

Size matters: diversity in swimbladders and Weberian ossicles affects hearing in catfishes

Walter Lechner and Friedrich Ladich*

Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

*Author for correspondence (e-mail: friedrich.ladich@univie.ac.at)

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SUMMARY

Otophysine fish possess Weberian ossicles, which connect the swimbladder to the inner ear and improve hearing ability. There is a high diversity in the morphology of the swimbladder and Weberian apparatus in catfishes, which might affect hearing. We have examined these structures in representatives of six families with large, single bladders (Ariidae, Auchenipteridae, Heptapteridae, Malapteruridae, Mochokidae, Pseudopimelodidae) and five subfamilies from two families (Callichthyidae, Loricariidae) having small, paired, encapsulated bladders. We tested their hearing abilities utilizing the non-invasive auditory evoked potential recording technique. Species with single, non-encapsulated, free airbladders possess one, three or four ossicles, whereas species with encapsulated bladders possess one or two. The relative sizes of the bladders and ossicles were significantly smaller in the latter group. All species were able to detect sound stimuli between 50 Hz and 5 kHz. Interspecific differences in hearing sensitivity varied at most by 24 dB below 1 kHz, whilst this variation increased to more than 50 dB at higher frequencies. Catfishes with free bladders had lower thresholds above 1 kHz than those having encapsulated ones. The relative lengths of swimbladders and of ossicular chains were correlated with hearing sensitivity above 1 and 2 kHz, respectively. The number of ossicles affected hearing at 4 and 5 kHz. These results indicate that larger bladders and ossicles as well as higher ossicle numbers improve hearing ability at higher frequencies in catfishes. We furthermore assume that the tiny bladders have minimized their hydrostatic function but were not completely lost because of their auditory function.

Key words: auditory evoked potential (AEP), Weberian apparatus, Siluriformes.

INTRODUCTION

Within the bony fish, the hearing generalists or non-specialists lack accessory hearing structures. They are sensitive to particle motion and only capable of detecting low-frequency sounds. On the other hand, hearing specialists possess accessory structures that connect gas-filled chambers acoustically to the inner ear. This enables specialists to detect sound pressure and improves their hearing ability by enhancing the detectable frequency range and by lowering their hearing threshold (von Frisch, 1936; Hawkins and Myrberg, 1983; Hawkins, 1993; Popper and Fay, 1993; Ladich and Popper, 2004). Otophysans, a group of hearing specialists comprising Cypriniformes, Siluriformes, Characiformes and Gymnotiformes, possess the Weberian apparatus. This consists of fused anterior vertebrae, known as ‘complex vertebra’ (Wright, 1884), and a paired chain of one to four ossicles and ligaments joining the swimbladder to the inner ear (Weber, 1820). The Weberian apparatus facilitates sound transmission to the inner ear and generally improves the hearing sensitivity of otophysines (Ladich, 1999; Ladich and Wysocki, 2003). The catfish order, currently consisting of approximately 3100 species within 36 families (Ferraris, 2007), exhibits a huge variability in the morphology of the Weberian ossicles and the gasbladder. Numerous catfish families have large single, free swimbladders. On the other hand, many groups have highly reduced, tiny and paired swimbladders located to the left and right of the vertebral column, directly behind the cranium. Additionally, these tiny bladders are surrounded by bony capsules formed by the skull and anterior vertebrae. These conspicuous structures prompted Bridge and Haddon (Bridge and Haddon, 1889;

Bridge and Haddon, 1892; Bridge and Haddon, 1893) to split catfish into two groups, the ‘siluridae normales’ with well-developed bladders and the ‘siluridae abnormales’ with reduced bladders.

Several investigators have described the structures of the Weberian apparatus in numerous species of catfishes (e.g. Sørensen, 1895; Chranilov, 1929; Alexander, 1964; Alexander, 1965; Mahajan, 1967; Chardon, 1968; Coburn and Grubich, 1998; Chardon et al., 2003), but its function was debated in the 1800s. While Weber (Weber, 1820) originally assumed that ossicles facilitate hearing, Bridge and Haddon (Bridge and Haddon, 1889; Bridge and Haddon, 1892; Bridge and Haddon, 1893) denied this contention, which was then contradicted by Sørensen (Sørensen, 1895). The effect of reduced and encapsulated swimbladders continued to be discussed ambivalently. Chranilov (Chranilov, 1929) assumed that swimbladder encapsulation and its reduction in size as well as reduction in the number of ossicles improves the sensitivity due to free, moveable ossicles. Ladich and Bass (Ladich and Bass, 2003) expected reduced hearing because a reduction in size of the swimbladder walls reduces the ability of the swimbladder to oscillate.

Studies on hearing in catfishes are rather sparse and have been carried out in only a few members of a few families (for a review, see Ladich and Bass, 2003). Most studies have been conducted in ictalurids (von Frisch, 1923; von Frisch, 1936; von Frisch, 1938; Stetter, 1929; Poggendorf, 1952; Kleerekoper and Roggenkamp, 1959; Weiss et al., 1969; Fay and Popper, 1975; Moeng and Popper, 1984; Plassmann, 1985) and a few in ariids (Tavolga, 1977; Popper and Tavolga, 1981; Tavolga, 1982). More recently, doradids,

pimelodids and callichthyids (Ladich, 1999; Ladich, 2000) have been investigated. Among all the species tested, only the callichthyid *Corydoras paleatus* possesses tiny, paired and bony encapsulated bladders and a single Weberian ossicle (Coburn and Grubich, 1998). This species had the lowest hearing ability, and Ladich (Ladich, 1999) assumed that this was due to the small relative size of its swimbladder.

The aim of this study was to demonstrate that the morphology of the Weberian apparatus (swimbladder size, size and number of Weberian ossicles) affects the hearing sensitivity in catfishes. Eleven species were chosen based on the size of their swimbladders. Six species belonging to the families Ariidae, Auchenipteridae, Heptapteridae, Malapteruridae, Mochokidae and Pseudopimelodidae have well-developed, free bladders, whereas five species belonging to the callichthyid subfamilies Callichthyinae and Corydoradinae, and to the loricariid subfamilies Hypoptopomatinae, Hypostominae and Loricariinae have reduced, paired and bony encapsulated bladders. Dissections along with clearing and staining techniques were used to examine and measure swimbladders and ossicles. Hearing thresholds between 50 Hz and 5 kHz were determined in all species using the auditory evoked potential (AEP) recording method.

