ACOUSTIC DETECTION BY SOUND-PRODUCING FISHES (MORMYRIDAE): THE ROLE OF GAS-FILLED TYMPANIC BLADDERS

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Summary

Mormyrid electric fish use sounds for communication and have unusual ears. Each ear has a small gas-filled tympanic bladder coupled to the sacculus. Although it has long been thought that this gas-filled structure confers acoustic pressure sensitivity, this has never been evaluated experimentally. We examined tone detection thresholds by measuring behavioral responses to sounds in normal fish and in fish with manipulations to one or to both of the tympanic bladders. We found that the tympanic bladders increase auditory sensitivity by approximately 30 dB in the middle of the animal's hearing range (200–1200 Hz).

Normal fish had their best tone detection thresholds in the range 400–500 Hz, with thresholds of approximately 60 dB (re 1 μPa). When the gas was displaced from the bladders with physiological saline, the animals showed a dramatic loss of auditory sensitivity. In contrast, control animals in which only one bladder was manipulated or in which a sham operation had been performed on both sides had normal hearing.

Key words: Mormyridae, electric fish, hearing, tympanic bladder, auditory sensitivity, communication.

Introduction

Mormyrid fish utilize specialized electric and acoustic sensory systems for nocturnal communication in the murky floodplains of West Africa. Much of the recent research on mormyrids has been devoted to the role of the electric organ discharge (EOD) and electroreceptors in electrolocation, foraging and communication (Bell et al., 1995; Crawford, 1992; Kramer, 1996; Moller, 1995; von der Emde and Bleckmann, 1998). Less attention has been given to the equally remarkable specializations of the mormyrid auditory system. Each ear contains a gas-filled tympanic bladder coupled to one of the hair-cell-based sensory organs, the sacculus (Fig. 1A). It has been postulated that this bladder plays a role in acoustic pressure detection (von Frisch, 1938; Stipetic, 1939; Werns and Howland, 1976) and communication (Crawford, 1997), but this hypothesis has not been tested directly. The mormyrids have sensitive auditory systems (Kramer et al., 1981; McCormick and Popper, 1984; Marvit and Crawford, 2000a), and several species produce sounds during agonistic encounters and courtship (Bratton and Kramer, 1989; Crawford et al., 1997b; Crawford, 1997; Rigley and Marshall, 1973). Here, we provide the first experimental evidence for the function of the tympanic gas bladder in the hearing of sound-producing mormyrids.

von Frisch and others observed that mormyrids have an acute sense of hearing and reasoned that vibrations of the tympanic bladder, induced by sound pressure waves, would be transferred directly to the adjacent sacculus (Stipetic, 1939; von Frisch, 1938; Werns and Howland, 1976). The gas-filled

bladder is compressible relative to the water and surrounding tissue. Consequently, variations in pressure due to sound should modulate the volume of the bladder and activate the mechanosensory hair cells in the adjacent sensory epithelium by moving their apical hairs. Thus, the acoustic pressure stimulus is amplified and transformed into a mechanical signal by these bladders. Subsequent theoretical studies led to the prediction that the bladders should extend the sensitivity of this auditory system to higher frequencies (Werns and Howland, 1976).

Early conditioning experiments have shown that mormyrids respond behaviorally to low-intensity sounds (Diesselhorst, 1938; Stipetic, 1939; von Frisch, 1938). More recently, complete audiograms have been measured for two sound-producing mormyrid species, *Gnathonemus petersii* and *Pollimyrus adspersus* (see Fig. 1B,C). The *G. petersii* audiogram reveals best sensitivity between 300 and 1000 Hz, with acoustic detection up to 3 kHz (McCormick and Popper, 1984). *P. adspersus* also have excellent auditory sensitivity to low frequencies (Marvit and Crawford, 2000a) and are capable of discriminating between small differences in naturalistic sounds (Marvit and Crawford, 2000b).

