

How does tripus extirpation affect auditory sensitivity in goldfish?

Friedrich Ladich *, Lidia Eva Wysocki

Institute of Zoology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Received 21 April 2003; accepted 27 May 2003

Abstract

Otophysine fishes are characterized by Weberian ossicles connecting the swimbladder to the ear acoustically. In order to determine the degree to which these ossicles contribute to auditory sensitivity, the tripus was unilaterally or bilaterally extirpated in goldfish and hearing thresholds determined. The auditory evoked potential (AEP) recording technique was used to measure auditory sensitivity between 100 and 4000 Hz. Bilateral extirpation resulted in a hearing loss at all frequencies ranging from 7 dB at 100 Hz to 33 dB at 2 kHz; no AEPs were detectable at 4 kHz. In contrast to bilateral extirpation, unilateral tripus removal caused no sensitivity change. Pre-exposure to intense white noise caused different threshold shifts in unilaterally versus bilaterally extirpated goldfish. Thresholds increased at all frequencies in unilaterally extirpated goldfish but only at 100 and 200 Hz after bilateral extirpation. The comparison between the hearing generalist *Neolamprologus brichardi* (family Cichlidae) and the tripus-extirpated otophysine revealed that the latter is still more sensitive than the cichlid. Higher sensitivity in the goldfish after bilateral extirpation as compared to swimbladder elimination indicates that swimbladder oscillations might partly be transmitted to the inner ear independently of the ossicular chain. This suggests that the auditory system in otophysines improves with increasing frequency due to a more efficient connection between the swimbladder and inner ear ensured by the Weberian ossicles.

© 2003 Elsevier B.V. All rights reserved.

Key words: Auditory sensitivity; Weberian ossicle; Hearing loss; Accessory hearing structure; Auditory evoked potential; Noise exposure

1. Introduction

Fishes have a diversity of hearing abilities with regard to frequency range and auditory thresholds (von Frisch, 1936; Hawkins and Myrberg, 1983; Fay, 1988). Hearing generalists (non-specialists) such as cichlids, salmonids, sunfishes and toadfishes are able to detect the kinetic component of low frequency sounds (< 1 kHz) at relatively high sound intensities. Hearing specialists are characterized by accessory hearing structures (specializations) such as the Weberian apparatus in otophysines (Weber, 1820; Bridge and Haddon, 1889, 1892; Chranilov, 1927, 1929; Alexander, 1962; Char-

don, 1968; Rosen and Greenwood, 1970), suprabran- chial chambers in labyrinth fishes (Bader, 1937; Schneider, 1941; Yan, 1998) or the auditory bullae in mormyrids (Stipetić, 1939; Fletcher and Crawford, 2001). Common to all hearing specializations are air-filled cavities, which are acoustically advantageous for sound detection (and also sound production) under- water. Membranes in an acoustic field can move more freely with higher amplitudes when surrounded by air (such as the tympanum in terrestrial vertebrates and orthopteran insects) than membranes totally enclosed by fluids. This is particularly important at higher fre- quencies, where particle displacement declines rapidly. Essentially, the swimbladder and other gas-filled cav- ities are pressure-to-displacement transformers, which transform the high sound pressure underwater into membrane motion (von Frisch, 1938; van Bergeijk, 1967; Sand and Enger, 1973). Oscillations of their walls are transmitted in various ways to the inner ear, result- ing in sound pressure detection, a broadening of the

* Corresponding author. Tel.: +43 (1) 4277 54227; Fax: +43 (1) 4277 9544.

E-mail address: friedrich.ladich@univie.ac.at (F. Ladich).

Abbreviations: AEP, auditory evoked potentials; SPL, sound pressure level

frequency range (up to several kHz) and an increase in hearing sensitivity (down to 50–60 dB re 1 μ Pa) (Hawkins and Myrberg, 1983; Ladich and Yan, 1998; Ladich, 1999, 2000).

In contrast to all other hearing specialists the endolymph of the inner ear in otophysines is not directly stimulated by the wall of the gas-filled space or intervening tissue but indirectly via moveable bones (one to four Weberian ossicles – tripus, intercalarium, scaphium, claustrum). Thus, hearing enhancement could be due to the swimbladder, the Weberian ossicles, as well as to differences in the inner ear anatomy or physiology (von Frisch, 1936; Fay and Edds-Walton, 1997) or to various combinations of these. Removing or deflating the swimbladder, an approach applied by several investigators (von Frisch and Stetter, 1932; Kleerekoper and Roggenkamp, 1959; Fay and Popper, 1974, 1975; Laming and Morrow, 1981; Yan et al., 2000), therefore reveals nothing about ossicle function. Theoretically, the high sensitivity in otophysines could be due to the swimbladder, with only a minor contribution by the ossicles. Previously, only Poggendorf (1952) extirpated tripodes, namely in the catfish *Ictalurus nebulosus*. Utilizing a positive food reward method and playing back sound from an underwater speaker, he observed a constant, not frequency-dependent decrease in auditory sensitivity of 30–40 dB.

We pursue three goals in our study of Weberian ossicle function. First, we investigate their influence on hearing sensitivity by removing both tripodes in the goldfish *Carassius auratus*, a representative of an otophysine order (Cypriniformes) not investigated before. This is accompanied by a short anatomical description that compares the size of the tripus to the other Weberian ossicles. Data gained in the cypriniform will be compared to the siluriform *I. nebulosus* (Poggendorf, 1952), and to swimbladder elimination experiments in the goldfish, in particular to a study utilizing the same method as we did in determining hearing sensitivity (Yan et al., 2000).

Second, we investigate the effect of unilateral versus bilateral tripus extirpation because anatomical considerations led us to expect different effects on hearing. After unilateral extirpation, oscillations of the unpaired swimbladder could be transmitted via one set of ossicles to the unpaired perilymphatic sinus and from there via the transverse canal to both ears. This pathway would be lost after interruption of both chains of ossicles. Possible differences between unilaterally and bilaterally operated goldfish will be examined by exposing both groups to noise. Previous studies showed that the auditory sensitivity of hearing specialists such as cyprinids and catfish is affected by exposure to intense noise (142 dB re 1 μ Pa: Scholik and Yan, 2000; 158 dB: Amoser and Ladich, 2003), whereas a noise level of 142 dB did

not affect a fish lacking accessory hearing structures (hearing generalist) (Scholik and Yan, 2002).

Third, we determine the sensitivity of tripus-extirpated animals versus hearing generalists possessing a swimbladder. For this comparison we chose a cichlid, the Princess of Burundi *Neolamprologus brichardi*, because earlier studies revealed poor hearing sensitivity among members of the cichlid family and no influence of the swimbladder on hearing (Tavolga, 1974; Fay, 1988; Fay and Popper, 1975; Kenyon et al., 1998). Theoretically, differences in sensitivity might still exist because the otophysine inner ear differs anatomically and physiologically from that of generalists (von Frisch, 1938; Fay and Edds-Walton, 1997; Popper and Fay, 1999). Furthermore, swimbladders can affect hearing even in the absence of a direct anatomical connection to the inner ear (e.g. in pomacentrids and gadids; Sand and Enger, 1973; Myrberg and Spires, 1980).