MATERIALS AND METHODS

Animals

Eleven species of different families or subfamilies were chosen (Table 1). When possible, we used the same specimens of a species for morphological and auditory measurements; when this was not possible, we used specimens from the same source.

Fish were obtained from tropical fish suppliers: *Batrochoglanis raninus* from Ruinemans Aquarium, Montfoort, Netherlands; *Pimelodella* sp. from Amazon Exotic Import, Rosenheim, Germany; all other species from Transfish, Munich, Germany. Specimens of *Hemiodontichthys acipenserinus* were aquarium reared; all other species were wild caught. Fish were kept in planted aquaria with a sand bottom, equipped with roots and clay or bamboo tubes as shelters. Only external filters were used. In order to provide a quiet environment we did not use any internal filters or air stones. Temperature was kept

at 26±1°C and a 12 h:12 h L:D cycle was maintained. Depending on their specific requirements, fish were fed frozen chironomid larvae and artificial food (granulate and tablets) daily. All hearing experiments were performed with the permission of the Austrian Commission on Experiments in Animals (GZ 66.006/2-BrGT/2006).

Morphological measurements

Fish and swimbladder sizes were measured to the nearest 0.1 mm using digital callipers. Standard length (*SL*) was measured as 'standard length 2' following Holcik et al. (Holcik et al., 1989). A standardized swimbladder length (*SBL*) was determined following the formula $SBL=(l+w+h)/3$, where *l* is length, *w* is width and *h* is height. For morphological measurements, fish were killed using an overdose of tricaine methanesulphonate (MS-222); measurements were taken either immediately or after preserving the fish in alcohol (70%). Dissecting microscopes (Wild M7 and Wild M5, Wild Heerbrugg Ltd, Heerbrugg, Switzerland) equipped with a camera lucida were used for these measurements and morphological drawings. For investigation of the Weberian ossicles and swimbladder capsules, additional specimens were trypsin cleared and alizarin stained following Potthoff's method (Potthoff, 1984). Ossicles of *Corydoras sodalis* were additionally examined with a scanning electron microscope (SEM Philips XL 20, software: Microscope Control, Philips, Eindhoven, The Netherlands). Assuming these structures grow linearly with fish growth (Fine, 1975; Fine et al., 2007), 'relative swimbladder length' (rSBL) and 'relative length of the ossicular chain' (rCL) were calculated following the formulas $rSBL=SBL/SL$ and $rCL=CL/SL$, where *CL* is the ossicular chain length and *SL* is the standard length.

In *Malapterurus beninensis*, only the dimensions of the anterior chamber of the bladder (camera aerea Weberiana) were used for calculations and comparisons.

Auditory sensitivity measurements

Hearing thresholds were determined using the AEP recording technique following the protocol developed by Kenyon et al. (Kenyon et al., 1998) and modified by Wysocki and Ladich (Wysocki and Ladich, 2005a; Wysocki and Ladich, 2005b).

Table 1. Species, number of specimens and size ranges of catfish used in this study

Family Subfamily	Species	AM			SB		O SL
		N	SL	BM	N	SL	
Ariidae	<i>Ariopsis seemanni</i> (Günther 1864)	6	57.5–77	3.08–7.76	3	59.0–73.0	96.9
Pseudopimelodidae	<i>Batrochoglanis raninus</i> (Valenciennes 1840)	6	42.5–61.4	1.92–5.93	3	52.2–60.3	52.2
Malapteruridae	<i>Malapterurus beninensis</i> Murray 1855	6	95.6–150.3	13.75–75.9	3	81.9–132.8	81.9
Heptapteridae	<i>Pimelodella</i> sp.	7	75.9–101.6	5.07–13.7	3	72.0–101.6	70.7
Mochokidae	<i>Synodontis schoutedeni</i> David 1936	7	65.8–83.7	7.34–14.64	3	82.2–106.6	106.6
Auchenipteridae	<i>Trachelyopterichthys taeniatus</i> (Kner 1858)	6	123.4–143.3	16.91–26.96	3	121.3–140.3	139.7
Loricariidae							
Hypostominae	<i>Ancistrus ranunculus</i> Muller, Rapp Py-Daniel and Zuanon 1994	8	72.5–103.4	9.1–26.8	6	72.0–94.8	89.9
Callichthyidae							
Corydoradinae	<i>Corydoras sodalis</i> Nijssen and Isbrücker 1986	6	48.2–54.9	3.87–5.65	3	49.5–51.1	49.5
Callichthyidae							
Callichthyinae	<i>Dianema urostriatum</i> (Miranda-Ribeiro 1912)	8	63.3–76.6	5.64–11.1	3	72.1–74.8	57.5
Loricariidae							
Loricariinae	<i>Hemiodontichthys acipenserinus</i> (Kner 1853)	7	68.0–75.7	1.23–2.17	3	66.4–71.4	76.1
Loricariidae							
Hypoptopomatinae	<i>Hypoptopoma thoracatum</i> Günther 1868	7	43.5–49.7	1.05–1.78	3	45.3–51.5	48.7

AM, auditory measurements; BM, body mass (g); N, number of specimens; O, examination of ossicle(s); SB, morphological measurements of the swimbladders; SL, standard length (mm). The systematics of catfishes generally follows that of Ferraris (Ferraris, 2007); loricariid subfamilies systematics follows Armbruster (Armbruster, 2004). The nomenclature of the sea-catfish *Ariopsis seemanni* follows personal communications with R. Betancur, Auburn, AL, USA, and A. Acero, Santa Marta, Colombia. Other authors put this species into the genus *Sciades* (Marceniuk and Menezes, 2007).