The auditory capacities of mormyrids allow them to use sounds for communication. Male *P. adspersus* possess a repertoire of stereotyped vocalizations that are produced during courtship (Fig. 2C; Crawford et al., 1997a; Crawford, 1997). The acoustic energy of these courtship sounds matches the spectral regions of best auditory sensitivity (Marvit and
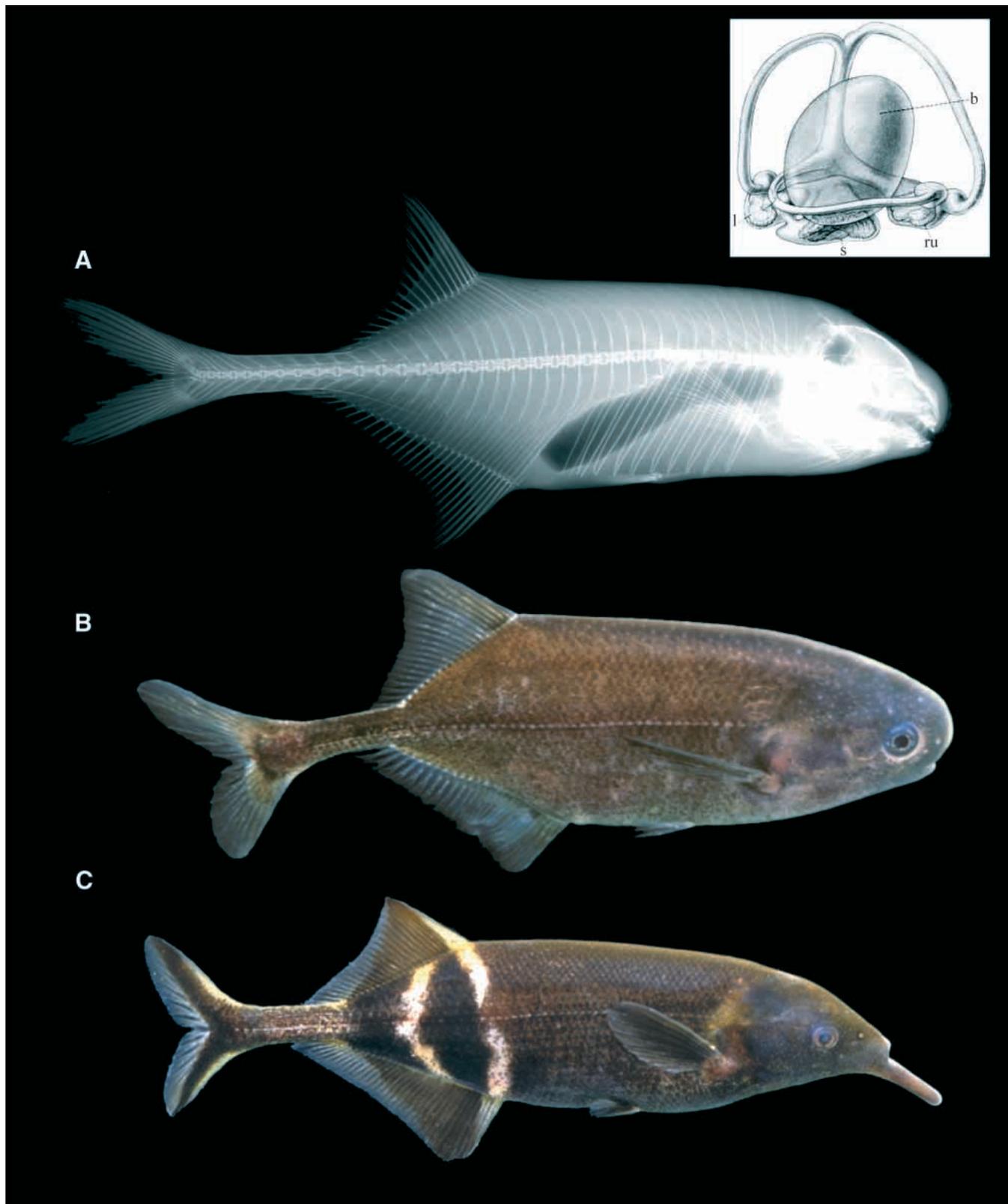
Fig. 1. Illustrations of *Pollimyrus adspersus* (A,B) and *Gnathonemus petersii* (C). (A) An X-ray image of a *P. adspersus*, revealing the elongate swim bladder and the small tympanic bladder in the head. The swim bladder extends from just behind the head, ventral to the vertebral column, to the anterior edge of the anal fin. The tympanic bladders are located just dorsal to the anterior-most end of the vertebral column. The inset above the fish's head shows a drawing of the tympanic bladder and associated sensory organs in the ear (from Stipetic, 1939). b, bladder; ru, utriculus; l, lagena; s, sacculus. The total length of the fish shown in X-ray image is 10 cm.