2. Material and methods

2.1. Animals

Fifteen specimens of the goldfish *C. auratus* (80–102 mm standard length, 16.6–35.7 g body weight) from a pond near Vienna, and six laboratory-reared Princess of Burundi *N. brichardi* were chosen (52–57 mm, 3.8–6.1 g) for this study. All animals were maintained in externally filtered aquaria equipped with plants and half flowerpots as hiding places, and sand. A 12:12 h light:dark cycle was maintained, and temperatures were kept at $25 \pm 1^\circ\text{C}$ in the laboratory. Fish were fed commercially prepared food (TetraMin[®] and TetraPond[®]) and various living foods such as *Tubifex* spp.

2.2. Auditory sensitivity measurements

The auditory sensitivity was measured using the auditory evoked potential (AEP) recording technique, following the recording protocol recently described by Ladich (1999), Kratochvil and Ladich (2000) and Wysocki and Ladich (2001, 2002).

Test subjects were positioned below the water surface (except for the contacting points of the electrodes, which were maximally 1 mm above the surface) in the center of a half-bowl shaped 11-l plastic tub (37 cm diameter, 8 cm depth, 1 cm of fine sand). This tub was lined inside with acoustically absorbent material (closed cell foam) in order to reduce resonances and reflections (see fig. 1 in Wysocki and Ladich, 2002). Animals were immobilized by galamine triethiodide (Flaxedil), with a required dosage of 0.5–1 $\mu\text{g/g}$ for *C. auratus* and 1–2.6 $\mu\text{g/g}$ for *N. brichardi*.

Both sound stimulus presentation and auditory evoked potentials (AEP) waveform recording were accomplished using a Tucker-Davis Technologies (Gainesville, FL, USA) modular rack-mount system (TDT System II and TDT System 3) controlled by Pentium PCs containing a TDT digital processing board and running TDT BioSig 2.2 or BioSigRP Software.

Sound stimuli consisted of tone bursts which were presented at a rate of 41 s^{-1} . Animals were tested at frequencies of 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0 and 4.0 kHz in random order. The duration of stimuli increased from two cycles at 100 and 200 Hz up to eight cycles at 4 kHz. Rise and fall were one cycle at 100 and 200 Hz and two cycles at all the other frequencies. All bursts were gated using a Blackman window. The maximum sound pressure level (SPL) applied was 136 dB re $1 \mu\text{Pa}$.

Sound stimulus waveforms were constructed using TDT SigGen software and fed through a power amplifier (Alesis RA 300). A dual cone speaker (Tannoy System 600, frequency response 50 Hz–15 kHz ± 3 dB, suspended in air, was mounted 1 m above the test subject. SPLs of stimuli were reduced in 4 dB steps until the AEP waveform disappeared. The lowest SPL which yielded a repeatable AEP trace to the presented tone pulses as determined by overlying replicate traces was considered the threshold (Kenyon et al., 1998). A hydrophone (Brüel and Kjaer 8101, frequency range: 1 Hz–80 kHz ± 2 dB; voltage sensitivity: -184 re $1 \text{ V}/\mu\text{Pa}$) was placed close to the right side of the animals (2 cm apart) in order to determine absolute SPLs underwater in close vicinity of the subjects. Control measurements showed that, in accordance with theoretical expectations (due to increasing distance from the loudspeaker), SPLs decreased with increasing distance from the center of the tub as well as with increasing depth. Our sound pressure-sensitive hydrophone responded exactly to any attenuation in SPL generated by the BioSig software and played back via the air loudspeaker.

Only sound pressure measurements were conducted because in any acoustic field, sound pressure is the adequate measure of the degree of auditory stimulation in goldfish (Fay and Popper, 1974) and because we wanted to compare our data to previous studies. Furthermore, Weeg et al. (2002) recently showed that in the generalist *Porichthys notatus*, sensitivity results gained in response to pressure stimuli are consistent with responses to whole-body displacement mimicking acoustic particle motion.

2.3. *Tripus* extirpation

Animals were mildly anesthetized in a solution of tricaine methane sulfonate (0.3 g/l MS 222) until the

respiratory movements ceased. Behind the opercle an incision was made in anterior–posterior direction through the skin and trunk musculature immediately dorsal to the lateral line nerve (Fig. 1A). The incision was spread open with forceps and the tripus carefully removed. Usually the connections of the tripus to the swimbladder and intercalarium detached readily; in a few cases the intercalarium was removed together with the tripus. The incision was sealed with a protective layer of parafilm and secured with Vetbond (3M, St. Paul, MN, USA). The animal was then returned to its home aquarium for recovery. The injuries usually healed within 2 weeks and after that animals were used for remeasurements.

No further examination of the surgical procedure could be performed for several reasons. A closer examination of the swimbladder wall (the insertion point is covered by the ribs – see Section 3) or ossicles would have required a distinctly larger incision; this would have caused a much longer healing process and endangered our study. Moreover, we thought it unnecessary to kill our fish (several weeks after the surgery) because several factors pointed to a minor influence of surgery on the function of the swimbladder and the perilymphatic sinus. First, none of the experimental goldfish died during surgery or anytime afterwards or exhibited aberrant behavior. Second, sham operations or unilateral extirpation had no measurable influence on the hearing sensitivity in our animals. In particular the latter indicates that the swimbladder and perilymphatic sinus remained intact.

In the bilaterally extirpated fish, the two tripodes were removed on consecutive days. In the unilateral control group, only the left tripus was removed. In the sham-operated control group the same surgery was performed, but tripodes were only manipulated and not removed.

2.4. Noise exposure

The tripus-extirpated goldfish were exposed singly to unfiltered white noise at 155 ± 1.8 dB SPL ($x \pm \text{S.D.}$) (range within the bucket: 152–157 dB) re $1 \mu\text{Pa}$ for 12 h in a plastic bucket (20 cm height, 18 cm diameter, 14 cm water depth). SPLs were measured using a sound level meter (Brüel and Kjaer Mediator 2238 – time weighting: root mean square slow; frequency weighting: linear between 20 and 16000 Hz), a power supply (Brüel and Kjaer 2804) and the hydrophone (Brüel and Kjaer 8101). Fish could move freely within the bucket. White noise was generated by a noise generator (IVIE Electronics IE 20B), sent to a 24 band equalizer (Alesis MEQ 230) to obtain as flat a noise spectrum as possible (± 6 dB from 100 to 4500 Hz), and fed to a power amplifier (Brüel and Kjaer 2713) that drove an under-

water loudspeaker (University Sound UW 30) situated on the bottom of the bucket.

After the end of the 12 h noise exposure period, hearing thresholds were tested immediately.

Each measurement session was completed within 3 h.