Table 2. Morphological measurements of specimens, swimbladders and ossicles

Species	N	SB	O	Mean SL (mm)	Mean TL (mm)	Mean SBL (mm)	rSBL	rCL
<i>A. seemanni</i>	3	Free	4	64.48±4.32	78.38±6.24	7.84±1.45	0.120	0.034
<i>B. raninus</i>	3	Free	4	55.04±2.64	69.15±3.28	7.96±0.41	0.145	0.033
<i>M. beninensis</i>	3	Free	4	104.62±14.96	123.27±17.38	8.86±1.45	0.084	0.050
<i>Pimelodella</i> sp.	3	Free	4	89.57±8.98	104.10±9.8	10.43±0.89	0.117	0.044
<i>S. schoutedeni</i>	3	Free	3	87.31±9.99	113.20±9.14	13.19±1.47	0.152	0.053
<i>T. taeniatus</i>	3	Free	1	132.80±5.84	146.40±7.00	14.39±0.54	0.109	0.035
<i>A. ranunculus</i>	6	Enc.	2	81.98±3.97	97.82±4.88	4.68±0.36	0.057	0.016
<i>C. sodalis</i>	3	Enc.	1	50.77±0.70	64.73±1.20	1.92±0.16	0.038	0.019
<i>D. urostriatum</i>	3	Enc.	1	73.17±0.83	92.07±1.71	3.15±0.14	0.043	0.011
<i>H. acipenserinus</i>	3	Enc.	2	68.10±1.65	74.46±2.08	1.09±0.03	0.016	0.011
<i>H. thoracatum</i>	3	Enc.	2	48.33±1.79	60.03±2.30	1.46±0.10	0.030	0.013

Mean SL, TL and SBL values are given ± s.e.m. Enc., encapsulated swimbladder; Free, non-encapsulated swimbladder; N, number of specimens examined; O, number of ossicles in chain; rCL, relative length of chain of ossicles; rSBL, relative swimbladder length; SB, swimbladder; SBL, maximum swimbladder length; SL, standard length; TL, total length.

The catfish were mildly immobilized with Flaxedil (gallamine triethiodide; Sigma Aldrich Handels GmbH, Vienna, Austria) during these experiments. The dosage used was 2.9–3.5 µg g⁻¹ for *Ancistrus ranunculus*, 2.13–2.95 µg g⁻¹ for *Ariopsis seemanni*, 39.6–45.3 µg g⁻¹ for *Batrochoglanis raninus*, 0.62–0.74 µg g⁻¹ for *Corydoras sodalis*, 1.20–1.85 µg g⁻¹ for *Dianema urostriatum*, 1.13–3.27 µg g⁻¹ for *Hemiodontichtys acipenserinus*, 1.21–1.43 µg g⁻¹ for *Hypoptopoma thoracatum*, 6.17–7.7 µg g⁻¹ for *Malapterurus beninensis*, 1.61–1.96 µg g⁻¹ for *Pimelodella* sp., 3.57–10.81 µg g⁻¹ for *Synodontis schoutedeni* and 20.40–37.53 µg g⁻¹ for *Trachelyopterichthys taeniatus*. The lowest dosage that immobilized fish while enabling slight movement of the opercula during the experiments was applied. All auditory measurements were carried out in a bowl-shaped plastic tub (diameter 33 cm, water depth 13 cm, 1 cm layer of gravel), which was lined inside with acoustically absorbent material (air-filled packing wrap) to decrease resonances and reflections. For a more detailed description see Wysocki and Ladich (Wysocki and Ladich, 2002). The tub was positioned on an air table (TMC Micro-g 63-540, Technical Manufacturing Corporation, Peabody, MA, USA), which rested on a vibration-isolated plate of concrete. A sound-proof chamber, constructed as a Faraday cage (interior dimensions: 3.2 m×3.2 m×2.4 m), enclosed the whole setup.

Test subjects were positioned in the centre of the tub, so that the nape of the head was just above the water surface. For respiration a pipette was inserted into the fish's mouth and respiration was effected by a simple, temperature-controlled (25±1°C), gravity-fed water circulation system. The area of the head above the water surface was covered with a small piece of Kimwipes® tissue paper to keep it moist. Silver wire electrodes (diameter 0.38 mm) were used for recording AEPs. The recording electrode was placed in the midline of the skull over the region of the medulla, the reference electrode cranially between the nares. Both electrodes were pressed firmly against the skin.

Both presentation of sound stimuli and AEP waveform recording were achieved using a modular rack-mount system [Tucker-Davis Technologies (TDT) System 3, Gainesville, FL, USA] controlled by a PC containing a TDT digital signal processing board and running TDT BioSig RP software.

Sound stimuli

Hearing thresholds were determined at the following frequencies: 0.05, 0.1, 0.3, 0.5, 0.8, 1, 2, 3, 4 and 5 kHz. *Ariopsis seemanni* was additionally tested at 0.2 kHz. Sound stimuli waveforms were

created using TDT SigGen RP software and fed through a power amplifier (Alesis RA 300, Alesis Corporation, Los Angeles, CA, USA). For presentation of stimuli during testing, a dual-cone speaker (Warfedale Pro Twin 8, Huntingdon, UK), installed 0.5 m above the test subjects, was used. The different frequencies were presented in random order. A hydrophone (Brüel and Kjaer 8101, Naerum, Denmark; frequency range 1 Hz to 80 kHz ±2 dB; voltage sensitivity -184 dB re 1 V µPa⁻¹) was placed 2 cm from the right side of the animal to determine absolute sound pressure levels (SPLs) under water in the immediate vicinity of the test subjects. A second, custom-built preamplifier was used to boost the hydrophone signal. Sound stimuli consisted of tone bursts played at a repetition rate of 21 s⁻¹ and at opposite polarities (90° and 270°). One-thousand stimuli of each polarity were presented and the corresponding AEPs averaged by BioSig RP software to eliminate stimulus artefacts. The SPL was reduced in 4-dB steps until the AEP waveform was no longer identifiable. By overlaying replicate traces, the lowest SPL yielding a repeatable AEP trace was determined and regarded as threshold.

Data analysis

All data were tested for normal distribution utilizing Shapiro–Wilk's test. When data were normally distributed, parametric statistical tests were applied. The Mann–Whitney *U*-test was used to test for differences in the relative length of the ossicular chain between species with free and encapsulated bladders. Mean hearing thresholds were determined for each species and at each frequency. A mean threshold curve for each frequency was calculated for both types of species. The Mann–Whitney *U*-test was used to analyse differences in the number of ossicles and mean hearing thresholds at each frequency of all species with free bladders from those of all species with bony encapsulated bladders. Pearson's correlation was calculated for comparing mean hearing thresholds to morphological measurements (rSBL, rCL, number of Weberian ossicles) of each species. SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used to run statistical tests.

RESULTS

Morphological analysis

Considerable variation was observed in the size and shape of both swimbladders and Weberian ossicles within both groups – single and free *versus* paired and bony encapsulated bladders (Table 2, Figs 1 and 2). Intraspecific variation of morphological factors was marginal in the specimens examined.