A
Gnathonemus petersii
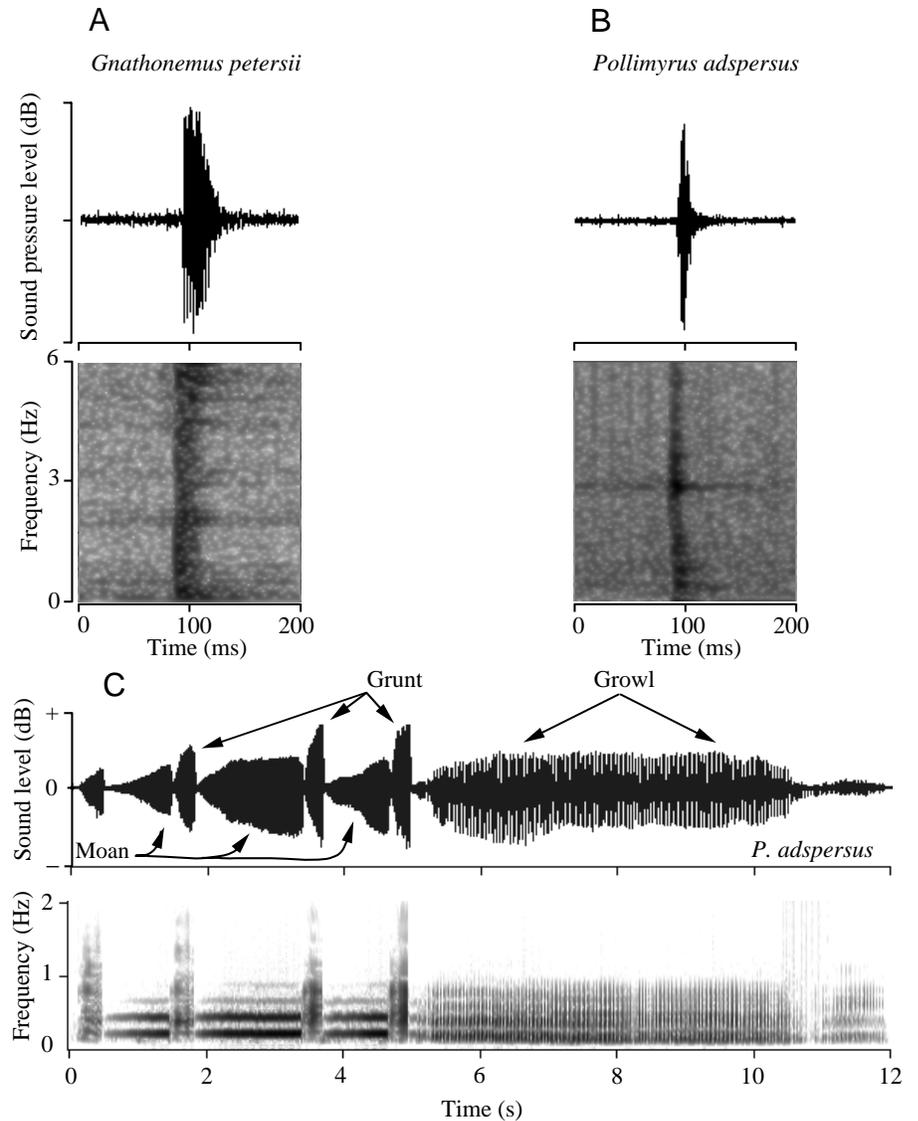
B
Pollimyrus adspersus

C

Fig. 2. Sounds of mormyrid electric fish. Both *Gnathonemus petersii* (A) and *Pollimyrus adspersus* (B) produce clicks during agonistic interactions. The upper panels show the waveforms and the lower panels show the corresponding sonograms. These impulsive sounds have acoustic energy distributed evenly across the animal's hearing range. *P. adspersus* also use sounds during courtship communication (C). Males court females with an alternating sequence of grunts and moans while females are near, and then terminate the display with a growl after the female has left the territory. The courtship sounds have energy peaks near 250 Hz and 500 Hz. Pressure levels for these sounds are typically 130 dB peak (re 1.0 μPa) when recorded near the fish (i.e. 10 cm from the fish).

Crawford, 2000a). In addition, *P. adspersus* and *G. petersii* both produce sounds (clicks) during agonistic encounters (Fig. 2A,B). The clicks are brief sounds with spectral energy spanning the audiograms of these fish (Rigley and Marshall, 1973). Werns and Howland (1976) suggested that the tympanic bladder may allow *G. petersii* to detect the higher-frequency components of these broad-band clicks.

In this paper, we present the results of new behavioral experiments on the role of the tympanic bladder in sound detection by *G. petersii* and *P. adspersus.* We compare acoustic pressure sensitivity in fish with gas displaced from the bladders with hearing in normal fish. We report that fish with both tympanic bladders deflated are approximately 30 dB less sensitive to sounds than normal or control fish. We conclude that the tympanic bladder plays a critical role in hearing in mormyrid fish. A preliminary account of this work has been presented in abstract form (Fletcher et al., 2000).

Materials and methods

We compared the hearing of normal and control fish with that of fish with the gas removed from their tympanic bladders. Experiments were carried out with three sound-producing mormyrids, *Gnathonemus petersii* (Gunther), *Pollimyrus adspersus* (Gunther) and *Pollimyrus isidori* (Valenciennes), and a non-sound producer, *Brienomyrus niger* (L.). We used behavioral methods to measure auditory thresholds for tones. Sound detection was measured using changes in the rate of the animal's electric organ discharge (EOD). This technique has been detailed in several previous papers (e.g. Marvit and Crawford, 2000a).

Animals

Gnathonemus petersii (*N*=16), *Pollimyrus adspersus* (*N*=17) and *P. isidori* (*N*=4) were collected in Nigeria and imported to the United States. The animals were 6–8 cm in standard length, and both males and females were used. Previous studies have

not revealed sex differences in tone detection thresholds (Marvit and Crawford, 2000a). The *G. petersii* we used were all juveniles. This species does not reach sexual maturity until they are quite large (20 cm or more). Fish were housed in laboratory aquaria at 25–28 °C, with a water conductivity of 50–200 μS cm^{-1}.

Fish were divided into four treatment groups. The first group consisted of normal fish (normals; *G. petersii*, N=7; *P. adspersus*, N=6). In the second group, the tympanic bladders were surgically exposed, and the gas was displaced with physiological saline in both ears (bilateral experimentals; *G. petersii*, N=3; *P. adspersus*, N=6). For the third group, the gas was expelled from just one ear (unilateral controls; *G. petersii*, N=6). In the fourth group, we carried out surgery on both ears, but this merely involved injecting saline into the exposed ears without penetrating the tympanic bladders (shams; *P. adspersus*, N=5). Apart from the surgery, the animals in all four groups were treated in the same manner.