2.5. Data analysis

The goldfish were divided into three groups, depending on the number of tripodes removed. Three animals were sham-operated, in six animals tripodes were extirpated unilaterally and in six bilaterally. Auditory sensitivities were measured prior to surgery (first and second pre-surgical groups), and after sham operation, tripus extirpation and noise exposure.

Peak-to-peak amplitudes of AEPs were measured in four animals and compared across frequencies and conditions (for details see Wysocki and Ladich, 2002).

Audiograms of the different experimental groups (uni- and bilateral baseline (=pre-surgical) group of goldfish, uni- and bilaterally extirpated, uni- and bilaterally extirpated noise-exposed goldfish, baseline of *N. brichardi*) were compared by two-factor analysis of variance (ANOVA) using a general linear model where one factor was treatment/experimental group and the other was frequency. The treatment/experimental group factor alone should indicate overall differences between differently treated/experimental groups of animals, and in combination with the frequency factor if different tendencies exist at different frequencies of the audiograms.

In order to determine at which frequency thresholds differ, paired (in the case of within-goldfish comparisons) or unpaired (in the case of goldfish versus cichlid comparisons) *t*-tests were calculated at each frequency.

3. Results

3.1. Anatomy of Weberian ossicles in *C. auratus*

The goldfish possesses four Weberian ossicles, which are aligned in a dorsorostral–caudal direction (Fig. 1). The caudal tripus, which is by far the largest ossicle, lies medial of the first two ribs. In our goldfish the tripodes were 5.5–6.7 mm long, which is equivalent to approximately two thirds of the distance covered by Weberian ossicles. Tripodes were moon-shaped and had three processes with different functions (Fig. 1C). The posterior process (PP) inserted at the dorsocranial surface of the swimbladder and the dorsal process (PR) articulated with the vertebral column. The anterior process (AP) was connected to the stick-like intercalarium via a small ligament. The intercalarium itself was joined to the scaphium by another small ligament. The scaphia

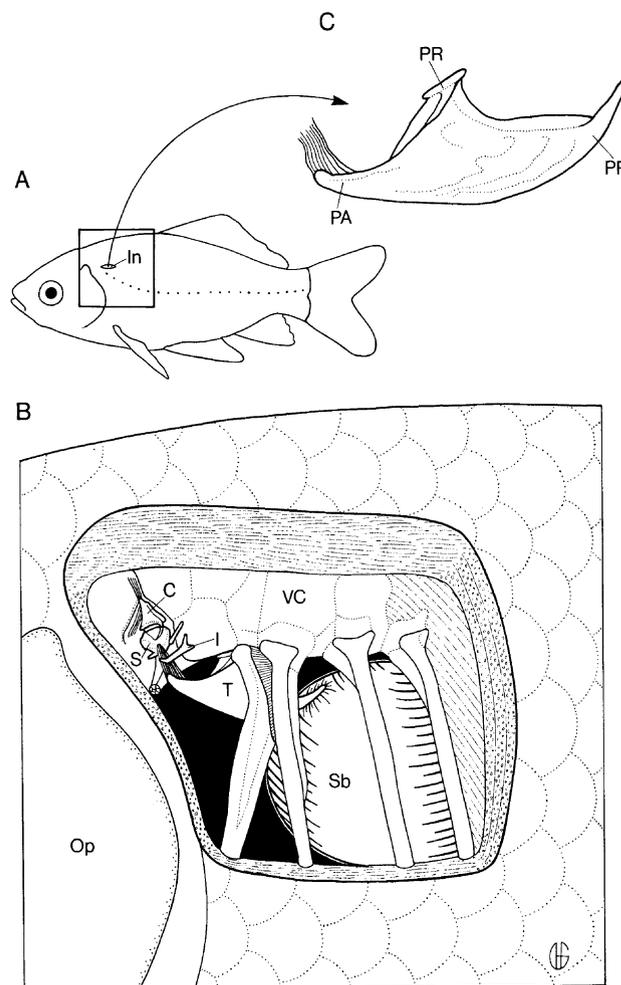


Fig. 1. (A) Line drawing of a lateral view of the goldfish showing the incision (In) for the surgical removal of the tripus. (B) Postcranial region (inset in A) illustrating the position of all four Weberian ossicles (C, claustrum; I, intercalarium; S, scaphium; T, tripus; Op, operculum; VC, vertebral column), anterior ribs and the swimbladder (Sb). (C) Enlarged dorsolateral view of the tripus with an attached cranial ligament. PA, anterior process; PP, posterior process; PR, articulation process. Drawing by H.C. Grillitsch.

were round and, together with the dorsally situated claustrum, formed two shelf-halves which enclosed the perilymphatic extensions.

3.2. AEP waveforms

AEP waveforms in response to 300, 500 and 1000 Hz tone bursts presented at 30 dB above threshold consisted of a series of positive and negative peaks. A much smaller number of peaks was observed at 2000 Hz (Fig. 2). After bilateral tripus removal, peak-to-peak amplitudes of AEPs clearly diminished at all frequencies (Fig. 2). The extirpation decreased maximum amplitudes by 256 nV at 300 Hz and by up to 900 nV at

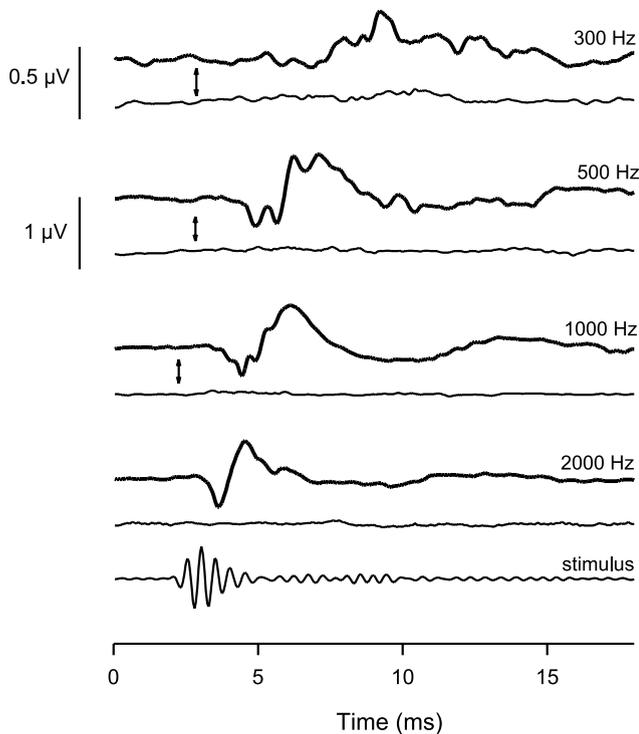


Fig. 2. AEP waveforms obtained at different frequencies 30 dB above hearing threshold in an intact goldfish (bold traces) and at the same SPL after bilateral tripus extirpation in the same individual. Amplitudes of AEPs in response to 300 Hz tone bursts were doubled for better illustration of the waveform. The waveform of the 2000 Hz sound stimulus is given. Arrows indicate approximate onsets of sound stimuli at 300, 500 and 1000 Hz.

