

## EVIDENCE FOR AN AUDITORY FUNCTION OF THE SWIMBLADDER IN THE COD

BY OLAV SAND AND PER S. ENGER

*Institute of Zoophysiology, University of Oslo, Oslo, Norway*

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### INTRODUCTION

Fish flesh has nearly the same acoustic properties as sea water. In a sound field a fish will thus be effectively acoustically transparent, and vibrate with the same phase and amplitude as the surrounding water particles. The denser otoliths, which are in close contact with the sensory epithelium in the ear, will lag behind, thereby creating a shear movement of the sensory hairs (Pumphrey, 1950; Griffin, 1955; de Vries, 1956). This is the adequate stimulus for the hair cells in the acoustico-lateralis system in vertebrates. The otolith/hair-cell system in fish thus responds to the kinetic part of a sound wave (particle velocity or particle displacement) rather than the sound pressure.

Because of the high compressibility of gas compared to water, the surface of a gas-filled swimbladder will show much larger displacement amplitudes when exposed to sound than the water particles in the absence of a bladder. If these swimbladder displacements are converted into an amplified movement of the otolith, the swimbladder may improve hearing by functioning as a pressure/displacement transformer in a sound field. The system will then respond to sound pressure, although the end organ itself is still sensitive to particle motion.

The swimbladder no doubt plays a role as an accessory hearing organ in the Ostariphysi. In these fish an anterior chamber of the swimbladder is linked to the sacculi through a chain of small bones, the Weberian ossicles. Poggendorf (1952) showed that the hearing sensitivity decreased drastically when this bony connexion was destroyed, and he furthermore demonstrated that *Ictalurus* (= *Amiurus*) *nebulosus* is sensitive to sound pressure, and not to the kinetic part of the sound. The function of the Weberian apparatus has been reviewed by Alexander (1966).

Other forms of close association between the swimbladder and the ear have been reported for the families Anabantidae, Balistidae, Clupeidae, Engraulidae, Holocentridae, Moridae, Mormyridae, Notopteridae, Ophiocephalidae, Sciaenidae and Sparidae (Jones & Marshall, 1953; Alexander, 1966; van Bergeijk, 1967; Tavalga, 1971). From anatomical considerations it is likely that the swimbladder has an auditory function in these groups.

In fish lacking a mechanical linkage between the swimbladder and the ear the situation is less clear. Poggendorf (1952), van Bergeijk (1964) and Alexander (1966) have suggested that even in these cases the swimbladder might be important for hearing. Sound-induced swimbladder pulsations might then be transmitted to the ear through the intervening body tissues, but of course with a greater mechanical loss than within the more specialized groups. However, experimental evidence supporting this theory

is sparse. Enger & Andersen (1967) compared the hearing of cod (*Gadus morhua*) and sculpin (*Cottus scorpius*) by recording microphonic potentials from the labyrinth. They varied the sound-source distance and suggested that the sculpin, which lacks a swimbladder, was sensitive to particle motion since saccular microphonic potentials were only obtained in the near-field, where the displacement amplitude is relatively large. In the cod, on the other hand, microphonic potentials were recorded in the far-field, where the displacements are relatively small. These differences were attributed to an accessory role of the swimbladder in the hearing of cod. Their data from cod, however, did not show that this fish was sensitive to sound pressure rather than to the kinetic part of the sound (Chapman & Hawkins, 1973), and the difference in sensitivity in the two species could thus not be attributed conclusively to the functioning of a pressure/displacement transformer in cod.

Chapman & Hawkins (1973), on the other hand, found sound pressure, and not particle motion, to be the relevant auditory stimulus in cod for frequencies above about 50 Hz. These authors measured acoustic thresholds in cod, and varied the ratio between displacement and sound pressure by changing the distance to the sound source. Their data indicate, though indirectly, that the swimbladder in cod is important for hearing over the frequency range from 50 Hz to the upper frequency limit. Measuring threshold values, Poggendorf (1952) had previously shown *Ictalurus* to be pressure-sensitive even with the Weberian ossicles destroyed, and this also indicates that the swimbladder might be utilized in hearing in fish without a specialized apparatus like the Weberian ossicles.