One *Brienomyrus niger* was also imported from Nigeria and tested without any surgical manipulation. We have observed *B. niger* under conditions that would reveal acoustic behavior in *Pollimyrus* spp., but have detected no sound production (L. B. Fletcher and J. D. Crawford, unpublished observations).

Surgery

Fish were anesthetized by immersion in 0.5 g l^{-1} tricaine methanesulfonate (MS222) for 90 s. The immobilized specimen was wrapped in damp gauze, placed on a Plexiglas platform and respirated with oxygenated water through a tube. A 0.05 ml injection of 0.96 mg ml^{-1} Baytril (enroflaxin; Bayer) in saline was delivered to the back muscle to reduce the risk of post-operative infection. With the aid of a dissecting microscope, an incision was made around the perimeter of the paper-thin supratemporal bone that covers the tympanic cavity containing the bladder (Heusinger, 1826; Taverne, 1973). The bone was then lifted to reveal the tympanic bladder lying just underneath. The bladder was penetrated with a hypodermic needle, and the gas was displaced by injecting physiological saline with a 27.5 gauge hypodermic needle and 1.0 ml syringe. Since the thin bladder wall is taught and elastic, the hole created by the hypodermic enlarged so that there was sufficient space for the gas to escape as saline was delivered. An antibacterial ointment, Terramycin (oxytetracycline hydrochloride with polymyxin B sulfate, Pfizer Inc.), was dabbed on the underside of the bone before returning the bone to its normal position and sealing the wound with a tissue adhesive (Vetbond, 3M). In the unilateral controls, we performed the surgery on one side only. For the bilaterals, the surgery was repeated on the other ear after a second immersion in the MS222 solution. For shams (*P. adspersus* only), physiological saline was injected into the space surrounding the tympanic bladder, but the bladder was left intact.

The fish were allowed to recover for 7 days in a 114 l hospital tank. Tank water was continuously treated with an ultraviolet sterilizer (Aquanetics: Quartz, 15 IL), a carbon/ammonia filter (Marineland: 75 % carbon, 25 % zeolite) and a

power micron filter (Marineland Magnum 350). NaCl was added to increase water conductivity to 7.0–7.5 mS cm^{-1}, and water temperature was maintained at 26–28 °C. Animals began behaving normally within a few minutes of being placed in the hospital tank. After approximately 5 days, the Vetbond adhesive came off the healed wound, and after 2 weeks it was difficult to discern any evidence of the surgery.

Follow-up dissection revealed that the operated tympanic bladders did not re-inflate with gas during the testing period. Thirteen operated ears were examined, in eight fish (three were unilaterals), 269–376 days post-surgery. In every case, the bladder was completely collapsed and sitting adjacent to the sacculus, retaining its normal coupling to the sacculus. The intact ears in unilaterals appeared normal.

Behavioral training and testing

The apparatus for sound presentation, calibration and acoustic testing was the same as that described for previous behavioral studies of hearing in *P. adspersus* (Marvit and Crawford, 2000a). A review of the methods is presented here with modifications pertaining to the present study. Microcomputers and hardware from Tucker-Davis Technologies (TDT) were used for stimulus generation and data acquisition.

Sounds were presented through an underwater speaker in an acoustic tank. A fish holder was suspended from a stand attached to a vibration-isolation table and centered 25 mm below the surface of the water. Fish were able to move freely in the holder, but they were confined to the central area of the tank, which was acoustically calibrated. The entire apparatus was placed in a sound-attenuating chamber (IAC or Acoustic Systems). EOD activity was recorded with three Ag/AgCl electrodes built into the walls of a fish holder and with a differential amplifier (BMA 202). A TDT spike discriminator (SD1) and event timer (ET1) were used to record the time of each EOD to the nearest 1 μs. EOD rates were monitored by the computer during testing and were used to determine whether the fish heard a particular sound.

The holder was constructed from a section of polyvinyl chloride (PVC) tube (length 80 mm, diameter 38 mm). A fine fiberglass mesh cloth (1 mm×1 mm squares) covered elongate windows that were machined from the length of the tube. Two electrodes were also positioned on either side of the holder for delivery of a weak aversive current used during training and testing (unconditioned stimulus; described below).

Acoustic stimuli were played through an underwater speaker positioned at the bottom of the tank and projecting upwards. Tones were synthesized on a computer and then output at 50 kHz by a 16-bit digital-to-analog converter (TDT DA1) and low-pass-filtered at 10 kHz (TDT FT4). The analog signals were attenuated with programmable attenuators (TDT PA4), amplified (Crown D-75) and delivered to the speaker.

For calibration, a hydrophone (B&K 8103) was positioned in the tank at the center of the fish holder. The output of the hydrophone was passed to a BMA amplifier and digitized by a 16-bit analog-to-digital converter (TDT AD1). Tones were calibrated (dB rms re 1 μPa) from their amplitude spectra.

Prior to training, fish were familiarized with experimental conditions for two 120 min adaptation sessions (sessions without the acoustic stimulus or unconditioned stimulus) on separate days. In addition, fish were allowed 30 min in the testing apparatus before each training or testing run.

