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Auditory role of the suprabranchial chamber in gourami fish

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Abstract Fish hearing specialists (e.g., goldfish, holocentrids, clupeoids, mormyrids) have evolved specialized structures (e.g., Weberian ossicles, swimbladder diverticulae, gas-filled bullae) to enhance their auditory frequency range and threshold sensitivity. The inner ears of anabantoid fish are encased in membranous cranial bones and are protruded into air-filled suprabranchial chambers. This research was intended to test the hypothesis that the gas bubbles inside the suprabranchial chambers may modulate the hearing abilities of anabantoid fish because of their proximity to the membranous bone-encased inner ears. Three species of gourami (blue gourami *Trichogaster trichopterus*; kissing gourami *Helostoma temminckii*; dwarf gourami *Colisa lalia*) were examined. Using the auditory brainstem response recording technique, baseline audiograms tested at 300, 500, 800, 1500, 2500, 4000 Hz were obtained. The air bubbles in the suprabranchial chambers were replaced by water, and the audiograms were remeasured. Thresholds were elevated in all three species. When three blue gouramis were allowed to replenish air into the suprabranchial chambers their hearing abilities returned to baseline levels. These results support the hypothesis that air bubbles in the suprabranchial chambers can affect hearing abilities of gouramis by lowering the thresholds.

Key words Auditory brain stem response · Gouramis · Hearing · Suprabranchial chamber · Ear

Abbreviations *ABR* auditory brainstem response · *BW* body weight · *SL* standard length

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Introduction

In terms of overall underwater hearing capacities, different species of fish have been classified either as a hearing generalist or a specialist. The generalists such as squirrelfish *Adiorxy xantherythrus* (Coombs and Popper 1979), pinfish *Lagodon rhomboides* (Tavolga 1974) and oscar *Astronotus ocellatus* (Yan and Popper 1992; Yan 1995) have narrower frequency ranges of hearing as well as higher auditory thresholds than specialists, e.g., goldfish *Carassius auratus* (Jacobs and Tavolga 1967), squirrelfish *Myripristus kuntee* (Coombs and Popper 1979).

In all examples hearing specialists have evolved a peripheral mechanical specialization that increases sensitivity to underwater sound. These specializations include three forms (Popper and Platt 1993). The first is found in the otophysans (e.g., goldfish, catfish) in which the first three vertebrae of the vertebral column have been modified as the Weberian ossicles. These ossicles physically connect the rostral end of the swim bladder to the fluid system of the inner ear at the midline between the two saccules. Fishes with Weberian ossicles clearly detect sounds of wider bandwidths and lower thresholds than those without ossicles (Fay and Popper 1975; Blaxter 1981; Blaxter et al. 1981; Fay 1988). Swimbladder mutilation or removal of the ossicles causes loss of threshold sensitivity and reduction of bandwidth in a bullhead catfish *Ameiurus nebulosus* (Poggendorf 1952; Kleerekoper and Roggenkamp 1959). The second type of specialization involves rostral projections of the swim bladder directly to the ear. This specialization can be found in a squirrelfish (*Myripristis kuntee*; Family Holocentridae) in which the rostral end of the swim bladder terminates on the wall of the saccule (Coombs and Popper 1979). This species hears considerably better than the confamilial species (*Adioryx xantherythrus*), which has no coupling between the ear and the swim bladder (Coombs and Popper 1979). The third form of specialization is the presence of a separate gas-contain-

ing bullae in the head close to the inner ear. These specialized structures are found in the mormyrids and clupeoids which also possess excellent hearing (Blaxter et al. 1981; Kramer et al. 1981; McCormick and Popper 1984). Chapman and Sand (1974) demonstrated that even an artificial gas bubble (a balloon) placed near the head decreased the hearing threshold and widened the frequency range in a fish lacking a swimbladder, the flatfish (*Pleuronectes platessa*).

The anabantoids are unique in possessing suprabranchial chambers positioned behind and above the gills. The bones of the skull and operculum form the roof and walls, and muscles of the jaw compose the floor of suprabranchial chamber. Valves at chamber apertures largely isolate the suprabranchial chamber's from the adjacent buccal and pharyngeal cavities. Each suprabranchial chamber encloses an air-breathing organ called the labyrinth (Das 1928; Liem 1963, 1980; Lauder and Liem 1983; Wolf and Kramer 1987). The labyrinth apparatus is a bony structure formed by the epibranchial (processus articularis) segment of gill arch 1. Both the walls of the suprabranchial chamber and labyrinth are covered with a highly vascularized respiratory epithelium (Peters 1978). All anabantoids engulf air bubbles into the suprabranchial chamber and undergo gas exchange. Therefore, the labyrinth is used as an accessory breathing apparatus to aid gills in respiration (Burggren 1979; Graham 1997). In addition, the suprabranchial chamber serves as an aid to buoyancy and as a sound radiator (Kratochvil 1985; Schuster 1986). In behavioral experiments Schneider (1941) first demonstrated that the paradise fish (*Macropodus opercularis*), an anabantoid, could detect sounds up to 4500 Hz. The close proximity of air bubbles to the ear prompted Schneider (1941) to speculate that the suprabranchial chamber might serve as a pressure-displacement transducer that enhanced hearing. Dissections in blue gourami (H.Y. Yan, unpublished data) revealed that the roof of the suprabranchial chambers consists of the cranial floor, and a portion of the roof, which protrudes into the suprabranchial chambers, contains the saccule of the inner ear. The cranial floor that covers the saccule is made up of thin bony materials. It is likely that any pressure displacement of air inside suprabranchial chamber can transmit directly into the inner ears.

