus-Hills and Johnstone 1970; Loftus-Hills 1973). Compared to that, it is surprising that the threshold of *O. schmackeri* is very low (< 42 dB SPL) for the peak at 3.5–4 kHz, and similar to the thresholds at 2 kHz. Our results are not consistent with the notion that animals in noisy environment elevate auditory thresholds to change their relative sensitivity to both signals and ambient noise (e.g. in fish: Wysocki and Ladich 2005). In order to improve acoustic signals detectability, frogs living in an intensely noisy environment likely reduce the thresholds in the frequency range at which the dominant frequency of their calls is possibly located.

Animals change frequency spectrum and loudness of acoustic signals as an adaptation in noisy environments. Our results suggest that frogs' signal-receiving system may have evolved at least in two aspects, frequency shift of the lower peak and thresholds decrease of the upper peak, to avoid the masking noise and enhance the communication in an intense, low-frequency noise environment. Frogs living in this

environment would benefit from these changes since high-level noise would mask any low-amplitude signal that would potentially be detected by a high-frequency-sensitive auditory system.

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References

- Bradbury JW, Vehrencamp SL (1998) Principles of animal communication. Sinauer Associates, Sunderland
- Brumm H (2004) The impact of environmental noise on song amplitude in a territorial bird. *J Anim Ecol* 73:434–440
- Brumm H, Todt D (2002) Noise-dependent song amplitude regulation in a territorial songbird. *Anim Behav* 63:891–897
- Brumm H, Voss K, Köllmer I, Todt D (2004) Acoustic communication in noise: regulation of call characteristics in a New World monkey. *J Exp Biol* 207:443–448
- Fei L (1999) Atlas of amphibians of China. Henan Science and Technical Publisher, Zhengzhou
- Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX (2006) Ultrasonic communication in frogs. *Nature* 440:333–336. DOI:10.1038/nature04416
- Frishkopf LS, Geisler CD (1966) Peripheral origin of auditory response recorded from the eighth nerve of the bullfrog. *J Acoust Soc Am* 40:469–472

- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans. University of Chicago Press, Chicago
- Hödl W, Amézquita A (2001) Visual signaling in anuran amphibians. In: Ryan MJ (eds) Anuran communication. Smithsonian Institution Press, Washington DC, pp 121–141
- Kime NM, Turner WR, Ryan MJ (2000) The transmission of advertisement call in Central American frogs. *Behav Ecol* 11:71–83
- Lewis ER, Narins PM (1999) The acoustic periphery of amphibians: anatomy and physiology. In: Fay RR, Popper AN (eds) Comparative hearing: fish and amphibians. Springer, Berlin Heidelberg New York, pp 101–154
- Loftus-Hills JJ (1973) Comparative aspects of auditory function in Australian anurans. *Aust J Zool* 21:353–367
- Loftus-Hills JJ, Johnstone BM (1970) Auditory function, communication, and the brain-evoked response in anuran amphibians. *J Acoust Soc Am* 47:1131–1138
- Narins PM (1982) Effects of masking noise on evoked calling in the Puerto Rican Coqui (Anura: Leptodactylidae). *J Comp Physiol A* 147:438–446
- Narins PM, Benedix JH Jr, Moss F (1997) Does stochastic resonance play a role in hearing? In: Lewis ER, Lyon R, Long GR, Narins PM (eds) Diversity in Auditory Mechanics, World Scientific Publishers, Singapore, pp 83–90
- Narins PM, Feng AS, Lin WY, Schnitzler HU, Denzinger A, Suthers RA, Xu CH (2004) Old World frog and bird vocalizations contain prominent ultrasonic harmonics. *J Acoust Soc Am* 115:910–913
- Penna M, Solis R (1998) Frog call intensities and sound propagation in the South American temperate forest region. *Behav Ecol Sociobiol* 42:371–381
- Penna M, Pottstock H, Velásquez N (2005) Effect of natural and synthetic noise on evoked vocal responses in a frog of the temperate austral forest. *Anim Behav* 70:639–651
- Penna M, Márquez R, Bosch J, Crespo EG (2006) Non-optimal propagation of advertisement calls of midwife toads in Iberian habitats. *J Acoust Soc Am* 119:1227–1237
- Pettigrew A, Chung SH, Anson M (1978) Neurophysiological basis of directional hearing in amphibia. *Nature* 272:138–142
- Rabin LA, McCowan B, Hooper SL, Owings DH (2003) Anthropogenic noise and its effect on animal communication: an interface between comparative psychology and conservation biology. *Int J Comp Psychol* 16:172–192
- Ryan MJ, Wilczynski W (1988) Coevolution of sender and receiver: effect on local mate preference in cricket frogs. *Science* 240:1786–1788
- Ryan MJ, Cocroft RB, Wilczynski W (1990) The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. *Evolution* 44:1869–1872
- Ryan MJ, Wilczynski W (1991) Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). *Biol J Linn Soc* 44:249–271
- Slabbekoorn H, Peet M (2003) Birds sing at a higher pitch in urban noise. *Nature* 424:267
- Smotherman MS, Narins PM (2003) Hair cells, hearing and hopping: a field guide to hair cell physiology in the frogs. *J Exp Biol* 203:2237–2246
- Sun JWC, Narins PM (2005) Anthropogenic sounds differentially affect amphibian call rate. *Biol Conserv* 121:419–427
- Wilczynski W, Rand AS, Ryan MJ (2001) Evolution of calls and auditory tuning in the *Physalaemus pustulosus* species group. *Brain Behav Evol* 58:137–51
- Wiley RH, Richards DG (1978) Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behav Ecol Sociobiol* 3:69–94
- Wysocki LE, Ladich F (2005) Hearing in Fishes under Noise Conditions. *J Assoc Res Otolaryngol* 6:28–36
- Witte K, Farris HE, Ryan MJ, Wilczynski W (2005) How cricket frog females deal with a noisy world: habitat-related differences in auditory tuning. *Behav Ecol* 10:571–578
- Ye CY, Fei L (2001) Phylogeny of genus *Odorrana* (Amphibia: Ranidae) in China. *Acta Zool Sin* 47:528–534
- Zakon HH, Wilczynski W (1988) The physiology of the Anuran VIII-Ith nerve. In: Fritzsche B, Wolkowiak W, Ryan MJ, Wilczynski W, Hetherington T (eds) The Amphibian Auditory System, Wiley, New York, pp 125–155