Training followed the classical conditioning paradigm described previously (Marvit and Crawford, 2000a); the acoustic stimulus (conditioned stimulus) was paired with a mild electric shock (unconditioned stimulus) on every trial, to prevent habituation (see Figs 1–2 in Marvit and Crawford, 2000a).

On each training trial, a tone frequency was randomly selected from a set of 10 frequencies spanning the range 100–1700 Hz. During a 3 s peri-stimulus period, the tone burst was presented (30 ms rise/fall ramps), and the EOD rate was compared with that during the preceding 3 s pre-stimulus period. The pressure level of the tone was set at random for each trial, within the range 105–125 dB. An EOD rate increase of 25 % was used as a criterion for tone detection. The tone continued for 500 ms into a post-stimulus period (duration 3.5 s), where it overlapped with the unconditioned stimulus. The unconditioned stimulus consisted of five consecutive 80 ms direct current pulses (3 mA) with an inter-pulse interval of 40 ms. Training included 20 trials, with an average inter-trial interval (ITI) of 720 s. Each inter-trial interval was randomly set between 660 and 780 s (720±60 s). Most fish acquired a conditioned response to the tone bursts within the first few trials.

Two days after training, we began threshold determinations using a one-up two-down adaptive staircase procedure. A single tone, randomly selected from a set of 12 in the range 100–2900 Hz, was presented during 40 consecutive trials (ITI=720±60 s). The sound pressure level was determined by the fish's EOD response to the tone. Individuals that indicated tone detection by giving a criterion response were presented with the same stimulus level in the next trial. If the fish responded a second time, the stimulus level was decreased for the following trial (two down). When the fish did not detect the stimulus, stimulus intensity was increased during the following trial (one up). A change in the direction of the stimulus level was termed a reversal. For the first four reversals, stimulus levels changed in steps of 6 dB. Thereafter, stimulus levels changed in steps of 3 dB. The threshold and standard deviation were calculated after excluding the first four reversals and then averaging the sound levels corresponding with the last even number of reversals. Occasionally, the stimulus reached levels that exceeded the behaviorally natural range of sounds (threshold greater than 135 dB), and the test session was aborted.

Fish were tested at 100, 200, 300, 400, 500, 600, 900, 1200, 1400, 1700, 2350 and 2900 Hz. They were tested twice at every frequency, and the lowest threshold was used in the audiogram. In a few cases, our criteria for an acceptable threshold estimate were not met for the first two estimates at a particular frequency, so the fish was tested a third time.

We used two criteria to evaluate each threshold estimate. First, the fish's false alarm rate was estimated, and the threshold was not used if the false alarm rate exceeded 35 %. False alarm rate was determined by measuring the rate at which the fish spontaneously reached the 25 % acceleration criterion during the pre-stimulus period relative to the 3 s preceding the pre-stimulus period (i.e. relative to the pre-pre-stimulus interval). Second, dispersion among the reversals was considered, and thresholds were excluded if the standard deviation was more than 5.0 dB. Staircases for which the standard deviation was high appeared erratic and did not level off at a clear threshold. Fish were tested no more than once every 3 days.

Analysis of variance (MGLH: ANOVA) was used (SYSTAT version 5.2 on a Macintosh Quadra 840AV) to determine whether the main effect of treatment group (normal, unilateral and bilateral for *G. petersii*; normal, sham and bilateral for *P. adspersus*) was statistically significant.

Values are presented as means ± S.E.M.

Results

Displacing the gas from the tympanic bladders of mormyrids produced a profound reduction in auditory sensitivity. The tone detection thresholds for fish with gas displaced from both ears were elevated by approximately 30 dB compared with normal fish (Fig. 3). Normal and unilateral control *G. petersii* were most sensitive between 200 and 1200 Hz, and their lowest thresholds (60 dB) were in the range 400–500 Hz (Fig. 4A). The mean mid-audiogram difference between normals and unilaterals (uni-normal) was only −8.3±2.3 dB (mean ± S.E.M.; 200–600 Hz, thresholds at five frequencies). In contrast, the mean mid-audiogram threshold difference between normal and
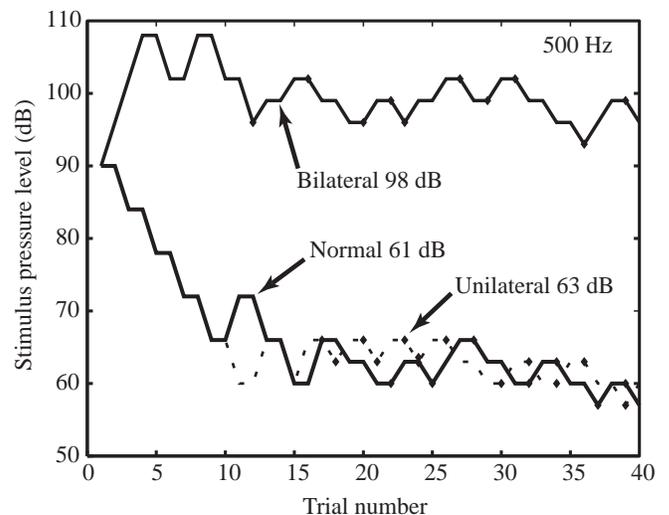