It is hypothesized that the air bubbles inside the suprabranchial chambers may serve as a device to enhance peripheral hearing. The possible function of air bubbles within the suprabranchial chamber in aiding overall hearing ability in gouramis, however, has never been examined physiologically. The present study tested this hypothesis by using three anabantoids, the blue, kissing and dwarf gouramis as subjects. A newly developed auditory brainstem response (ABR) recording technique for fish audiometry (Kenyon et al. 1998; Ladich and Yan 1998) was used to evaluate differences of auditory thresholds before and after air bubble removal from the suprabranchial chamber.

Materials and methods

Animals and preparation

Five specimens of each of the following species obtained from a local fish dealer were used: blue gourami, *Trichogaster trichopterus* (Belontiidae) standard length (SL) 46.0–62.2 mm; body weight (BW): 3.2–8.1 g, kissing gourami, *Helostoma temminckii* (Helostomatidae) SL 39.3–51.9 mm; BW 2.1–4.8 g, and dwarf gourami, *Colisa lalia* (Belontiidae) SL 41.5–48.6 mm; BW 3.2–4.9 g. They were maintained in filtered aquaria at 25 ± 1 °C with a light cycle of 14L:10D and fed commercial flake food. Animal use protocol in this study was approved by the University of Kentucky (IACUC # 93005L).

Hearing threshold determination

The ABR recording protocol was used for hearing threshold determinations. Since the protocol has been described in detail by Kenyon et al. (1998) and Ladich and Yan (1998), only a summary of the protocol is provided here. In order to reduce myogenic noise, test subjects were first lightly anesthetized in 50 ppm 3-aminobenzoic acid ester (MS222, Sigma, St. Louis) and then immobilized with gallamine triethiodide (Flaxedil, Sigma) injected intramuscularly close to the base of dorsal fin. The dosage used was 0.28 μg g^{-1} for *T. trichopterus*, 0.56 μg g^{-1} for *C. lalia* and 0.20 μg g^{-1} for *H. temminckii*. This dosage allowed fish to retain light opercular movement but without significant myogenic noises to interfere with the recording. After sedation the test subject was wrapped in a wet tissue paper to prevent skin injury and placed in a small sling made from a rectangle of nylon mesh. The tissue paper and the nylon mesh secured the fish behind the opercular flap, allowing for light opercular movement. The ends of the mesh were clamped together by a clip attached to a glass rod. The rod was then placed in a micro-manipulator and the animal was positioned in a 15-l plastic tub (38 cm × 24.5 cm × 14.5 cm) filled with water. The position of the fish was adjusted accordingly so that the nape of the head was 0.5–1 mm above the surface of the water. A respiration pipette was inserted into the subject's mouth when necessary. The respirator consisted of a temperature-controlled (25 ± 1 °C), gravity-feed, aerated water system. A small piece of tissue paper (10 mm × 2 mm) was placed on the exposed portion of the head in order to keep the skin moist, and electrodes were pressed firmly through the tissue paper and against the top surface of the skin. The recording electrode (a Teflon-coated silver wire, 0.25 mm diameter with 1 mm of exposed tip) was placed on the midline of the skull, over the medulla region. The reference electrode was positioned 5 mm anteriorly to the recording electrode, also on the midline and slightly caudal to the eyes. The point of contact of both electrodes was right against the skin of the skull above the water surface. The recorded signal was amplified 100 times by an a.c. preamplifier (Grass P-15, band pass: 10–10 000 Hz). The ground terminal of the preamplifier was connected via a wire to the water in the tub. A hydrophone (Celesco LC-10) was placed adjacent to the head region to monitor the sound pressure level of the stimulus. The hydrophone input was amplified by a second Grass P-15 (100 × amplification, band pass 10–10 000 Hz). The entire apparatus rested on a vibration-free air table (Kinetic Systems, model 1201) in a walk-in soundproof chamber (2 m × 3 m × 2 m, Industrial Acoustics Company). Speakers were suspended from the ceiling of the sound proof chamber 1 m above the test subject. For frequencies below 2500 Hz a 30-cm-diameter speaker (Pioneer frequency response 19–5 kHz) was used and a 12-cm midrange speaker (Pyle MR 516, frequency response 500–11 kHz) was used for frequencies of 2500 Hz and above. The position of the fish was adjusted so that the subject was placed in the center of the projected sound field.

Presentation of the sound stimulus and recording of the ABR waveform utilized a Tucker-Davis Technologies (Gainesville, Fla. USA) modular rack-mount system controlled by an optically-linked

66-MHz 486 PC containing a TDT AP2 board and running TDT "Bio-Sig" software. Using TDT "Sig-Gen" software, sound stimuli waveforms were created and fed through a DA1 digital-analog converter, a PA4 programmable attenuator, and a power amplifier (QSC Audio Products, Model USA 370) that drove the speaker. The hydrophone preamplifier output cable was fed to one channel of an AD1 analog-digital converter, and the electrode preamplifier output was passed through a PC1 spike conditioner, providing an additional 100 times amplification before reaching the AD1.