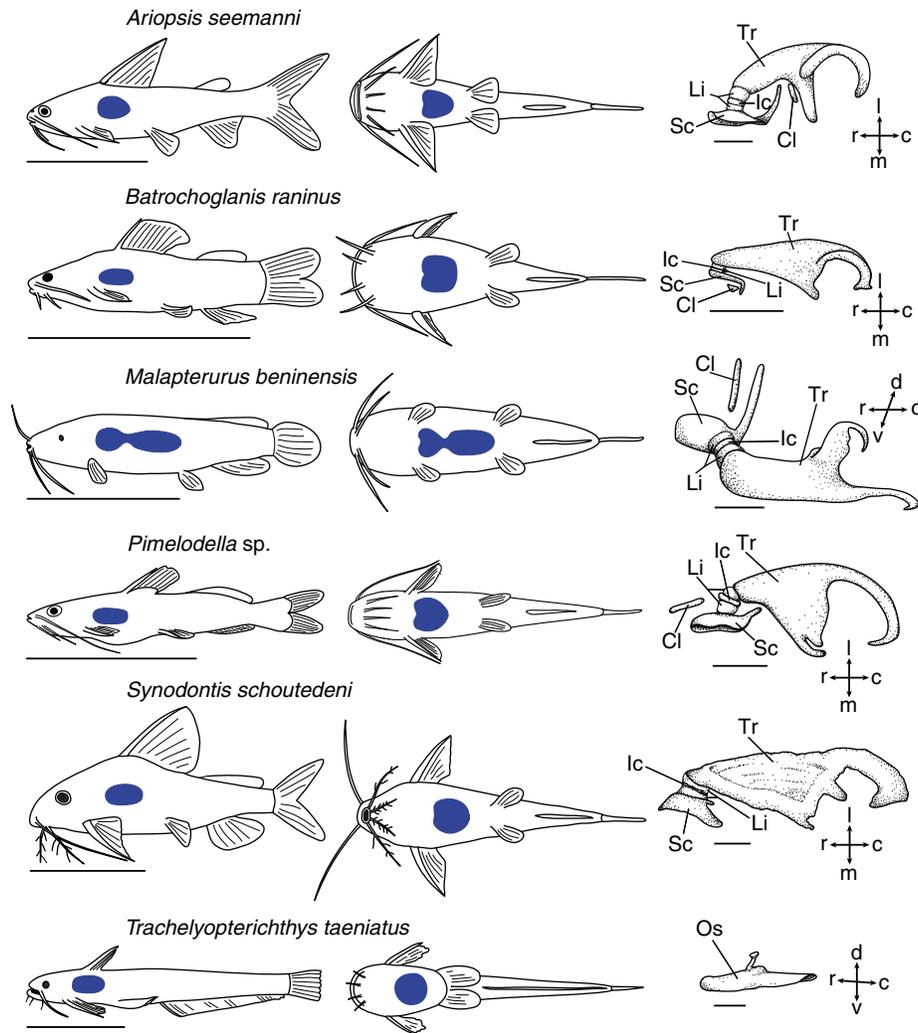


Fig. 1. Lateral and ventral view of the six species possessing free swimbladders and ventral, dorso-lateral (*Malapterurus*) or side (*Trachelyopterichthys*) view of the Weberian ossicles. Swimbladders are in blue. Ossicle sizes were standardized and correspond to individuals of the same standard length. Scale bars, 5 cm for fish and 1 mm for ossicles. c, caudal; Cl, claustrum; d, dorsal; Ic, intercalarium; l, lateral; Li, ligamentum interossiculare; m, median; Os, ossicle; r, rostral; Sc, scaphium; Tr, tripus; v, ventral.

Swimbladders

Free swimbladders were ellipsoid or slightly heart shaped, whereas the shapes of encapsulated bladders varied from conical in *A. ranunculus* to nearly spherical in *H. thoracatum* (Figs 1 and 2). Mean swimbladder length ranged from 7.84 to 14.39 mm in the first group and from 1.09 to 4.68 mm in the second group. Free bladders were absolutely and relatively larger than encapsulated ones (rSBL: Student's *t*-test: $t=-11.51$, $N=36$, $P<0.001$). None of the species with paired bladders had a connection between the two lateral compartments.

Ossicles

Species with free bladders had generally four Weberian ossicles – tripus, intercalarium, scaphium and claustrum, except for *S. schoutedeni*, which lacked the latter. Interestingly, the auchenipterid *T. taeniatus* had just one well-developed ossicle (Fig. 1). The tripus was the largest ossicle in all species, whereas the intercalarium usually was the smallest one, mainly consisting of a thin plate. In species with free bladders the scaphium had a typically patelliform concha. The scaphium in all species showed a well-developed

processus ascendens, except in *S. schoutedeni*, where it was degenerated and only rudimentarily present. In species with encapsulated bladders the scaphium, when present, showed no processus and was reduced to the concha scaphii, which was bowl shaped as in the group with free bladders (Fig. 2). Claustra, when present, were thin sticks with no connection to the rest of the chain. They were positioned opposite the scaphium towards the midline in *B. raninus* and *M. beninensis*, towards the caudal end in *A. seemanni* and towards the rostral end in *Pimelodella* sp. Representatives with encapsulated bladders had fewer ossicles, usually one or two (Mann–Whitney *U*-test, $U=4$, $N_1=6$, $N_2=5$, d.f.=9, $P<0.02$). *Ancistrus ranunculus*, *H. acipenserinus* and *H. thoracatum* exhibited a tripus and scaphium whereas *C. sodalis* and *D. urostriatum* had only a single ossicle (Fig. 3). SEM photographs of the ossicle of *C. sodalis* (Fig. 4) revealed no sutures that would indicate a possible fusion of several ossicles. *Dianema urostriatum* had a single bipartite ossicle. These two parts were fused with a connecting shaft, which was still surrounded by a ligament.