Fig. 3. Behavioral responses (staircases) for normal, unilateral and bilateral *Gnathonemus petersii* tested with 500 Hz tones. Normal and unilateral fish had similar detection thresholds near 62 dB, but fish with the gas displaced from both ears (bilaterals) only detected sounds that were very intense (98 dB in this case). The diamonds indicate reversals.

bilateral experimental fish was 27.7±2.7 dB. The threshold differences between bilateral and unilateral controls were similarly large, usually near 35 dB in the center of the audiogram. The mean mid-audiogram difference between the unilateral and bilateral fish was 37.5±2.9 dB. Threshold differences were somewhat smaller at extreme frequencies, where none of the fish hears well (Fig. 4B). An analysis of variance (ANOVA: mixed-effects model, with group as a fixed variable and frequency a random variable; Zar, 1996) revealed a highly significant effect (F=79; d.f.=2,22; P<0.001) of group membership (normal, unilateral and bilateral).

The mean normal audiogram we obtained for *G. petersii*, using the EOD response, was virtually identical to that obtained previously (McCormick and Popper, 1984) using an entirely different method; the mean threshold difference was only 6±2.4 dB for the 12 frequencies we examined.

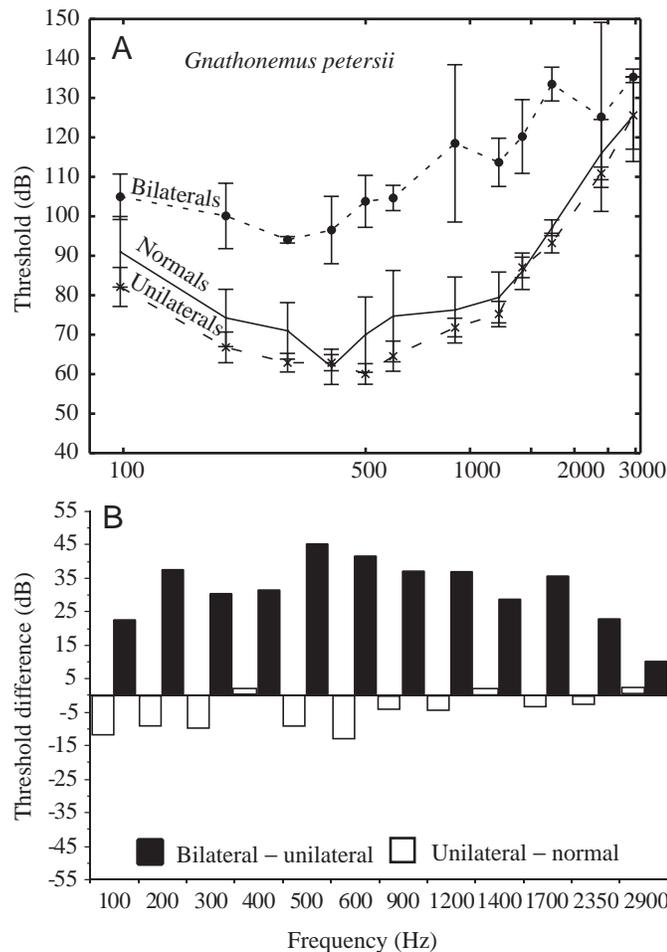Our manipulations to the tympanic gas bladders had a similar pronounced influence on tone detection in *P.*

adspersus. *P. adspersus* were of particular interest because they are known to use relatively elaborate sounds for courtship communication. The audiogram for normal fish is similar to that of *G. petersii*, although sensitivity above approximately 750 Hz is roughly 10 dB poorer in *P. adspersus*. Bilateral gas displacement again elevated thresholds by approximately 30 dB (Fig. 5). The mean mid-audiogram (200–600 Hz) difference between bilaterals and normals was 22.7±3.8 dB. When we examined detection thresholds following a sham operation performed on both ears, we found no loss of auditory sensitivity, with a mean mid-audiogram difference between shams and normals of only −6.3±2.1 dB. Again, an analysis of variance revealed a highly significant effect (F=100; d.f.=2,18; P<0.001) of group membership (normal, sham and bilateral).