Sound stimuli consisted of repeated 20-ms tone bursts (2000 sweeps per test). Tone bursts were presented to a test subject at a particular frequency beginning with the highest sound pressure level which was then attenuated in 5-dB (for frequencies below 2500 Hz) or 3-dB (for frequencies of 2500 Hz and above) steps (re: 1 μPa). The number of cycles in the tone burst stimuli affected the clarity of the elicited ABR waveform. Thus, cycle number was adjusted to provide the best ABR response while still providing acceptable power spectra (i.e., sharp peaks at the dominant frequency, as verified by FFT analysis using the BioSig software. See Fig. 2 of Kenyon et al. 1998, for details). Data in human ABR audiometry (Hall 1992) indicated that shorter-duration tone bursts elicited the clearest ABRs; therefore, bursts of two plateau cycles and two rise and fall cycles (3.3 ms/cycle) were used at 300 Hz. Middle frequencies (400–3000 Hz) were presented using five plateau cycles with two rise and fall cycles (e.g., 2 ms/cycle for 500 Hz). High frequency (3000–5000 Hz) tone bursts were eight plateau cycles, also with two rise and fall cycles (e.g., 0.25 ms/cycle for 4000 Hz; see Davis et al. 1984; Silman and Silverman 1991; and Hall 1992 for technical details). Earlier work with such a setup and parameters showed that spectral "side lobes" were greatly reduced (see Fig. 2 of Kenyon et al. 1998 for details). It also provided a ramped onset/decay, thereby preventing speaker transients (Silman and Silverman 1991; Hall 1992). Two replicates of ABR waveforms were obtained from each subject for each tested sound pressure level. In ABR audiometry, the lowest stimulus level that elicits a repeatable waveform is commonly taken as the threshold and is based on visual inspection (Kilney and Shea 1986; Gorga et al. 1988; Warren 1989; Hall 1992; also see Kenyon et al. 1998 and Ladich and Yan 1998 for technical details). In order to statistically quantify the threshold value, two replicates of waveforms from each sound pressure level were compared by Spearman correlation test (Zar 1996). From the pilot study it was found that when a correlation coefficient (r) between two replicates was less than 0.3, the two replicated waveforms showed very little resemblance. Based on visual inspection criteria this sound pressure level would be considered one attenuation level below threshold. Therefore, one attenuation level above that particular sound pressure level which had r less than 0.3 was defined as the threshold level (see Fig. 1 for details). The use of correlation coefficient to determine threshold values agreed well with the traditional visual inspection method. The correlation coefficient method allows a quantitative mean of threshold determination that avoids potential bias using visual inspection. The root mean square (RMS) sound pressure level at threshold was determined by analyzing the hydrophone recording according to the method used by Burkhard (1984, 1997) which was implemented in the BioSig software. The threshold levels of the test animals were determined for the frequencies of 300, 500, 800, 1500, 2500, and 4000 Hz both before and after removal of air bubbles from the suprabranchial chambers.

Ambient noise levels in the test tank were also measured using the hydrophone and preamplifier. Samples of full-spectrum ambient noise were recorded into the TDT system and filtered with digital band-pass filter. Filtered noise root mean square levels were measured using the BioSig software, and spectrum levels were calculated by applying filter corrections and calibration factors.

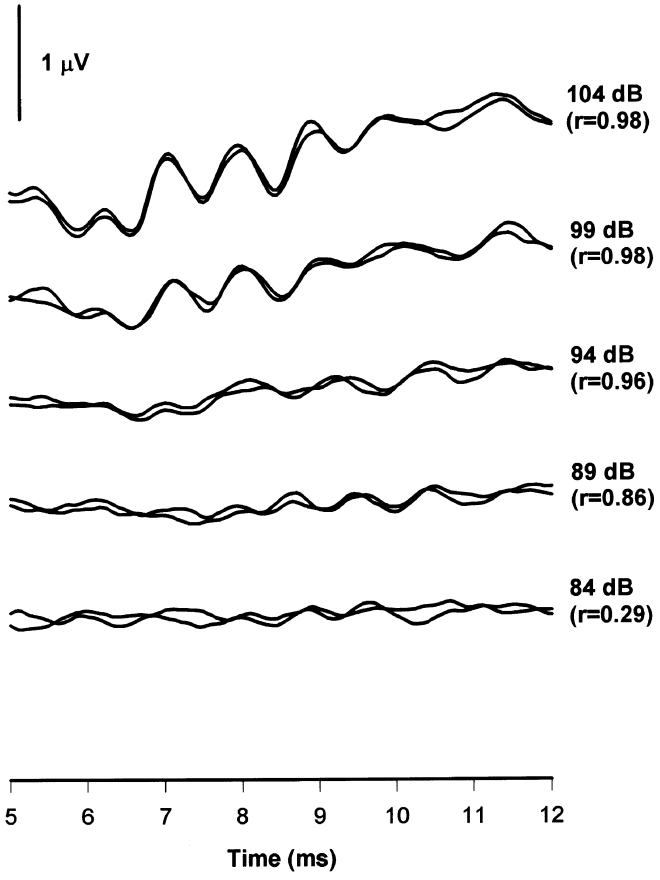

Fig. 1 Auditory brainstem response (ABR) waveforms of *Trichogaster trichopterus* in response to 500-Hz tone bursts with attenuation in 5-dB steps. Averaged traces of two different runs (2000 sweeps each) for each level is overlaid. The r values indicate Spearman Rank Order correlation coefficient. Based on visual inspection, at 84 dB two ABR traces are not reproducible and r is less than 0.3. The threshold is therefore determined as 89 dB. See text for detailed explanation of threshold determination