In addition to the difference in ossicle number, the relative length of the ossicular chain differed between groups. Catfishes with free

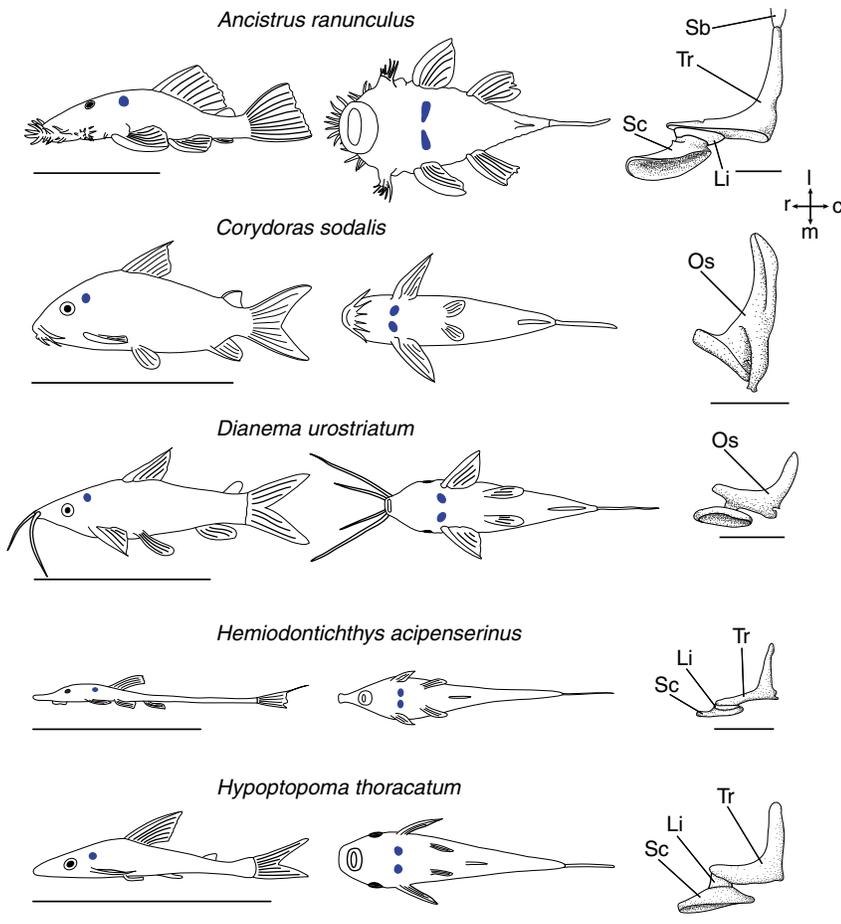


Fig. 2. Lateral and ventral view of the five species with paired and encapsulated swimbladders and ventral view of the Weberian ossicles. Swimbladders are in blue. Ossicle sizes standardized as in Fig. 1. Scale bars, 5 cm for fish and 0.5 mm for ossicles. c, caudal; l, lateral; Li, ligamentum interossiculare; m, median; Os, ossicle; r, rostral; Sb, swimbladder; Sc, scaphium; Tr, tripus.

Correlations between morphological and physiological data

The relative swimbladder length (rSBL) was negatively correlated to the hearing threshold at frequencies above 1 kHz (Pearson's correlation, 2 kHz: $r=-0.71$, $P=0.015$; 3 kHz: $r=-0.80$, $P=0.003$; 4 kHz: $r=-0.77$, $P=0.006$; 5 kHz: $r=-0.79$, $P=0.004$; $N=11$). Species with larger swimbladders had lower thresholds. Similarly, the relative length of the Weberian ossicular chain (rCL) was significantly correlated with hearing at frequencies above 2 kHz. Species with longer ossicular chains had better hearing (Pearson's correlation, 3 kHz: $r=-0.67$, $P=0.025$; 4 kHz: $r=-0.64$, $P=0.033$; 5 kHz: $r=-0.61$, $P=0.045$; $N=11$). The number of ossicles had a significant impact on hearing ability only at the highest frequencies tested. The number of ossicles was positively correlated with auditory sensitivity (Pearson's correlation, 4 kHz: $r=-0.69$, $P=0.018$; 5 kHz: $r=-0.61$, $P=0.011$; $N=11$).

DISCUSSION

Morphology of swimbladders and Weberian ossicles in cattfishes

Among Otophysi, Siluriformes encompass the largest number of bottom-dwelling representatives.

This life history reduces the necessity to maintain a gas bladder for swimming and hovering although it would still contribute to buoyancy (Fine et al., 1995). Furthermore, in those groups that possess aerial respiratory organs (Clariidae, Loricariidae and Callichthyidae) a reduced size might compensate for the hydrostatic effect of an accessory air-filled respiratory cavity (Alexander, 1964). Nevertheless, contrary to many other bottom-dwelling bony fishes such as sculpins and some gobies, otophysines in general and cattfishes in particular never completely lose their swimbladders and Weberian ossicles. At the same time they reduce swimbladder and ossicle size and ossicle number. Extreme reductions (or perhaps even absence) have been reported for cave-dwelling troglomorphic North American ictalurids (Hubbs and Bailey, 1947; Lundberg, 1982; Langecker and Longley, 1993). Unfortunately none of these authors state clearly whether bladders are totally absent or only extremely reduced and whether there are really cattfish without swimbladders.

The structures of the Weberian apparatus and the morphology of the swimbladder in cattfishes have been described by several authors since the late 19th century (Sagemehl, 1885; Bridge and Haddon, 1889; Bridge and Haddon, 1892; Bridge and Haddon, 1893; Sørensen, 1895; Chranilov, 1929; Alexander, 1964; Alexander, 1965; Mahajan, 1967; Chardon, 1968; Schaefer, 1987; Aquino and Miquelarena, 2001; Chardon et al., 2003). Our morphological findings that free bladders are larger than encapsulated ones and are accompanied by more ossicles are consistent with most of their observations. We have shown that free single swimbladders are heart shaped or ellipsoid, whereas paired bladders are tiny and in some species hardly detectable, such as those in loricariids and

swimbladders had relatively large ossicles and large ossicular chains (rCL: Student's t -test, $t=-6.5$, $N=11$, $P<0.001$).

Auditory sensitivities

All cattfish examined showed AEPs in response to tone bursts from 50 Hz to 5 kHz. Only in three out of four larger individuals of *Ancistrus ranunculus* did we fail to get any response at 5 kHz (possibly because our equipment could not deliver SPLs beyond 129 dB re 1 μ Pa). (A conservative calculation approach was used; accordingly, the data from these three specimens were omitted in calculations at 5 kHz, and so the average underestimates the threshold of *A. ranunculus* at this frequency.) The lowest absolute auditory threshold was found in the sea cattfish *Ariopsis seemanni* at 3 kHz with a mean threshold of 67 dB, whereas in *A. ranunculus* and *Corydoras sodalis* the highest thresholds were 117 and 121 dB at 4 and 5 kHz (Table 3A,B; Fig. 5). Differences in hearing threshold increased rapidly at higher frequencies. While thresholds varied maximally by 18 to 24 dB from 50 to 1000 Hz among all species, at higher frequencies the differences ranged from 29 to 51 dB. The largest difference (51 dB) was found between *A. seemanni* and *A. ranunculus* at 4 kHz (Table 3A,B; Fig. 5).