2000 Hz (Table 1). The differences in amplitude increased at higher frequencies.

3.3. Auditory sensitivity after bilateral tripus extirpation

Audiograms obtained from sham-operated fish showed no change in hearing thresholds before and after surgery. Therefore, data on sham-operated fish are omitted here.

The baseline audiogram of the goldfish was rather flat between 100 and 1000 Hz, whereas the auditory sensitivity dropped quickly at 2 and 4 kHz. The sensi-

Table 1
Peak-to-peak amplitudes of AEP waveforms (nV) at particular frequencies 30 dB above hearing level obtained in intact goldfish and after extirpation of both tripodes

Frequency (Hz)	Amplitude (nV)		
	Intact	Bilat. ext.	Difference
300	336	80	-256
500	868	62	-806
1000	881	73	-808
2000	976	77	-900

Means of data from four fish and the mean drop in amplitudes are given (see also Fig. 2).

tivity maximum was located between 500 and 1000 Hz (Fig. 3).

Removal of both tripodes dramatically lessened auditory sensitivity at all frequencies, and no response could be detected at 4 kHz, except for one individual (threshold at 4 kHz: 131 dB re 1 μPa). Comparisons between audiograms of the pre-surgical and bilaterally extirpated groups by a two-factor ANOVA revealed significant overall differences ($F_{1,75} = 395.13, P < 0.001$) and a significant interaction between group and frequency ($F_{6,75} = 9.75, P < 0.001$). Thus, changes in auditory sensitivity showed different trends at different frequencies.

The shape of the audiogram after tripus removal was ramp-like, i.e. the sensitivity maximum switched to the lower end of the hearing range. The hearing loss was more pronounced at higher frequencies; it increased from 7.3 dB at 100 Hz to 32.5 dB at 2 kHz (Fig. 3). In accordance with the significant interaction between treatment/group and frequency (two-factor ANOVA), the loss in hearing ability was positively correlated to the frequency tested (Pearson correlation coefficient: $r = 0.99, n = 7, P < 0.01$) (Fig. 4).

3.4. Auditory sensitivity after unilateral tripus extirpation and effects of noise exposure

In contrast to bilateral tripus extirpation, unilateral extirpation did not affect auditory sensitivity. A comparison between the audiogram of the baseline group and unilaterally extirpated fish revealed no significant difference (two-factor ANOVA, $F_{1,79} = 0.105, n.s.$) and no interaction between group and frequency (Fig. 3).

In order to determine whether the auditory sensitivity

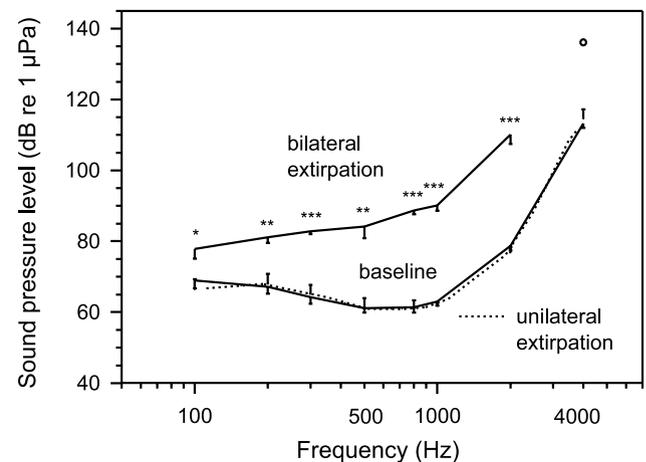


Fig. 3. Audiograms (mean ± S.E.M.) of the pooled baseline data and after bilateral and unilateral tripus extirpation. Significance of the differences at particular frequencies between the bilateral extirpation and the baseline data as calculated by a paired sample *t*-test is given: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars are drawn in only one direction to minimize overlap. Circle indicates the highest SPL tested without evoking an AEP.

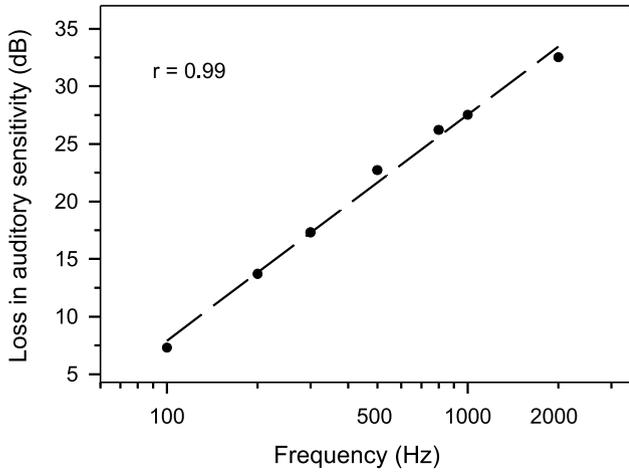


Fig. 4. Relationship between the sound frequency and the hearing loss relative to baseline thresholds after bilateral extirpation of tripodes in the goldfish. Regression equation: hearing loss = \log sound frequency $\times 19.6 - 31.4$, $P < 0.001$.

of unilaterally and bilaterally extirpated goldfish is differently affected by intense noise, all animals were compared before and after noise exposure. While the unilateral extirpation per se did not influence hearing sensitivity as mentioned previously, noise exposure significantly shifted the overall hearing curve (two-way ANOVA: $F_{1,80} = 92.76$, $P < 0.001$). The decrease in sensitivity was significant at all frequencies and ranged from 8.5 dB at 4 kHz to 24.9 dB at 500 Hz (Fig. 5).

In goldfish that lacked both tripodes, intense noise exposure revealed a quite different pattern of threshold change. The change in auditory sensitivity ranged from 10.2 dB at the lowest frequency tested to less than 2 dB

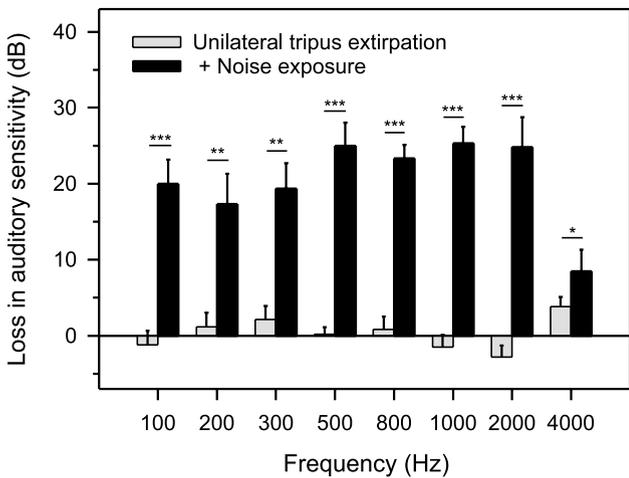


Fig. 5. Loss in auditory sensitivity (mean \pm S.E.M.) relative to pre-surgical thresholds after extirpation of one tripus and after noise exposure. Differences in hearing thresholds at particular frequencies after the bilateral extirpation and the additional intense noise exposure were calculated by a paired sample t -test: * $P < .05$, ** $P < 0.01$, *** $P < 0.001$.