Removal of air bubbles from the suprabranchial chambers

After hearing thresholds of each fish were obtained, the air bubbles inside the suprabranchial chambers were removed with a 60-ml syringe (containing charcoal-filtered water) with approximately 23 cm of polyethylene tubing (Clay Adams, inner diameter 1.19 mm) attached. A fine plastic pipette tip (inner diameter 300 μm; USA/Scientific Plastics) was secured onto the anterior end of the tubing. The animal was lightly anesthetized with 50 ppm MS222 (Sigma) when necessary, and held ventral side up while totally submerged in water and viewed under a Topcon OMS-50 motorized dissecting microscope mounted next to the ABR recording setup. The pipette tip was then inserted into one side of the suprabranchial chamber by carefully lifting the opercular flap and entering the chamber via the exhalent aperture between the first gill arch and the operculum (Prasad et al. 1982). A slow stream of water was then injected into the chamber, driving out air bubbles. Injection of water continued until no more air bubbles were observed. The same procedure was repeated on the opposite side of the chamber and also through the mouth to flush out any air bubbles trapped in the buccal cavity. The animal was submerged at all times during air removal so that no fresh air bubbles could be gulped. Thresholds for each air-bubbles-deprived fish were remeasured. Upon the completion of measurement, the animal was again checked for air bubbles under the dissecting microscope in situ. If air bubbles were found, data from that fish was discarded. After the threshold measurement fish were preserved in 10% buffered formalin to determine the suprabranchial chamber volume.

To further validate the auditory role of air inside the suprabranchial chamber, audiograms of three blue gourami (mean SL 53.5 mm; mean BW 5.4 g) were obtained before and after air removal following the same protocol described earlier. These three fish were allowed to recover in its holding tank for 3 days so that air inside the suprabranchial chamber was replenished and audiograms were remeasured.

Comparison of audiograms

Threshold values from all individuals were averaged and frequency versus mean threshold sound pressure level was plotted to construct audiograms for each species before and after air bubbles removal. A one-way ANOVA on threshold data was used to determine significant differences between audiograms. A paired *t*-test was used to compare differences of each specific frequency to understand the effects of air removal on threshold change.

Suprabranchial chamber volume determination

Preserved animals were dissected so as to expose a small opening in the suprabranchial chamber on both sides of the fish. The weight of a 1-ml syringe containing distilled water (density = 1.0 at 25 °C) was recorded. Water from the syringe was then carefully injected into the suprabranchial chamber through the opening on one side of the fish via a 27 1/2 gauge needle under direct observation of a dissecting microscope. When the chamber appeared full, i.e., water filled to the surface of the chamber and no bubbles were visible, the weight of the syringe was again recorded and subtracted from the initial weight. Since 1 cm^3 = 1 g, the difference was considered to be equal to the volume of the chamber. The procedure was repeated five times on each side of suprabranchial chamber of each fish. The ten measurements for each fish were averaged to represent the suprabranchial chamber volume of that particular fish. Five specimens of blue, kissing and dwarf gouramis were measured.

Results

ABR Threshold Determinations

Figure 1 shows a series of ABR waveforms of a blue gourami in response to 500-Hz tone bursts at five sound pressure levels. A typical ABR waveform exhibits a series of peaks. At higher sound pressure levels, the peaks are obvious (e.g., at 104 dB). As sound pressure levels attenuated, the peaks of acoustically evoked potentials become less prominent.

All subjects exhibited ABRs to tone bursts between 300 and 4000 Hz before and after removal of air bubbles from the suprabranchial chambers (Figs. 2, 3, 4). For the blue gourami, *T. trichopterus*, the lowest overall threshold sound pressure level was at 800 Hz before treatment, with a mean of 75.9 ± 3.9 dB (mean ± SD). After removal of air, the lowest sound pressure level remained at 800 Hz, but with a significantly increased value of 107.6 ± 7.4 dB (Fig. 2, Table 1a). For the kissing gourami, *H. temminckii*, the lowest threshold level, 87.7 ± 6.5 dB, occurred at 800 Hz before air bubbles removal. After treatment, the lowest threshold (110.2 ± 3.8 dB) was still at 800 Hz (Fig. 3, Table 1b). The lowest threshold for the dwarf gourami, *C. lalia*, was 89.0 ± 5.2 dB at 800 Hz before removal of air bubbles and 105.2 ± 2.6 dB, at 800 Hz, after air bubbles removal (Fig. 4, Table 1c).

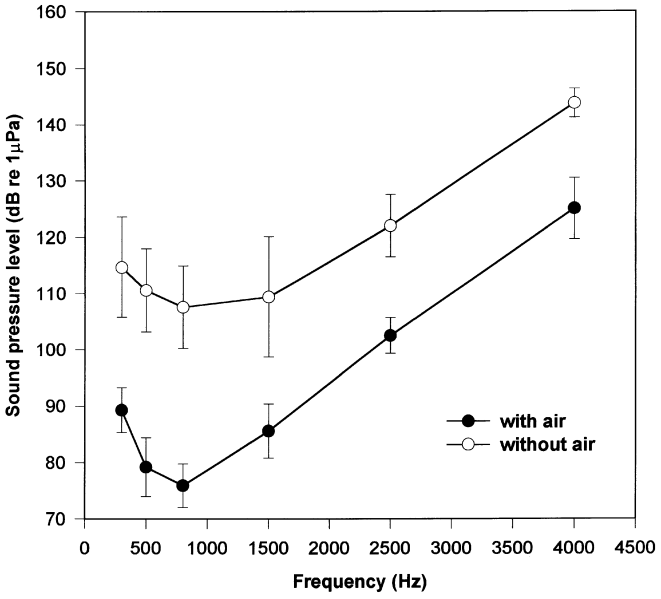

Fig. 2 The audiograms of blue gourami (*Trichogaster trichopterus*) before (*solid circles*) and after (*open circles*) removal of air bubbles. Each data point indicates mean ± SD ($n = 5$)