Hearing thresholds differed significantly between the group with free and the group with encapsulated bladders between 1 and 5 kHz, but not at lower frequencies (Fig. 6) (Mann-Whitney U -test, 1 kHz: $U=494.5$, $P=0.028$; 2 kHz: $U=83$, $P<0.001$; 3 kHz: $U=25.5$, $P<0.001$; 4 kHz: $U=29$, $P<0.001$; 5 kHz: $U=34$, $P<0.001$; $N_1=6$, $N_2=5$, d.f.=9).

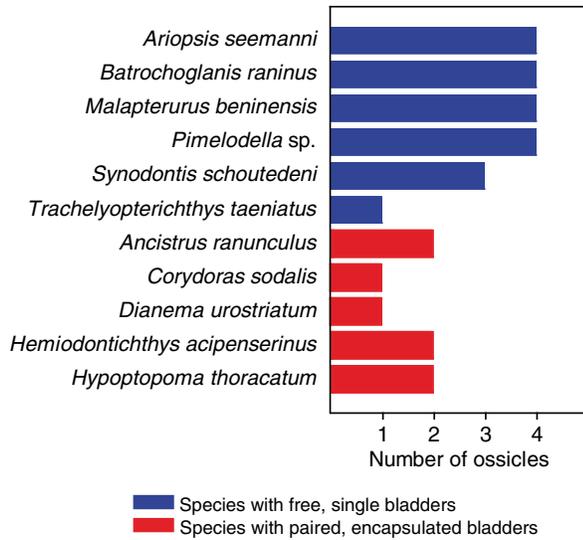


Fig. 3. Histogram showing the number of ossicles within the catfish species described.



Fig. 4. Scanning electron microscope micrograph of the single ossicle of *Corydoras sodalis*. Ventral view, scale bar 200 µm; c, caudal; l, lateral; m, median, r, rostral.

callichthyids. Fish possessing free single bladders had mostly four Weberian ossicles, and groups with small encapsulated bladders had two ossicles in loriciariids and a single one in callichthyids. Ariids, heptapterids, malapterurids and pseudopimelodids are regarded as basal catfish groups and we found a claustrum in all species of the families examined, which corresponds to the report of Britz and Hoffmann (Britz and Hoffmann, 2006). While some authors could not detect an intercalarium in mochokids of the genus *Synodontis* (Chranilov, 1929; Chardon, 1968) or stated that these fish possess only a highly reduced intercalarium (Bridge and Haddon, 1893), we undoubtedly found this ossicle in *Synodontis schoutedeni*. It is

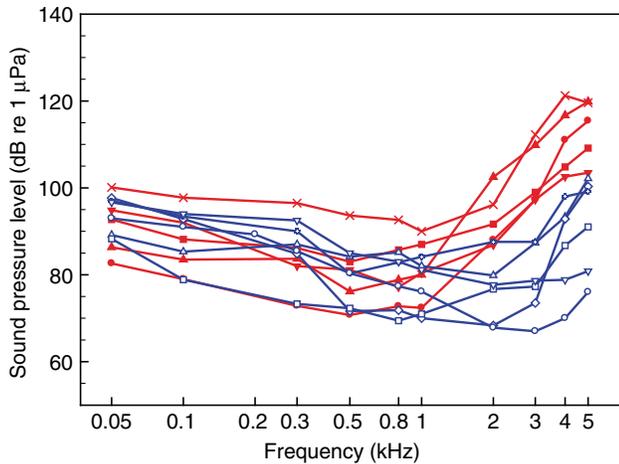
a very thin, small disc at which interossicular ligaments insert, recognizable only after multiple alizarin stainings. We do not know whether representatives of *Synodontis* exhibit a different development of Weberian ossicles or whether prior investigators simply overlooked the intercalarium.

Interestingly, the auchenipterid *Trachelyopterichthys taeniatus* has a single free bladder but possesses just one ossicle, the tripus. The tripus of *T. taeniatus* is, just like the tripus of *Pseudauchenipterus nodosus* (Bridge and Haddon, 1893), curled

Table 3. Hearing threshold values (dB re 1 µPa) of species with (A) free swimbladders and (B) paired, bony encapsulated bladders

A		<i>A. seemanni</i>	<i>B. raninus</i>	<i>M. beninensis</i>	<i>Pimelodella</i> sp.	<i>S. schoutedeni</i>	<i>T. taeniatus</i>
<i>f</i> (kHz)	0.05	93.00±0.52	96.67±1.31	89.17±1.08	97.00±0.53	88.29±0.71	97.67±0.56
	0.1	91.00±1.35	94.00±1.00	85.33±0.80	93.43±0.69	78.86±0.99	92.83±1.42
	0.2	89.29±1.18	–	–	–	–	–
	0.3	85.43±2.50	92.50±1.45	87.00±0	90.00±0.93	73.29±2.83	84.83±2.02
	0.5	80.33±2.66	85.00±1.13	84.17±1.40	80.29±1.46	72.29±2.12	71.67±1.86
	0.8	77.33±2.65	83.00±1.59	85.17±1.33	82.86±1.83	69.43±2.98	71.83±2.12
	1	76.14±1.87	81.17±1.62	82.00±2.45	84.14±1.40	71.00±2.88	70.00±2.53
	2	67.83±2.23	77.67±0.76	79.83±1.92	87.57±3.58	76.71±2.82	68.33±2.89
	3	67.00±1.54	78.67±2.86	87.33±1.89	87.57±3.05	77.29±3.09	73.50±2.25
	4	70.00±1.59	78.83±2.40	93.33±1.58	98.00±2.04	86.71±2.76	92.83±3.46
	5	76.00±0.85	80.83±3.90	102.17±0.65	99.14±1.92	91.00±1.91	100.33±2.08
B		<i>A. ranunculus</i>	<i>C. sodalis</i>	<i>D. urostriatum</i>	<i>H. acipenserinus</i>	<i>H. thoracatum</i>	
<i>f</i> (kHz)	0.05	100.13±0.95	86.33±0.71	82.63±1.67	92.71±1.21	94.86±0.67	
	0.1	97.75±1.26	83.50±1.48	79.00±1.73	88.17±0.75	92.00±0.85	
	0.3	96.50±1.78	83.67±0.92	72.86±1.10	86.17±0.87	82.00±1.91	
	0.5	93.63±1.87	76.17±1.49	70.75±1.71	83.00±1.81	81.14±1.08	
	0.8	92.63±1.94	78.83±1.22	72.75±2.05	85.71±0.61	77.17±1.60	
	1	90.00±2.10	80.00±1.79	72.38±1.99	87.00±1.59	80.43±1.70	
	2	96.13±2.12	102.50±0.89	88.00±0.85	91.67±0.92	86.83±1.25	
	3	112.25±1.31	109.83±1.01	97.25±0.62	99.00±0.87	97.29±1.55	
	4	121.25±3.22	116.67±1.12	111.00±1.40	104.83±0.83	102.57±1.29	
	5	119.60±2.18	119.83±1.49	115.43±1.02	109.14±1.20	103.50±0.72	