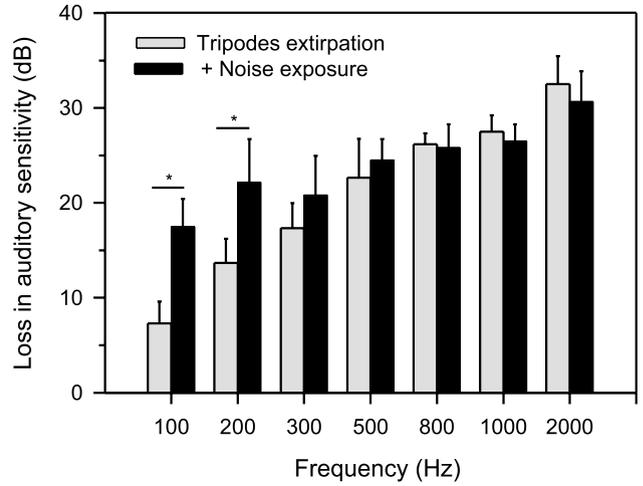


Fig. 6. Loss in auditory sensitivity (mean \pm S.E.M.) after extirpation of both tripodes and after intense noise exposure. Differences in hearing thresholds at particular frequencies after the bilateral extirpation and the additional noise exposure were calculated by a paired sample t -test: * $P < 0.05$.

above 800 Hz. The decrease was only significant at 100 Hz and 200 Hz (Fig. 6).

3.5. Tripus extirpation versus lack of specialization

How does the surgical disconnection of the swimbladder from the inner ear in the goldfish compare to the lack of a specialization in a hearing non-specialist? Bilaterally extirpated goldfish still possessed a higher auditory sensitivity than the cichlid (two-way ANOVA: $F_{1,70} = 179.1$, $P < 0.001$; Fig. 7), while the sensitivity difference varied at different frequencies ($F_{6,70} = 7.23$, $P < 0.001$). Hearing was significantly better at all fre-

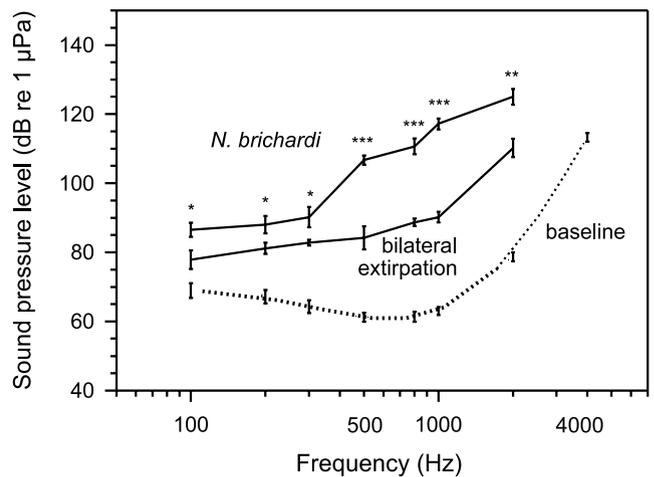


Fig. 7. Audiograms (mean \pm S.E.M.) of the goldfish after bilateral tripus extirpation as compared to the cichlid *N. brichardi*. Significance of the differences at particular frequencies between the bilateral extirpation and the cichlid thresholds calculated by an unpaired t -test are given: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

quencies and differences in thresholds ranged from 6.8 dB at 300 Hz to 27 dB at 1000 Hz. The sensitivity difference was more pronounced between 500 Hz and 2 kHz than between 100 and 300 Hz (unpaired *t*-test: $t = -4.62$, $df = 5$, $P < 0.01$). No AEPs could be detected at the highest frequency tested in bilaterally extirpated goldfish or in cichlids (4 kHz).

4. Discussion

4.1. Effects of tripus extirpation versus swimbladder manipulation in otophysines

To our knowledge only two studies (Poggendorf, 1952, and the present one) have investigated the effects of Weberian ossicles on hearing in otophysines. Both revealed a clear decrease in auditory sensitivity at all frequencies tested and thus the importance of these ossicles for sound transmission. However, whereas Poggendorf observed a rather similar shift in hearing thresholds of about 30 and 40 dB between 60 and 6000 Hz in the catfish, our threshold change was clearly frequency-dependent, increasing from 7 dB at 100 Hz to 32 dB at 2 kHz. This difference might be due to different methodological approaches or species tested. Anatomical differences are unlikely to be responsible for this result because *I. nebulosus* possesses a well-developed swimbladder and the full set of Weberian ossicles including a prominent tripus (Chardon, 1968). A reduction of swimbladders and ossicles and possible effects on hearing have been described in other siluriforms, in particular callichthyids, which possess only one ossicle (Chrnilov, 1929; Jenkins, 1977; Ladich, 1999).

Theoretically, tripus extirpation should eliminate the contact between the swimbladder and the inner ear and thus result in a loss of pressure sensitivity. Audiograms determined in our and Poggendorf's study would then reflect responses to the acoustic particle motion. Differences in hearing loss in the catfish and goldfish (e.g. no frequency dependence in the catfish) might then be explained by differences in tank acoustics in particular when using loudspeakers in different media. Thus, Poggendorf utilized a behavioral method and played back sounds from an underwater speaker below the animals, whereas we measured AEPs in response to sound stimuli played back in air 1 m above the water. According to Enger (1966), stimulation with an air speaker can simulate a far field, whereas close to an underwater sound source the near-field water displacement is considerable and sound pressure thresholds are dependent on the distance to the speaker.

Because the auditory sensitivity in the extirpated catfish was independent of water depth, Poggendorf concluded that catfish were still pressure-sensitive. This in-

dicates that the swimbladder plays some role in sound detection in catfish lacking a Weberian connection. An influence of swimbladders on hearing was also mentioned for gadids and pomacentrids, families having no known accessory hearing structure. Sand and Enger (1973) described pressure sensitivity in the cod *Gadus morhua* and argued that the ear responds to displacements re-radiated from the swimbladder. In the damselfish *Eupomacentrus partitus*, sound pressure controls sensitivity at frequencies above 200 Hz, while particle motion was the relevant stimulus below that frequency (Myrberg and Spires, 1980).

If, in our study, goldfish respond only to acoustic particle motion after tripus extirpation, then thresholds should be similar to swimbladder elimination experiments under comparable acoustic conditions. Swimbladder deflation in *C. auratus* by removal of the gas resulted in a much larger hearing loss (33–55 dB; Yan et al., 2000) than tripus elimination. The additional hearing loss ranges from approximately 30 dB at 300 Hz to about 20 dB at 2 kHz. This comparison is interesting because the present study and Yan et al. (2000) utilized the same AEP recording technique, each yielding quite similar pre-surgical audiograms. The pronounced hearing loss shown after swimbladder deflation can probably be explained by a complete cessation of swimbladder wall oscillations and thus no stimulation of the perilymphatic sinus even in the presence of Weberian ossicles.