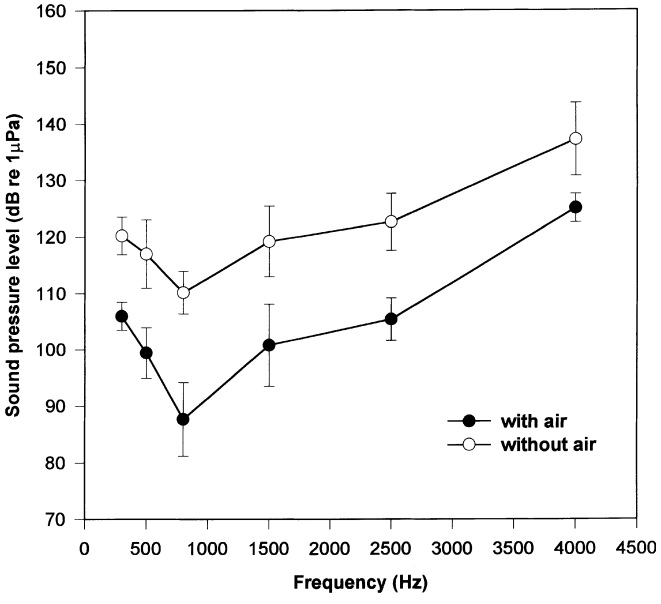

Fig. 3 The audiograms of kissing gourami (*Helostoma temminckii*) before (*solid circles*) and after (*open circles*) removal of air bubbles. Each data point indicates mean ± SD ($n = 5$)

Ambient noise levels inside the tank ranged from 56 dB to 40 dB and were at least 20–36 dB below thresholds of blue gourami (the most sensitive species among the three) at all frequencies tested. Therefore, the ambient noise should have no effect on threshold determinations. The results were quite similar to those reported earlier (see Fig. 6 of Kenyon et al. 1998 for ambient noise spectrum).

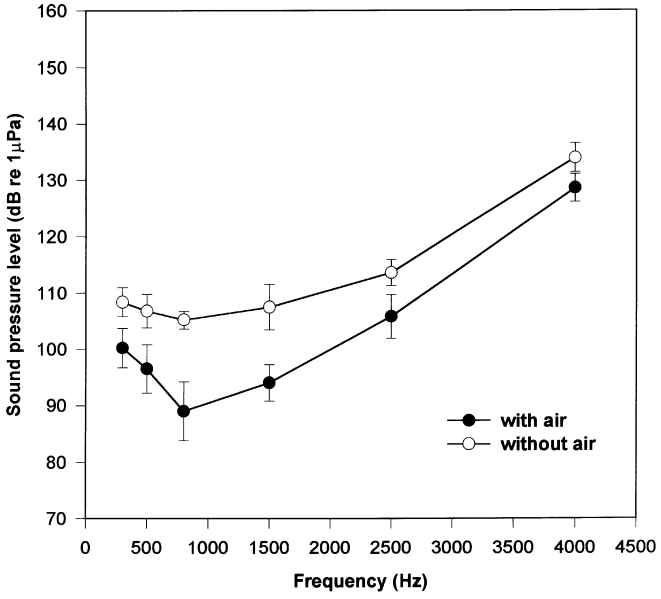

Fig. 4 The audiogram of dwarf gourami (*Colisa lalia*) before (*solid circles*) and after (*open circles*) removal of air bubbles. Each data point indicates mean ± SD ($n = 5$)

Comparison of thresholds before and after removal of air bubbles from the suprabranchial chambers

An increase in threshold sound pressure levels occurred at all frequencies for all species tested after flushing the air bubbles out of the suprabranchial chambers (Fig. 5). One-way ANOVA revealed significant differences in the audiograms obtained before and after removal of air bubbles from the suprabranchial chambers for *T. trichopterus* ($F = 93.03$, $P < 0.0001$), for *H. temminckii* ($F = 38.08$, $P < 0.0001$), and *C. lalia* ($F = 21.53$, $P < 0.0001$). Paired *t*-test (Table 1a, b, c; $P < 0.05$) indicated that the threshold levels increased significantly at all frequencies for all species tested (Table 1a, b, c). Among the three species, the largest shift of thresholds were 31.7 dB (at 800 Hz), 22.5 dB (at 800 Hz) and 16.2 dB (at 800 Hz) for *T. trichopterus*, *H. temminckii*, and *C. lalia*, respectively (Table 1; Fig. 5).