Hearing threshold values (dB) are means ± s.e.m. *f*, frequency.



- Species with free, single swimbladders:
- *Ariopsis seemanni*
 - ▽ *Batrochoglanis raninus*
 - △ *Malapterurus beninensis*
 - ◇ *Pimelodella* sp.
 - *Synodontis schoutedeni*
 - ◇ *Trachelyopterichthys taeniatus*
- Species with bony encapsulated, paired swimbladders:
- × *Ancistrus ranunculus*
 - ▲ *Corydoras sodalis*
 - ◆ *Dianema urostriatum*
 - *Hemiodontichthys acipenserinus*
 - ▼ *Hypoptopoma thoracatum*

Fig. 5. Auditory evoked potential audiograms of the catfish species investigated.

inward along the medial edge, but in contrast to the tripus in *P. nodosus* does not form a thickened ridge but remains hollow at this medial end. Bridge and Haddon did not mention whether further ossicles are present or absent in this species (Bridge and Haddon, 1893). A description of the Weberian ossicles in the auchenipterid *Centromochlus albescens* (probably *Glanidium albescens* in the current nomenclature) given by Chranilov shows the presence of all four ossicles (Chranilov, 1929), while the respective description of *Auchenipterus nigripinnis* by Chardon lacks any information on ossicle number (Chardon, 1968). Based on the present investigation of *T. taeniatus*, we assume that the intercalarium, scaphium and claustrum are missing.

Catfishes with tiny paired bladders have reduced ossicle size and number. The callichthyid catfishes *Corydoras sodalis* and *Dianema urostriatum* have a single pair of ossicles. Coburn and Grubach state that all ossicles except the tripus are reduced in *C. paleatus* (Coburn and Grubach, 1998), while other authors (e.g. Chranilov, 1929; Chardon, 1968) suppose the scaphium and tripus to be fused in callichthyids. Comparing the shapes of these two parts in *D. urostriatum* with those in the other groups indicates that the caudal part was apparently a tripus and the cranial part resembled a scaphium (concha scaphium). This supports the theory of Chranilov, Chardon and others that scaphium and tripus fused into a single ossicle in callichthyid catfish. However, three Weberian ossicles were found in some loricariids and astroplebids according to Schaefer (Schaefer, 1990) as well as in certain sisorids (Mahajan, 1967).

As expected, *H. acipenserinus* had the smallest ossicles and bladders compared to body length, clearly due to the slender and elongate body shape of the loricariid subfamily Loricariinae.

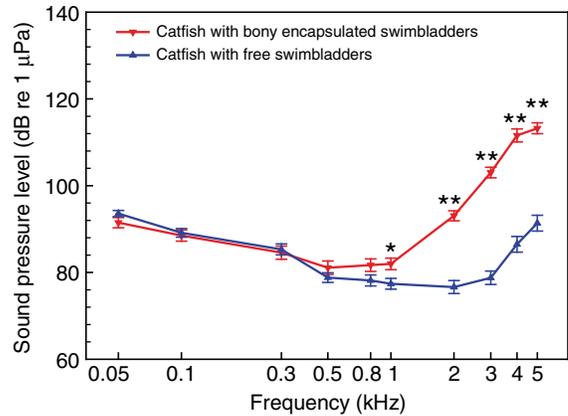


Fig. 6. Mean hearing thresholds (\pm s.e.m.) of all species with free swimbladders (blue) and paired, bony encapsulated bladders (red). Asterisks indicate statistically significant differences (Mann–Whitney *U*-test; for details see text). 1 kHz: * $P < 0.05$, $N = 75$; 2–5 kHz: ** $P < 0.001$, $N = 70–74$.

Auditory sensitivities

The sensitivities described in the present study differ only slightly from the findings of Ladich, who detected sensitivity maxima between 300 and 1000 Hz in all catfish species tested and a flat curve in species with free swimbladders (Ladich, 1999). Four out of six species with free swimbladders tested in the present study exhibited lowest hearing thresholds at higher frequencies, at 2 kHz (*B. raninus*, 77.7 dB; *M. beninensis*, 79.8 dB; *T. taeniatus*, 68.33 dB). The lowest thresholds of *A. seemanni* occurred at 3 kHz (67 dB), which was the best value among all species we examined. Moreover, catfishes with free bladders showed rather flat hearing curves, similar to those in *Pimelodus blochii*, *Pimelodus pictus* and *Platydoras costatus* tested previously (Ladich, 1999). *Batrochoglanis raninus* exhibited a difference of only 7 dB across its hearing range from 500 to 5000 Hz.

The audiogram of the ariid catfish *Ariopsis seemanni* used in the current study differs from that in the sea catfish *Ariopsis* (formerly *Arius felis*) (Popper and Tavolga, 1981). Although we also observed a large utricular otolith as described previously in *Ariopsis*, the hearing curve reveals no particularly well-developed low-frequency hearing ability. Popper and Tavolga found lowest hearing thresholds at 200 Hz and no response above 1 kHz in behavioural tests (Popper and Tavolga, 1981). Our data on *A. seemanni* show very good hearing ability at several kilohertz. The well-developed low frequency sensitivity in *A. felis* could be an adaptation for the primitive echolocation abilities of this species (Tavolga, 1976). Another explanation might be that the previous study used behavioural techniques, which occasionally yield lower thresholds at lower frequencies (Kenyon et al., 1998).