Despite the different methodological approaches in *I. nebulosus* and *C. auratus*, both studies indicate that tripus elimination does not totally eliminate the acoustic function of the swimbladder. Thus, Weberian ossicles are only partly responsible for the total hearing enhancement in otophysines. One explanation is that swimbladder wall oscillations are picked up by the surrounding tissue and then transmitted to cranial ossicles (intercalaria, scaphia, claustra) and/or the perilymphatic sinus. The perilymphatic sinus can be regarded as a posterior extension of the inner ears, functionally similar to the anterior extension of swimbladders in holocentrids and clupeids (Blaxter et al., 1981; Hawkins, 1993). Thus, Weberian ossicle removal apparently does not result in a complete switch from the sound pressure detection mode of hearing via the swimbladder to particle motion detection.

4.2. Is the enhancement of hearing by air-filled cavities frequency-dependent?

Experiments eliminating air-filled cavities in otophysines and non-otophysines resulted in a diversity of hearing losses but do not show unequivocally that the increase in auditory sensitivity is frequency-dependent. Theoretically, the effect of the 'tympanum'-like struc-

tures in hearing specialists should increase with increasing frequency, with almost no effect at lower frequencies. [Fay and Popper \(1974\)](#), 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. After swimbladder removal, the sensitivity declined by 20–35 dB, with a smaller drop below 100 Hz. Note, however, that [Yan et al. \(2000\)](#) observed no frequency-dependent increase after swimbladder deflation. A loss in sensitivity was found in the minnow by [von Frisch and Stetter \(1932\)](#) and in the roach by [Laming and Morrow \(1981\)](#) using quite different techniques. [Laming and Morrow \(1981\)](#) observed a greater decline of the AEP amplitudes at higher frequencies after extracting the gas from the swimbladder. [Kleerekoper and Roggenkamp \(1959\)](#) found an increasing drop of sensitivity after gas extraction in *I. nebulosus*, ranging from 9 dB at 210 Hz up to 35 dB at 1840 Hz (correlation coefficient calculated from their data: $r=0.96$).

An unusual form of accessory hearing structure has been described in cobitids. In these cypriniforms the anterior part of the swimbladder is encapsulated by bone to varying extents ([Kratochvil and Ladich, 2000](#)). This, however, does not diminish the auditory sensitivity as compared to other otophysines ([Ladich, 1999](#)), perhaps because special channels stretch laterally from the swimbladder to the outer body wall. Filling these lateral trunk channels (not related to lateral line canals) with damping material resulted in a loss of sensitivity of 14–18 dB ([Kratochvil and Ladich, 2000](#)). The experiments demonstrated that the intact lateral trunk channels enhance the hearing sensitivity of cobitid fishes by approximately the same degree.

Interestingly, strong correlations between the sound frequency and sensitivity loss after extirpation of accessory hearing structures have so far only been described by [Kleerekoper and Roggenkamp \(1959\)](#) in a catfish and in the present study on a cyprinid. A hearing loss at higher frequencies was mentioned by [von Frisch and Stetter \(1932\)](#) in the minnow and in the present work, although this could be due to difficulties in presenting intense sound stimuli.

The current data on otophysines do not reveal whether the diversity in hearing losses is due to different methodological approaches such as differences in surgery (deflation versus removal), threshold determinations (behavioral versus electrophysiological techniques), sound projectors (underwater versus air), tank acoustics et cetera.

Non-otophysine hearing specialists add additional variability. An early study on anabantoids by [Schneider \(1941\)](#) showed that the upper hearing range dropped by more than two octaves in the paradise fish *Macropodus*

opercularis (from 4.5 kHz down to 800 Hz) when the suprabranchial organ was filled with water. [Yan \(1998\)](#) found the greatest decline of hearing sensitivity at 500 and 800 Hz, the most sensitive frequencies, again with a smaller decline at higher frequencies. Similar results were described in mormyrids, where [Yan and Curtsinger \(2000\)](#) and [Fletcher and Crawford \(2001\)](#) replaced the gas of the tympanic bladders with physiological saline. Threshold differences were maximal at mid-audiogram frequencies (400–800 Hz), with smaller threshold shifts at extreme frequencies (100 Hz and 2–4 kHz).

In summary, there is no clear picture of which frequencies are most affected by eliminating accessory hearing structures. Whereas several investigations found a frequency-dependent effect and in two cases strong positive correlations (between hearing loss and frequency tested), others revealed no such dependence or a maximum effect at low and middle frequencies.

4.3. Unilateral versus bilateral elimination

Most hearing specializations are developed bilaterally, making it instructive to study the effects of unilateral versus bilateral elimination. In otophysines the anatomical situation suggests different outcomes of such experiments. As we expected, the unilateral extirpation of the tripus had no detectable effects on hearing thresholds in goldfish, in contrast to bilateral extirpation. Our data showed that oscillations of the unpaired swimbladder in the sound field can be transmitted via one set of ossicles to the unpaired perilymphatic sinus and from there to the transverse canal. Subsequently, endolymph movements within this unpaired canal are conducted to both ears. We therefore assume that both ears in our goldfish are stimulated after unilateral tripus extirpation, similar to intact fish. In bilaterally operated goldfish, however, the swimbladder vibrations are only minimally transmitted to the ears.

In mormyrids the anatomical situation is quite different but the results are similar. Filling the auditory bullae of mormyrids on one side changed auditory sensitivity only insignificantly ([Yan and Curtsinger, 2000](#); [Fletcher and Crawford, 2001](#)). One ear is clearly sufficient to maintain auditory sensitivity.

In summary, unilateral extirpation does not deteriorate hearing thresholds in either family. This, however, does not rule out an effect on other hearing characteristics such as time, frequency or intensity discrimination.

4.4. Effect of noise on unilaterally and bilaterally extirpated goldfish

Noise of a particular intensity affects the auditory system of hearing specialists more than that of non-

specialists. Exposure to white noise of 142 dB shifted thresholds in the minnow *Pimephales promelas* but not in the bluegill sunfish *Lepomis gibbosus* (Scholik and Yan, 2000, 2002) whose baseline hearing thresholds are close to the noise level. Amoser and Ladich (2003) demonstrated an auditory sensitivity loss of up to 26 dB in *C. auratus* and 32 dB in the catfish *Pimelodus pictus* immediately after white noise exposure (approx. 159 dB SPL), with the greatest hearing loss in the range of their most sensitive frequencies. The present study revealed that in unilaterally extirpated goldfish, hearing sensitivity dropped similarly to intact fish. The hearing loss in intact fish ranged from 14 to about 23 dB after 12 h noise exposure (Amoser and Ladich, 2003). In tripus-less animals, the respective values were 10.2 and 8.5 dB at 100 and 200 Hz. No loss occurred at higher frequencies at the same white noise level. This might be explained by the fact that the extirpation effect on auditory sensitivity is small at lower frequencies and gradually increases with increasing frequency, whereas the noise exposure affects hearing abilities at all frequencies more evenly in goldfish.