The data in Fig. 6 showed that hearing thresholds at all frequencies were significantly elevated after air removal when compared to baseline data ($P < 0.05$). However, when air bubbles were allowed to be replenished through normal intake of air into the suprabranchial chambers, no significant difference in thresholds was found between baseline and recovery data ($P > 0.05$; Fig. 6). This finding further supports

Table 1 Threshold sound pressure levels, changes in sound pressure level before and after removal of air from the suprabranchial chamber, and paired *t*-test results of mean sound pressure level difference (mean Δsound pressure level) for: (a) blue gourami, *T. trichopterus*; (b) kissing gourami (*H. temminckii*); and (c) dwarf gourami (*C. lalia*). $n = 5$ for each species (sound pressure levels in dB, re: 1 μPa)

Frequency (Hz)	Mean sound pressure level ± SD		Differences in sound pressure level	
	With air	Without air	Mean Δ sound pressure level	*t* values
(a) Blue gourami				
300	89.3 ± 4.0	114.7 ± 8.9	25.4	5.395*
500	79.2 ± 5.2	110.6 ± 7.4	31.4	7.875*
800	75.9 ± 3.9	107.6 ± 7.4	31.7	12.89*
1500	85.6 ± 4.8	109.4 ± 10.7	23.8	4.785*
2500	102.5 ± 3.2	122.0 ± 5.5	19.5	5.687*
4000	125.0 ± 5.4	143.8 ± 2.6	18.8	4.954*
Frequency (Hz)	Mean sound pressure level ± SD		Differences in sound pressure level	
	With air	Without air	Mean Δ sound pressure level	Significance
(b) Kissing gourami				
300	106.0 ± 2.5	120.2 ± 3.3	14.2	5.875*
500	99.4 ± 4.5	117.0 ± 6.0	17.6	6.585*
800	87.7 ± 6.5	110.2 ± 3.8	22.5	5.432*
1500	100.8 ± 7.3	119.2 ± 6.2	18.4	7.325*
2500	105.4 ± 3.8	122.6 ± 5.0	17.2	9.534*
4000	125.0 ± 2.5	137.2 ± 6.5	12.2	6.234*
Frequency (Hz)	Mean sound pressure level ± SD		Differences in sound pressure level	
	With air	Without air	Mean Δ sound pressure level	Significance
(c) Dwarf gourami				
300	100.2 ± 3.5	108.4 ± 2.6	8.2	5.785*
500	96.5 ± 4.3	106.8 ± 3.0	10.3	6.732*
800	89.0 ± 5.2	105.2 ± 1.6	16.2	7.435*
1500	94.0 ± 3.2	107.5 ± 4.1	13.5	6.832*
2500	105.8 ± 3.9	113.6 ± 2.3	7.8	5.973*
4000	128.5 ± 2.5	133.9 ± 2.6	5.4	6.241*

* Statistically significant differences determined at the 0.05 level

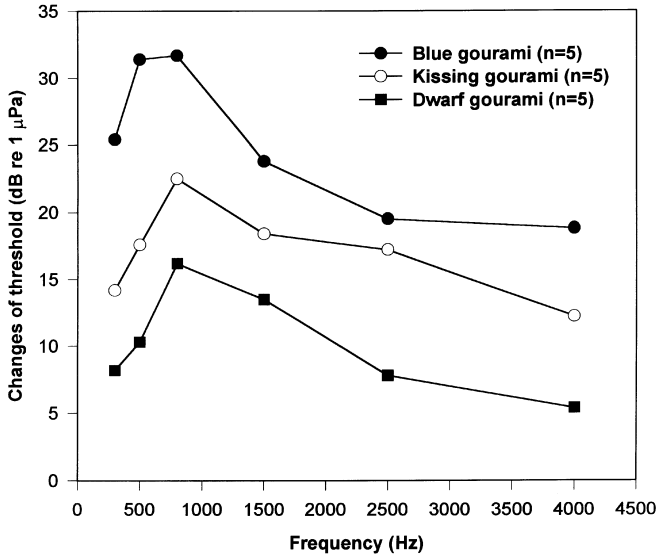

Fig. 5 Changes of mean threshold (dB) as a function of frequency after removal of air bubbles from the suprabranchial chambers in blue gourami (*open circles*), kissing gourami (*solid circles*) and dwarf gourami (*solid squares*). $n = 5$ for each species

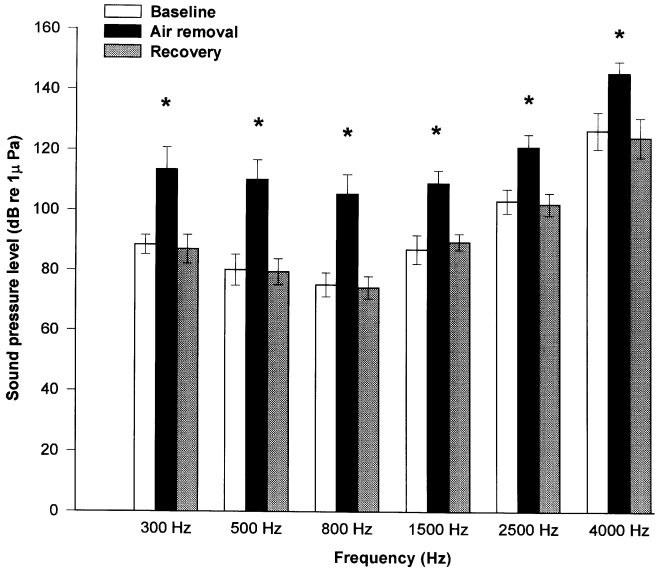

Fig. 6 Changes of hearing thresholds at six frequencies of three blue gourami before air removal (*baseline*; *open bars*), after air removal (*air removal*; *solid bars*) and air replenishment (*recovery*; *shaded bars*). * signs indicate significant difference ($P < 0.05$) in threshold of air removal treatment in each frequency group when compared with baseline and recovery group data

the hypothesis that the air inside the suprabranchial chambers plays auditory role enhancing hearing ability of anabantoid fish.