Chapman & Sand (1973) studied the hearing in flatfish, which lack a swimbladder. By supplying these fish with an artificial swimbladder consisting of a small gas-filled toy balloon placed just beneath the fish head, the hearing sensitivity was markedly increased and the audible frequency range was extended. However, a natural swimbladder may only improve the hearing if the sensory cells in the ear have a suitable orientation. These cells must be sensitive to vibrations radiating from the swimbladder, and this turned out to be the case in haddock (*Melanogrammus aeglefinus*) (Enger *et al.* 1973).

Although there is indirect evidence to show that the swimbladder might be utilized in the hearing of an unspecialized fish, direct confirmation is so far lacking. The present investigation was undertaken to obtain direct evidence of an auditory function of the swimbladder in cod. An electro-physiological technique, as described by Enger & Andersen (1967), was chosen, and saccular microphonic potentials were measured as a function of the swimbladder volume. The experiments were performed at 6 m depth in the sea. This was done to obtain reasonably good free-field conditions. The acoustics in small tanks are complicated (Parvulescu, 1964) and the displacement for a given sound pressure may be far above the values for free-field conditions. This will tend to decrease the importance of a swimbladder in hearing.

## Fish

## MATERIALS AND METHODS

Recordings were made from four cod (*Gadus morhua*) 31–36 cm long. The fish were caught in shallow water (< 10 m) to avoid damage to the swimbladder, and were subsequently kept in shallow tanks for at least 48 h before the experiments.

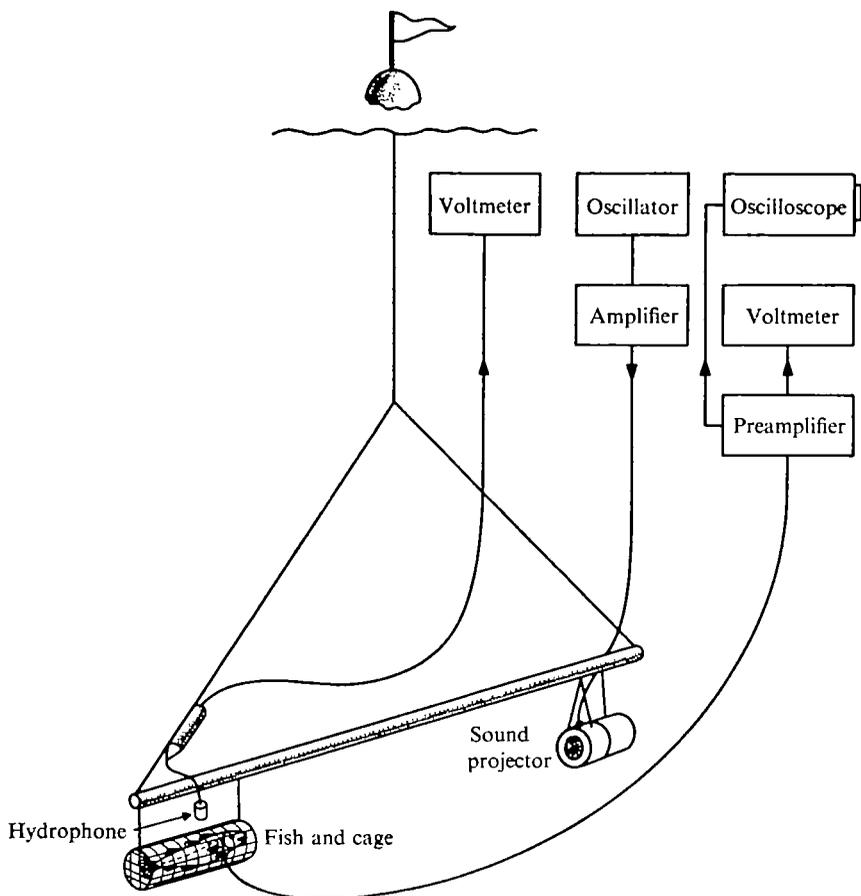


Fig. 1. Sketch of the experimental arrangement. See text for details.