Discussion

The gas-filled bladders in the ears of mormyrids have fascinated comparative anatomists (Heusinger, 1826; Stipetic, 1939; Taverne, 1973) and sensory physiologists (von Frisch, 1938; Werns and Howland, 1976) for many years, but hypotheses about the function of these bladders in sound pressure detection have so far escaped experimental testing. In recent years, interest in understanding this unusual auditory system has heightened with new discoveries about the function of species-specific communication sounds among mormyrids (Rigley and Marshall, 1973; Crawford, 1997; Crawford et al., 1986; Crawford et al., 1997a; Crawford et al., 1997b; Bratton and Kramer, 1989). Our results provide strong experimental support for the hypothesis that the mormyrid tympanic bladders function in sound pressure detection, and suggest that
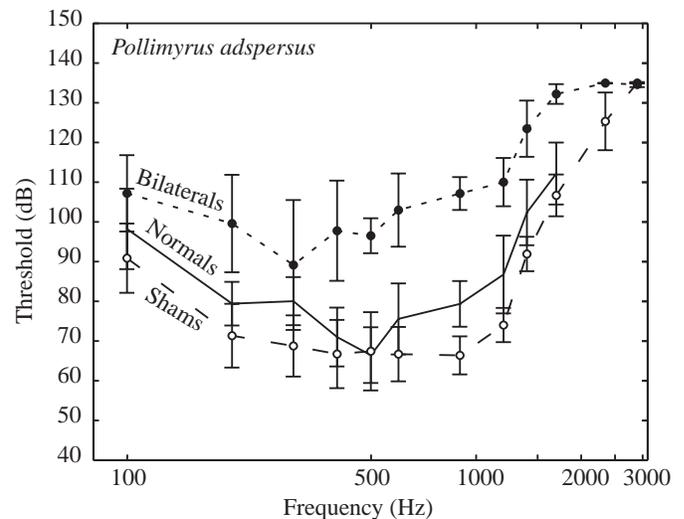

Fig. 4. Average audiograms for normal (*N*=7), unilateral (*N*=6) and bilateral (*N*=3) *Gnathonemus petersii* (A). Vertical bars show ±1 s.e.m. Unilaterals had normal hearing, but the thresholds of bilaterals were markedly elevated. (B) The sensitivity differences between bilaterals and unilaterals (black columns) and between unilaterals and normals (white columns) shown on a linear frequency scale.

Fig. 5. Audiograms for *Pollimyrus adspersus*. As was the case for *Gnathonemus petersii* (Fig. 4), bilateral gas removal (*N*=6) produced pronounced decrements in detection thresholds compared with normal fish (*N*=6). Fish that underwent a sham operation (*N*=5) had normal hearing. Data for the normals are replotted (from Marvit and Crawford, 2000a) as means with error bars of ±1 s.e.m.

the bladders may have evolved as an adaptation for acoustic detection.

The gas-filled bladders serve as pressure transducers because they are compressible compared with the water and surrounding tissues. The pressure changes due to an acoustic stimulus cause pulsation of the bladder, and this activates mechanosensory hair cells coupled to the bladder. Without the gas, the bladder can no longer serve as a pressure transducer, and auditory sensitivity is greatly reduced.

It is unlikely that our manipulations had any direct effect on the integrity of the sacculus or other parts of the inner ear. The ear bladder and sacculus are completely separated by the membrane that forms the wall of the bladder (Stipetic, 1939), so fluid inside the bladder will not come into contact with the sacculus. The experimental group with surgery to both ears (bilaterals) behaved normally, showing no evidence of vestibular dysfunction, indicating that the inner ear was healthy. Although the thresholds for these animals were substantially elevated, they did in fact detect sounds at most frequencies, revealing that the ears remained physiologically active. Shams and unilateral controls were handled just like the experimental fish and demonstrated normal hearing and locomotor behavior. It is possible that the prolonged deafness caused by bladder deflation may eventually lead to degeneration of the sensory epithelium, but this is not likely to have occurred during our testing shortly after surgery.

One of the most obvious advantages of sound pressure sensitivity is in acoustic communication. Four species of mormyrid are known to produce sounds (Crawford, 1997), and it seems likely that further investigation will reveal sound production among others of the many mormyrid species. Sensitivity to the pressure component of acoustic signals should increase detection range since pressure decreases much more slowly with distance from a source than does particle motion (Michelsen, 1983; Crawford et al., 1997b). In addition, prevailing theories about the mechanisms used by small aquatic animals for source direction determination involve simultaneous measurement of sound pressure and particle motion (Schuijf and Buwalda, 1980; Rogers et al., 1988). Increased hearing range and the ability to localize conspecific sound producers are two obvious benefits of pressure sensitivity potentially conferred by the tympanic bladders.

Despite the utility of the tympanic bladders in acoustic communication, it seems probable that additional selective forces may have contributed to the evolution of these auditory accessory structures and that the bladders could have been a pre-adaptation for acoustic communication (see Ladich and Yan, 1998). First, the auditory sensitivity of mormyrids (see also Marvit and Crawford, 2000a; McCormick and Popper, 1984) is sufficiently high that the fish should readily detect the incidental sounds made by other fishes while foraging, the sound of rain on the water surface (Kirschbaum, 1975; Kirschbaum, 1984; Kirschbaum, 1987), the pressure signals associated with lunging predators (Canfield and Eaton, 1990; Eaton et al., 1995) and perhaps even characteristics of the ambient noise that might serve as cues for acoustic spatial

orientation (Schellart and Popper, 1992; Lewis and Rogers, 1996). Second, all mormyrids possess tympanic bladders, although only some mormyrid species produce sounds (Crawford, 1997). Audiograms indicate that both sound-producing and non-sound-producing species (e.g. *Brienomyrus niger*) have similar hearing sensitivity (Fig. 6). Thus, high acoustic pressure sensitivity may be a relatively primitive condition among mormyrids, a condition that facilitated the evolution of acoustic communication in some species.