Suprabranchial chamber volumes

Preliminary *t*-test indicated no difference in the volume of air bubbles held on each side of the suprabranchial chamber. Therefore, volumes determined for each side were added to obtain the total volume of the suprabranchial chambers of each fish. The mean suprabranchial chamber volumes for blue gouramis, dwarf gouramis and kissing gouramis were 204 μl (range 126–290 μl), 151 μl (range 68–292 μl), and 100 μl (range 72–184 μl), respectively. The volume of the suprabranchial chamber varied positively with BW of the fish. The relationship of each species could be best described by the following linear regression equations:

1. Blue gourami: volume (μl) = 15.7 + (33.6 · BW) ($r^2 = 0.97$, $P < 0.01$; $n = 5$)
2. Dwarf gourami: volume (μl) = −195.3 + (96.8 · BW) ($r^2 = 0.94$, $P < 0.01$; $n = 5$)
3. Kissing gourami: volume (μl) = −11.1 + (40.4 · BW) ($r^2 = 0.97$, $P < 0.01$; $n = 5$)

Discussion

Hearing thresholds of gouramis

The waveforms in Fig. 1 clearly show how the strength of acoustic stimuli affects the overall ABRs. It is obvious that both amplitude and latency of the ABR are modulated by attenuated signal strength. By using tone-burst stimuli with cosine^2-gating windows, Gorga et al. (1988) demonstrate that there is a significant difference between ABR and behavioral thresholds from 20 normal hearing human subjects. The ABR thresholds are higher (i.e., worse) than behavioral thresholds with the differences increasing for the lower test frequencies and it ranges from 35 dB (at 250 Hz) to 15 dB (at 8000 Hz) with an average of 20 dB difference. If this 20-dB difference between ABR and behavioral audiograms were also applicable in gourami audiometry, then the best frequency hearing threshold of "presumed" behavioral thresholds of blue, kissing and dwarf gourami are likely to be 56 dB, 68 dB, and 79 dB, respectively. When compares with best frequency hearing thresholds of hearing specialists (60–80 dB; re: 1 μPa) and hearing generalists (80–110 dB), the hearing abilities of these three species of gouramis can be considered as hearing specialists. It is also important to note that gouramis have wider bandwidths of hearing than those of generalists (see Fay 1988 for detailed data). However, the designation of gouramis as hearing specialists remains inconclusive at the present time. The issue can not be solved until behavioral audiograms of gouramis are obtained with the same setup for ABR protocol so possible threshold differences between two methods can be documented.

The enhanced auditory capacity of otophysans is largely due to mechanical coupling between swimbladder and inner ears (Popper and Platt 1993). No anatomical data are available yet to show a physical coupling between swim bladder and inner ears or suprabranchial chambers in gouramis. Preliminary ana-

tomical data (H.Y. Yan, unpublished data) on these three gouramis show the protrusion of saccules (encased with thin membrane-like bone) into the upper part of the suprabranchial chamber. Since the gas inside the suprabranchial chamber is relatively compressible, the pressure component of the sound waves in the surrounding water will cause the air inside the suprabranchial chamber to undergo corresponding fluctuations in volume. Since the saccules are directly exposed to fluctuating air volume, the sensory hair cells inside the saccules are likely to be stimulated by the pressure component of the sound waves leading to enhanced hearing ability. The enhanced hearing abilities of three gourami species clearly show a parallelism in function between the suprabranchial chambers and the gas bullae of one weakly electric mormyrid fish (McCormick and Popper 1984; Crawford 1993) and clupeoids (Allen et al. 1976; Blaxter and Denton 1976; Blaxter et al. 1979, 1981; Denton and Gray 1979, 1980; Denton et al. 1979; Best and Gray 1980). However, is it important to note that there is a direct mechanical coupling between the clupeoid bulla and the utricular receptors through a bulla membrane and an elastic thread (Best and Gray 1980), while in gouramis membranous bone-covered saccules are directly exposed to air-filled chamber.

Incidentally, when male dwarf gourami defend their territories, an explosive type of croaking sound is produced with a maximum of energy between 860 and 990 Hz (Schuster 1986, 1989) which is within the lowest hearing thresholds between 800 Hz and 1500 Hz (Fig. 4) as observed in the present study. During chasing interaction between blue gouramis, a "pop" sound was produced with a maximal energy around 750 Hz (Demary 1997). It coincides with the best hearing frequency around 800 Hz measured in this study (Fig. 2). This type of good match between best hearing frequency and maximal energy of acoustical signals is also observed in another anabantoid fish, the croaking gourami *Trichopsis vittatus* (Ladich and Yan 1998).