### Operation

The fish were anaesthetized with an intraperitoneal injection of approximately 40 mg sodium-iso-amyl-ethyl-barbiturate per kg body weight (Keys & Wells, 1930), which gives an anaesthesia lasting about 10 h. The skull was exposed and the electrodes—a pair of stainless-steel wires (0.3 mm diameter) insulated with a diamel coating except for a tiny area at the tips—were implanted through small holes in the skull. One electrode tip was placed between the brain and the sacculus, as close as possible to the saccular sensory epithelium, whereas the other wire was shorter and acted as the indifferent electrode. The distal part of the electrode pair was bent at right angles and fixed to the skull by dental cement (Fleck, Mizzy, Inc.). The connecting wires were attached to the skin by sutures just behind the incision. Gas was removed from, or injected into, the swimbladder by use of a hypodermic syringe.

### Experimental

A sketch of the experimental equipment is shown in Fig. 1. The fish was placed in a cage of cotton net which was attached to one end of a plastic pipe. The sound projector (Chesapeake Instrument Corp., J 9) was tied to the other end and the pipe

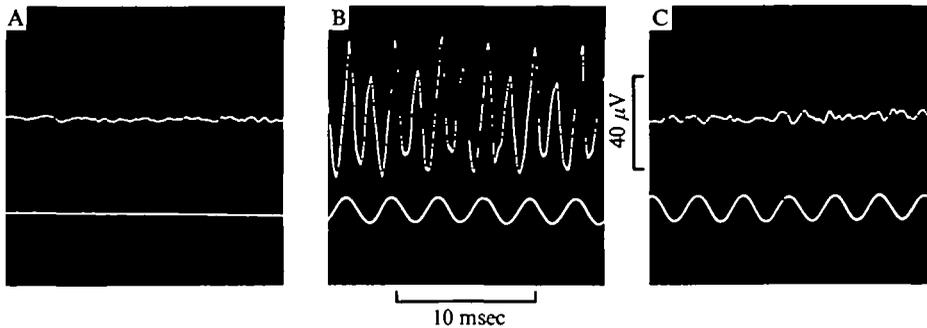


Fig. 2. Oscillographic recordings of the saccular microphonic potentials (upper trace) evoked by background noise (A) compared to the microphonic potentials generated by a 300 Hz tone of 22 dB with (B) and without (C) gas in the swimbladder. Sound recordings on lower beam. Note the pronounced decrease in the microphonic potentials caused by emptying the bladder.

was suspended from a surface float. The experiments were conducted at 6 m depth about 20 m from the shore. The fish was then 4 m above the bottom and the distance between the fish and loudspeaker kept at about 2 m. Signals from the fish were amplified by a pre-amplifier (Tektronix, 122) displayed on a storage oscilloscope (Tektronix, 564) and measured with a vacuum-tube voltmeter (Brüel & Kjaer, 2409). The microphonic potentials in this paper are given in dB referred to  $1 \mu\text{V}$  rms.

Sinusoidal sound stimuli were generated by an oscillator (Phillips, GM 2308), amplified through a power amplifier (Quad II) and fed into the sound projector.

Sound pressures were measured by a calibrated hydrophone (Atlantic Research Corp., LC 54) fitted with pre-amplifier and connected to a vacuum-tube voltmeter (Brüel & Kjaer, 2409). Sound pressures are given in dB referred to  $1 \mu\text{Bar}$  rms.

#### RESULTS

Saccular microphonic potentials for fish with normal swimbladder were obtained for frequencies from 50 to 600 Hz. The microphonic potentials showed a steep cut-off towards higher frequencies and we were unable to generate sound sufficiently intense to evoke microphonics at frequencies below 50 Hz. The frequency of the microphonic potentials were usually twice that of the sound. However, the two peaks corresponding to one cycle of the sound were frequently of different amplitude. In order to compare different microphonic potential amplitudes, these were therefore measured by an integrating a.c. voltmeter.