In summary, unilaterally and bilaterally extirpated goldfish differ in their response to noise exposure. The former responded similarly to intact fish, the latter showed changes only in the lower end of the hearing range.

4.5. Comparison between tripus extirpation and lack of specialization

Do otophysines become hearing generalists after tripus extirpation? In fishes lacking accessory hearing structures (non-specialists or generalists), air-filled cavities do not influence hearing. Fay and Popper (1975) showed that swimbladder removal in the cichlid *Tilapia macrocephala* does not affect the response to underwater sound stimuli. A comparison between the audiogram in the bilaterally extirpated goldfish and the cichlid *N. brichardi* revealed that the otophysine is still clearly more sensitive at all frequencies with regard to the same sound stimuli. Although sound pressure audiograms of generalists should be interpreted with caution, in particular those generated in the near field of an underwater sound source (see Section 4.1 and Enger, 1966), a similar conclusion can be drawn when comparing the (sound pressure) audiogram of extirpated goldfish to numerous other generalists from non-related families such as the toadfish *Opsanus tau*, the salmonid *Salmo salar* or the percid *Acerina cernua* (Wolff, 1968; Hawkins and Johnstone, 1978; Fish and Offutt, 1972).

The better hearing sensitivity in the tripus-extirpated goldfish as compared to generalists is most likely due to swimbladder vibrations picked up by the inner ear in a

non-ossicular way as in gadids and pomacentrids (Sand and Enger, 1973; Myrberg and Spires, 1980) and by additional anatomical and physiological differences. Otophysines possess, besides Weberian ossicles, a number of structural adaptations which facilitate transmission of oscillations from caudal regions of the body to the ear. The saccular otolith is stick-like and possesses flutes. In our opinion the flutes, which are located near the opening of the canalis transversus, register movements of the endolymph within this channel (von Frisch, 1936). The transverse channel connects both saccules to each other and touches the unpaired perilymphatic sinus, which reaches caudally to the cranial Weberian ossicles. Physiological differences have been described between generalists and specialists in the organization of the saccular sensory epithelium (macula) and frequency–response properties of the saccular nerve (Ladich and Bass, 2003). The goldfish saccular macula is crudely tonotopically organized with high frequency afferents originating primarily from the rostral region (Furukawa and Ishii, 1967). There is no evidence that the macula of the generalist *O. tau* is tonotopically organized. Furthermore, saccular afferents in the toadfish *O. tau* encode only low frequencies between 50 and 250 Hz, whereas in the goldfish, afferents had their best frequencies at up to 900 Hz (Fay and Edds-Walton, 1997; Popper and Fay, 1999).

5. Conclusion

Elimination of the Weberian connection by extirpation of both tripodes decreases auditory sensitivity in one cypriniform and – according to a prior study – one siluriform at all frequencies investigated. It is unclear whether the goldfish and catfish exhibit different degrees of hearing loss at different frequencies or whether the observed diversity is due to different methodological approaches. There are strong indications that both otophysine species studied utilized the swimbladder for sound detection after the extirpation. Unilateral tripus extirpation does not affect the acoustic linkage between the swimbladder and the inner ear.

Acknowledgements

We would like to thank Heidemarie Grillitsch for the line drawings in Fig. 1, Werner Timischl for statistical advice, Heinz Tunner for providing goldfish, and the native English speaker Michael Stachowitsch for proof-reading and correcting our English. The animal use protocol used in this study was approved by the Austrian Commission on Experiments in Animals (GZ 68.210/30-Pr/4/2001). This research was supported by

the Austrian Science Fund (FWF Grants 12411 and 15873 to F.L.).