Our results in catfish species with tiny and bony encapsulated bladders are similar to those obtained previously (Ladich, 1999). Both *Corydoras* species tested showed a decreased sensitivity at higher frequencies. On the other hand, the callichthyid *Dianema urostriatum* (encapsulated bladder) and *Synodontis schoutedeni* (free bladder) showed the best hearing ability of all species tested in the 50–300 Hz range. Therefore, encapsulation of the swim bladder may not affect hearing at lower frequencies. The loricariid *Hypoptopoma thoracatum* has the best hearing ability of all species with reduced and bony encapsulated bladders at the highest frequencies tested (4 and 5 kHz). In loricariids the capsules of the swimbladders and the pterotic-supracleithrum have pores. These pores are particularly

large in the ear-grid plecocs [subfamily Hypoptopomatinae (Schaefer, 1987)] and they probably facilitate sound transmission to the inner ear and might explain their – compared with other loricariids – enhanced hearing ability.

Relationship between accessory hearing structures and auditory sensitivity

As hearing specialists, otophysans exhibit a large diversity in hearing sensitivity (Ladich, 1999; Ladich and Bass, 2003). These differences may be based on various reductions of the Weberian system and swimbladders, but clear relationships had not been worked out. The present study clearly shows that reducing the size of air-filled bladders and reduced Weberian ossicles negatively affect hearing sensitivity, particularly at higher frequencies.

This relationship between larger swimbladders and better hearing at higher frequencies agrees with the work of Kleerekopper and Roggenkamp (Kleerekopper and Roggenkamp, 1959) and with that of Ladich and Wysocki, whose elimination studies showed that large swimbladders improve hearing especially at higher frequencies in catfish and goldfish (Ladich and Wysocki, 2003). Bilateral extirpation of the tripus in goldfish caused an increasing hearing loss at higher frequencies, ranging from 7 dB at 100 Hz to 33 dB at 2 kHz.

The basic factors improving hearing abilities at higher frequencies in ‘free bladder’ catfishes are mostly unknown. Alexander analysed the physics of free swimbladders and concluded that amplitudes of wall movements increase with swimbladder size (Alexander, 1966). Fay and Popper, recording microphonic potentials in a standing wave tube, calculated that the auditory system of the goldfish gains with increasing frequency due to the impedance transform characteristics of the fish’s accessory hearing structures (Fay and Popper, 1974). This agrees with our observation that the hearing sensitivity in catfishes with free bladders increases at higher frequencies. Alexander furthermore assumed that the encapsulation of the tiny bladders helps hold the surrounding tissue clear of the moving part of the bladder wall. This would allow larger oscillations of the wall because there is some free fluid-filled space between the bladder and the bony capsule. Therefore, the bony encapsulation might compensate for the loss in hearing sensitivity to some degree in groups with tiny swimbladders.

Our results revealed that the size and number of Weberian ossicles further influence hearing sensitivity. Obviously, larger and more ossicles transmit vibrations from the swimbladder to the inner ear more efficiently and improve hearing, in particular at higher frequencies. Improvement of the mechanical linkages between ossicles, the moveabilities of ossicles, the linkages to the swimbladder and leverages between ossicles might be responsible for this enhanced sound transmission. This correlation seems to be analogous to that found in terrestrial vertebrates. Mammals, which possess three middle ear ossicles (which are phylogenetically not related to Weberian ossicles), can detect much higher sound frequencies than frogs, reptiles and birds, which have just one ossicle (Fay, 1988). These results do not support prior assumptions. Chranilov (Chranilov, 1929) assumed that modifications such as swimbladder encapsulation and a reduction in the number of ossicles increase the general sensitivity of the Weberian apparatus.

Besides the size of the accessory hearing structures, a better linkage of oscillating bladders to the ear (i.e. a shorter distance between the two) is beneficial for hearing at higher frequencies. Examination of hearing thresholds in non-related squirrelfish (family Holocentridae) showed that hearing sensitivities varied between genera (Coombs and Popper, 1979; Hawkins, 1993). Squirrelfish of the genus *Myripristis* have a long anterior extension of the

swimbladder that directly couples the swimbladder to the otic bulla. *Myripristis* shows one of the best hearing abilities among fish, similar to that of carp (Hawkins and Myrberg, 1983). In contrast, the hearing sensitivity in *Adioryx* is 50 dB lower because they have no bladder–skull connection. *Holocentrus*, on the other hand, has an intermediate status and an intermediate sensitivity (Tavolga and Wodinsky, 1963). Atlantic sciaenids have a large variation in swimbladders and their relationship to the otic region (Ramcharitar and Popper, 2004). In the black drum *Pogonias chromis* and the spot *Leiostomus xanthurus*, the swimbladder is relatively far from the otic capsule, while in the Atlantic croaker *Micropogonias undulatus* and the weakfish *Cynoscion regalis*, anteriorly directed bladder–diverticula end close to the otic capsule (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006). Nevertheless, the two groups show no significant difference in hearing thresholds. Closer connection between swimbladders and the inner ear in the weakfish and croaker, however, improved their ability to detect higher frequency sound. Similarly, gouramis (Anabantoidei) and mormyrids show enhanced hearing due to gas-filled chambers in close proximity to the inner ear (Yan, 1998; Yan and Curtsinger, 2000). These observations support the general idea that a close connection of gas-filled chambers to the inner ear improves hearing at higher frequencies (Popper and Tavolga, 1981; Fay, 1988; Ladich and Popper, 2004).

Significance of swimbladder reductions

The fact that bottom-dwelling otophysines (Siluriformes as well as the examined Cypriniformes) have variously reduced swimbladders but never lack bladders indicates that this group of bony fishes has reached a compromise between reducing the buoyancy of an air-filled cavity within the body and keeping the auditory function of these air-filled cavities. The extremely small, paired bubbles directly behind or even within the head of loricariids and callichthyids have clearly lost most of their hydrostatic function and can no longer be called ‘swimbladders’. Despite their tiny size and thus tiny surface, the oscillations of the bladder walls in the sound field are still sufficient to enhance the hearing of these species far beyond that of hearing generalists. Retaining the auditory sensitivity for various purposes such as analysing the auditory scene for predators, prey or communicating conspecifics is the most likely explanation for why bottom-dwelling otophysans such as catfishes, which evolved and live in low ambient noise environments, never reduced their swimbladders, contrary to other bottom-dwelling teleosts belonging to hearing generalists.

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