Effects of air bubbles in suprabranchial chamber on hearing

The removal of air bubbles from the suprabranchial chambers resulted in a threshold increase for all three species tested. These data support the hypothesis that the air bubbles inside the suprabranchial chamber enhance the hearing abilities of these fishes. On the other hand, Chapman and Sand (1974) showed that a small air-filled balloon placed beneath the head of the dab (*Limanda limanda*) resulted in a gain of threshold of 3–19.8 dB (frequency dependent) and a wider hearing frequency range (increased from 200 Hz up to 350 Hz). The dab lacks a swimbladder and is known to be sensitive to acoustic particle displacement but not to sound pressure. The placing of the air-filled balloon clearly shows that a gas bubble near the head lowers hearing thresholds and widens the bandwidth of hearing. This finding corroborates the present study in that the removal of air from the suprabranchial chamber of three gouramis can lead to an increase of hearing threshold. Furthermore, results obtained in the present study corroborate those obtained by Schneider (1941) which showed hearing impairment when the suprabranchial chamber was filled with water. The suprabranchial chamber volumes obtained for *Colisa lalia* in this study are similar to those obtained by Schuster (1989). The close agreement of volumes of suprabranchial chamber obtained by Schuster (1989) and the present study further validates the accuracy of the water displacement method used here to measure suprabranchial chamber volume. Interestingly, among the three species tested, the kissing gourami has the smallest air volume (mean 100 μl) but the threshold changes are larger than those of dwarf gourami after air removal. This contrasts with the larger air bubble volume (mean 151 μl) and smaller threshold changes of dwarf gourami.

The air removal experiment results in significant elevation of hearing thresholds in all three gourami species. When three blue gourami are allowed to refill air bubbles into their suprabranchial chambers in a normal way, the hearing sensitivity is recovered (Fig. 6). This air bubble removal and replenishment experiment provides additional support for the hypothesis that the suprabranchial chamber indeed plays an auditory role in gouramis.

It is important to note that the exact volumes of air held inside the suprabranchial chamber at the time of hearing threshold measurement can not be accurately assessed due to the experimental needs. The volume is also likely to vary in some degree between treatments even in the same individual. This may explain the variations of threshold observed with the same individual fish. It is possible that the posthoc measurements of chamber volume may not reflect the actual volume of air that is in the chamber at the time of testing; however, published works show that anabantoids usually empty the suprabranchial chamber on each exhalation (Peters 1978; Burggren 1979; Schuster 1989). Therefore, the posthoc measurement at least can provide an estimated volume of air inside the suprabranchial chamber used for respiration and sound pressure detection. Burggren (1979) determined that *T. trichopterus* (8 g) inspired 27–32 μl g^{-1} and consumed 11–15% of this volume of air over the average time (4.7 min) the gas was held. How the gradual loss of air volume, albeit a small volume, inside the suprabranchial chamber would affect overall hearing ability (either in low- or high-frequency range) requires further study. The clarification of this issue also hinges upon the understanding of a fundamental question as to what kind of sounds (frequency range as well as sound pressure level) that gourami are tuning into. Future field work to document the acoustic characteristics of gouramis' natural habitats perhaps will help shed light on this issue.

Table 2 Comparison of threshold sound pressure levels (dB re 1 μPa) for *T. trichopterus* and *H. temminckii* obtained by ABR recording method (before and after removal of air from the suprabranchial chambers) and saccular microphonics (data from Saidel and Popper 1987). *w/air*: before air removal; *w/o*: after air removal

Frequency (Hz)	*T. trichopterus*			*H. temminckii*		
	ABR w/air	ABR w/o air	Microphonics	ABR w/air	ABR w/o air	Microphonics
300	86.3 ± 4.0	111.4 ± 8.9	117 ± 8	101.0 ± 2.5	115.2 ± 3.3	118 ± 6
500	79.2 ± 5.2	110.6 ± 7.4	128 ± 8	99.4 ± 5.5	117.0 ± 6.0	122 ± 7
800	75.9 ± 3.9	107.6 ± 7.4	136 ± 4	97.5 ± 6.5	120.0 ± 3.8	126 ± 7

Comparison of thresholds determined by different methods

Audiograms have previously been determined for *T. trichopterus* and *H. temminckii* using the saccular microphonics technique (Saidel and Popper 1987). The thresholds determined by both microphonics and ABR recording technique before removal of air bubbles differ significantly (Table 2). The difference, however, becomes less significant when comparing microphonics data with ABR recording data after removal of air bubbles from the suprabranchial chambers (see Table 2). This suggests that the difference in audiograms obtained for untreated fish lies in the technique used. Saccular microphonics recording is an invasive technique that involves partial removal of the cranium and exposure of the inner ear. The suprabranchial chambers could easily be damaged during the surgery preparing for microphonics recording (W. M. Saidel, personal communication; H. Y. Yan, unpublished data). This may help explain the fact that the microphonics data are comparable to the ABR data of fish without air bubbles inside the suprabranchial chambers, providing further evidence that the suprabranchial chamber when filled with air can serve as a hearing-enhancing device. There is, however, still a difference in thresholds obtained by microphonics and by ABR from fish without air bubbles in the suprabranchial chambers. This difference may be explained by the fact that microphonics measures the responses from only one hearing endorgan, the saccule (Saidel and Popper 1987), while the ABR measures responses from inputs of all six hearing endorgans: the saccules, lagenae, utricles and all the neurons involved in the ascending auditory pathways (Moller and Jannetta 1985; Hall 1992).

Based on manipulation of air bubbles, removal and replenishment of air to the suprabranchial chambers of three gouramis, and the measurement of threshold shifts using the non-invasive ABR protocol, this study has demonstrated that the hearing of anabantoid fishes is impaired when no air bubbles are present in the suprabranchial chambers. The results of this study support the hypothesis that the suprabranchial chamber functions as a hearing-enhancing device.

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