References

- Alexander, R.Mc.N., 1962. The structure of the Weberian apparatus in the cyprini. *Proc. Zool. Soc. Lond.* 139, 451–473.
- Amoser, S., Ladich, F., 2003. Diversity in noise-induced temporary hearing loss in otophysine fishes. *J. Acoust. Soc. Am.* 113, 2170–2179.
- Bader, R., 1937. Bau, Entwicklung und Funktion des akzessorischen Atmungsorgans der Labyrinthfische. *Z. Wiss. Zool. Leipzig* 149, 323–401.
- Blaxter, J.H., Denton, E.J., Gray, J.A.B., 1981. Acousticolateralis system in clupeid fishes. In: Tavolga, W.N., Popper, A.N., Fay, R.R. (Eds.), *Hearing and Sound Communication in Fishes*. Springer, Berlin, pp. 39–56.
- Bridge, T.W., Haddon, A.C., 1889. Contributions to the anatomy of fishes. I. The airbladder and Weberian ossicles in the Siluridae. *Proc. R. Soc. Lond.* 46, 309–328.
- Bridge, T.W., Haddon, A.C., 1892. Contributions to the anatomy of fishes. II. The airbladder and Weberian ossicles in the Siluridae. *Proc. R. Soc. Lond.* 184, 65–324.
- Chardon, M., 1968. Anatomie comparée de l'appareil de Weber et des structures connexes chez les Siluriformes. *Mus. R. Afr. Cent. Tervuren, Belg. Ann. Ser. 8, Sci. Zool.* 169, 1–273.
- Chranilov, N.S., 1927. Beiträge zur Kenntnis des Weber'schen Apparates der Ostariophysi I. Vergleichend-anatomische Übersicht der Knochelemente des Weber'schen Apparates bei Cypriniformes. *Zool. Jb. Anat.* 49, 501–597.
- Chranilov, N.S., 1929. Beiträge zur Kenntnis des Weber'schen Apparates der Ostariophysi: 2. Der Weber'sche Apparat bei Siluroidea. *Zool. Jb. Anat.* 51, 323–462.
- Enger, P.S., 1966. Acoustic threshold in goldfish and its relation to the sound source distance. *Comp. Biochem. Physiol.* 18, 859–868.
- Fay, R.R., 1988. *Hearing in Vertebrates: A Psychophysics Databook*. Hill-Fay, Winnetka, IL.
- Fay, R.R., Edds-Walton, P.L., 1997. Diversity in frequency response properties of saccular afferents of the toadfish, *Opsanus tau*. *Hear. Res.* 13, 235–246.
- Fay, R.R., Popper, A.N., 1974. Acoustic stimulation of the ear of the goldfish (*Carassius auratus*). *J. Exp. Biol.* 61, 243–260.
- Fay, R.R., Popper, A.N., 1975. Modes of stimulation of the teleost ear. *J. Exp. Biol.* 62, 379–387.
- Fish, J.F., Offutt, G.C., 1972. Hearing thresholds from toadfish, *Opsanus tau*, measured in the laboratory and field. *J. Acoust. Soc. Am.* 51, 1318–1321.
- Fletcher, L.B., Crawford, J.D., 2001. Acoustic detection by sound-producing fishes (Mormyridae): the role of gas-filled tympanic bladders. *J. Exp. Biol.* 204, 175–183.
- Furukawa, T., Ishii, Y., 1967. Neurophysiological studies on hearing in goldfish. *J. Neurophysiol.* 30, 1377–1403.
- Hawkins, A.D., 1993. Underwater sound and fish behaviour. In: Pitcher, T.J. (Ed.), *Behaviour of Teleost Fishes*. Chapman and Hall, London, pp. 129–169.
- Hawkins, A.D., Johnstone, A.D.F., 1978. The hearing of the Atlantic salmon, *Salmo salar*. *J. Fish. Biol.* 13, 655–673.
- Hawkins, A.D., Myrberg, A.A., 1983. Hearing and sound communication underwater. In: Lewis, B. (Ed.), *Bioacoustics: A Comparative Approach*. Academic Press, London, pp. 347–405.
- Jenkins, D.B., 1977. A light microscopic study of the sacculle and lagena in certain catfish. *Am. J. Anat.* 150, 605–630.
- Kenyon, T.N., Ladich, F., Yan, H.Y., 1998. A comparative study of hearing ability in fishes: the auditory brainstem response approach. *J. Comp. Physiol. A* 182, 307–318.
- Kleerekoper, H., Roggenkamp, P.A., 1959. An experimental study on the effect of the swimbladder on hearing sensitivity in *Ameiurus nebulosus* (Lesueur). *Can. J. Zool.* 37, 1–8.
- Kratochvil, H., Ladich, F., 2000. Auditory role of lateral trunk channels in cobitid fishes. *J. Comp. Physiol. A* 186, 279–285.
- Ladich, F., 1999. Did auditory sensitivity and vocalization evolve independently in otophysan fishes? *Brain Behav. Evol.* 53, 288–304.
- Ladich, F., 2000. Acoustic communication and the evolution of hearing in fishes. *Phil. Trans. R. Soc. Lond.* 355, 1285–1288.
- Ladich, F., Bass, A.H., 2003. Audition. In: Kapoor, B.G., Arratia, G., Chardon, M., Diogo, R. (Eds.), *Catfishes*, Vol. II. Oxford and IBH, New Delhi, pp. 701–730.
- Ladich, F., Yan, H.Y., 1998. Correlation between auditory sensitivity and vocalization in anabantoid fishes. *J. Comp. Physiol. A* 182, 737–746.
- Laming, P.R., Morrow, G., 1981. The contribution of the swimbladder to audition in the roach (*Rutilus rutilus*). *Comp. Biochem. Physiol. A* 69, 537–541.
- Myrberg, A.A., Spires, J.Y., 1980. Hearing in damselfishes: an analysis of signal detection among closely related species. *J. Comp. Physiol.* 140, 135–144.
- Poggendorf, D., 1952. Die absolute Hörschwelle des Zwergwelses (*Amiurus nebulosus*) und Beiträge zur Physik des Weberschen Apparates der Ostariophysen. *Z. Vergl. Physiol.* 34, 222–257.
- Popper, A.N., Fay, R.R., 1999. The auditory periphery in fishes. In: Fay, R.R., Popper, A.N. (Eds.), *Comparative Hearing: Fish and Amphibians*. Springer, New York, pp. 43–100.
- Rosen, D.E., Greenwood, P.H., 1970. Origin of the Weberian apparatus and the relationships of the ostariophysan and gonorynchiform fishes. *Am. Mus. Novit.* 2428, 1–25.
- Sand, O., Enger, P.S., 1973. Evidence for an auditory function of the swimbladder in the cod. *J. Exp. Biol.* 59, 405–414.
- Schneider, H., 1941. Die Bedeutung der Atemhöhle der Labyrinthfische für ihr Hörvermögen. *Z. Vergl. Physiol.* 29, 172–194.
- Scholik, A.R., Yan, H.Y., 2000. Effects of underwater noise on auditory sensitivity of cyprinid fish. *Hear. Res.* 152, 17–24.
- Scholik, A.R., Yan, H.Y., 2002. The effects of noise on the auditory sensitivity of the bluegill sunfish, *Lepomis macrochirus*. *Comp. Biochem. Physiol.* 133, 43–52.
- Stipetić, E., 1939. Über das Gehörorgan der Mormyriden. *Z. Vergl. Physiol.* 26, 740–752.
- Tavolga, W.N., 1974. Signal/noise ratio and the critical band in fishes. *J. Acoust. Soc. Am.* 55, 1323–1333.
- van Bergeijk, W.A., 1967. The evolution of vertebrate hearing. In: Neff, W.D. (Ed.), *Contributions to Sensory Physiology*. Academic Press, New York, pp. 1–49.
- von Frisch, K., 1936. Über den Gehörsinn der Fische. *Biol. Rev.* 11, 210–246.
- von Frisch, K., 1938. The sense of hearing in fishes. *Nature* 141, 8–11.
- von Frisch, K., Stetter, H., 1932. Untersuchungen über den Sitz des Gehörsinnes bei der Elritze. *Z. Vergl. Physiol.* 17, 687–801.
- Weber, E.H., 1820. *De Aure et Auditu Hominis et Animalium. Pars I. De Aure Animalium Aquatiliu*. Gerhard Fleischer, Leipzig, 134 pp.
- Weeg, M.G., Fay, R.R., Bass, A.N., 2002. Directionality and frequency tuning of primary saccular afferents of a vocal fish, the plainfin midshipman (*Porichthys notatus*). *J. Comp. Physiol. A* 188, 631–641.
- Wolff, D.L., 1968. Das Hörvermögen des Kaulbarsches (*Acerina cernua* L.) und des Zanders (*Lucioperca sandra* Cuv. und Val.). *Z. Vergl. Physiol.* 60, 14–33.

- Wysocki, L.E., Ladich, F., 2001. The ontogenetic development of auditory sensitivity, vocalization and acoustic communication in the labyrinth fish *Trichopsis vittata*. *J. Comp. Physiol. A* 187, 177–187.
- Wysocki, L.E., Ladich, F., 2002. Can fishes resolve temporal characteristics of sounds? New insights using auditory brainstem response. *Hear. Res.* 169, 36–46.
- Yan, H.Y., 1998. Auditory role of the suprabranchial chamber in gourami fish. *J. Comp. Physiol. A* 183, 325–333.
- Yan, H.Y., Curtsinger, W.S., 2000. The otic gasbladder as an ancillary structure in a mormyrid fish. *J. Comp. Physiol. A* 186, 595–600.
- Yan, H.Y., Fine, M.L., Horn, H.S., Colon, W.E., 2000. Variability in the role of the gasbladder in fish audition. *J. Comp. Physiol. A* 186, 435–445.