Gas-filled auditory specializations have evolved independently in other teleosts, and only a few of these species are known to produce sounds (Schellart and Popper, 1992). For example, all members of the superorder Otophysi have otophysic connections, but only some produce sounds. The goldfish *Carassius auratus* is an otophysan, in the order Cypriniformes, whose hearing has been studied particularly intensively (Fay, 1969; Fay, 1995; Fay, 1998; Lu and Fay, 1996), but which does not make communication sounds. In this respect, there is an interesting parallel between Cypriniformes and the Mormyriformes since, in both orders, all species have otophysic hearing specializations, but only some species in each group produce sounds. It seems likely that sound pressure detection is a primitive characteristic in both groups that was adaptive independent of acoustic communication and served as a pre-adaptation for the evolution of sound communication in certain lineages.

Among the best known auditory specializations are those of the otophysan fishes. The otophysans have a chain of small bones, Weberian ossicles, that couple the swim bladder to the inner ear. A number of behavioral (von Frisch, 1938;
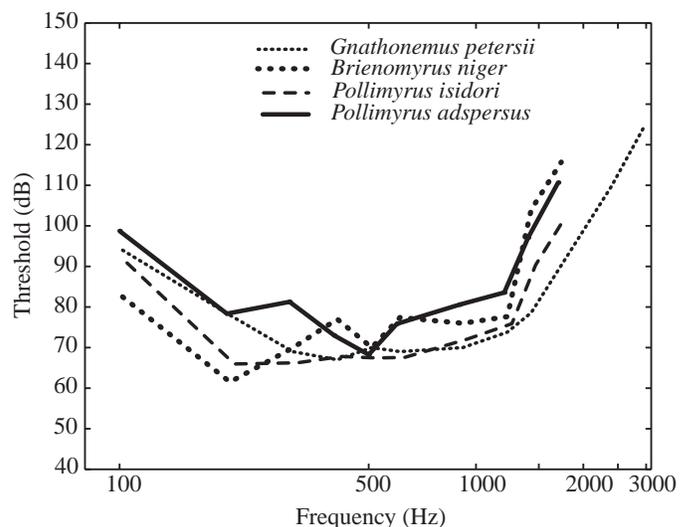

Fig. 6. Average audiograms for four mormyrid species. *Pollimyrus adspersus* (*N*=6), *P. isidori* (*N*=4) and *G. petersii* (*N*=4) are all sound producers, while *Brienomyrus niger* (*N*=1) is not known to make sounds. The tone detection thresholds of these four species are very similar. Each point represents the mean species threshold. The *G. petersii* data are reproduced from a previous publication (McCormick and Popper, 1984), and the *P. adspersus* data are also taken from a previous publication (Marvit and Crawford, 2000a).

Poggendorf, 1952; Kleerekoper and Roggenkamp, 1959) and electrophysiological (Fay and Popper, 1974; Fay et al., 1982) experiments have demonstrated the importance of this otophysic connection for increased auditory sensitivity and hearing range. Removing the gas from the goldfish swim bladder reduces auditory sensitivity by approximately 35 dB (Fay et al., 1982). Other swim-bladder-based specializations for pressure detection involve anterior protrusions of the swim bladder, which either contact the skull near the ear or actually penetrate the ear (Coombs and Popper, 1982; Schellart and Popper, 1992). This type of otophysic specialization has evolved among the herrings (Clupeiformes), squirrel fishes (Beryciformes), African knife fishes (Osteoglossiformes) and tarpons (Elopiformes).

The air-filled labyrinthine breathing organs of anabantid fishes (Perciformes) also serve to improve hearing through pressure transduction (Alexander, 1967; Yan, 1998). The gas-filled chambers are situated just above the branchial cavities and are specialized for respiratory gas exchange. However, the sacculus of the inner ear sits immediately adjacent to the chamber, separated from it by a thin membranous window. Removal of the gas reduces auditory sensitivity by 20–30 dB in the center of the audiogram (500–1500 Hz; Yan, 1998).

The tympanic bladders of mormyrids represent one example of a diversity of evolutionary inventions that enhance underwater hearing among fishes. The mormyrids appear unique in that tympanic bladders are apparently dedicated to sound pressure detection, having no physical continuity with the swim bladder. Swim bladders play an important role in regulating buoyancy (Denton, 1961), in the detection of hydrostatic pressure (Tytler and Blaxter, 1977) and in sound production (Fine and Pennypacker, 1986; Barimo and Fine, 1998). In mormyrids, the tympanic bladders pinch off completely from the swim bladder during development, allowing the swim bladder to function in buoyancy control and sound production (Crawford and Huang, 1999) independent of the hearing organs.

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Note in added proof

After the present paper had been submitted, a report appeared showing similar influences of tympanic bladder deflation in yet another species of mormyrid (Yan and Curtsinger, 2000). A non-sound-producing species was studied (*Brienomyrus brachyistius*) using physiological methods (the auditory brainstem response). As in our study, this report indicated that thresholds were elevated after tympanic bladder deflation, but only by approximately 12 dB in bilaterals relative to normals. We observed a substantially larger hearing decrement in the present study using our behavioral methods.

Yan, H. Y. and Curtsinger, W. S. (2000). The otic gasbladder as an ancillary auditory structure in a mormyrid fish. *J. Comp. Physiol.* A **186**, 595–602.