Fig. 2 compares the microphonic potentials evoked by background noise only (A) to the potentials generated by a 300 Hz tone with (B) and without (C) gas in the swimbladder. In this particular case the fish was first lowered directly to 6 m and the measurements with gas in the bladder were obtained. Thus, the swimbladder volume was then 63% of the surface volume, due to the increase in hydrostatic pressure. Thereafter, the fish was taken to the surface and the swimbladder was emptied. The measurements without gas in the bladder were then obtained, after re-lowering the fish to 6 m depth. It is evident from Fig. 2 that emptying the swimbladder has a dramatic effect on the saccular microphonic potentials, which in fact decrease to a level close to the electric background noise.

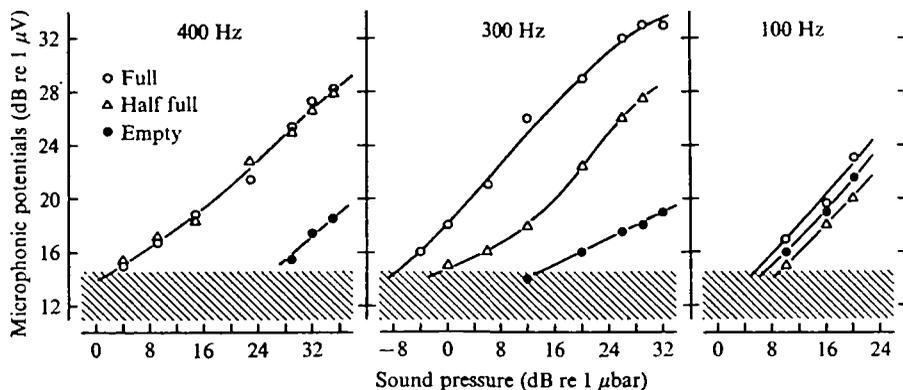


Fig. 3. Amplitude of saccular microphonic potentials as a function of sound pressure for three different frequencies and swimbladder volumes. Frequency and swimbladder state are given in the graph. Hatched area indicates the level of electric background noise. Note that the existence of gas in the swimbladder has no effect on the microphonic potentials at 100 Hz whereas this effect is marked at 300 and 400 Hz.

Fig. 3 gives data from another fish and shows the microphonic potentials as a function of sound pressure for three frequencies. In this fish the measurements at 6 m depth were first taken with an empty bladder, after 18 ml of gas had been removed at the surface. A diver then re-injected 18 ml gas, at 6 m depth, into the bladder, which thus gained its former volume. To ensure that the gas filled the bladder and not the body cavity, the needle was stitched in position after the bladder was initially emptied and the needle-tip remained in position inside the collapsed bladder. The needle was removed after re-filling the bladder, and microphonic potentials were then measured. Further recordings were taken with a swimbladder volume of 10 ml, after the diver had removed 8 ml of gas.

Fig. 3 clearly shows that the amplitude of the microphonic potentials depends on the volume of the swimbladder gas at the two highest frequencies. At 300 Hz the microphonic potentials evoked by a given sound pressure increased in proportion to the gas content of the swimbladder. However, at 400 Hz no difference in microphonic potential amplitudes was detected when the swimbladder volumes were 18 and 10 ml respectively, but with an empty bladder the microphonic potentials were far below these values. At 100 Hz, on the other hand, no significant difference in the microphonic potentials for the different swimbladder volumes was obtained.

Assuming that a definite potential amplitude corresponds to the auditory thresholds, independent of frequency, relative values of auditory thresholds can be obtained. To compare the effect of the gas volume on the microphonic potentials at different frequencies the sound pressure necessary to evoke a certain amplitude of the microphonic potentials was measured. These sound-pressure values will then indicate the relative auditory threshold at each frequency, and relative audiograms may be constructed.

Fig. 4 gives the sound pressure generating microphonic potentials 1.5 dB above the electric noise level (i.e. 16 dB re 1  $\mu$ V) as a function of frequency for the three different swimbladder states. It is evident that for all frequencies above 100 Hz the existence of gas in the swimbladder has a positive effect on the microphonic potentials, whereas

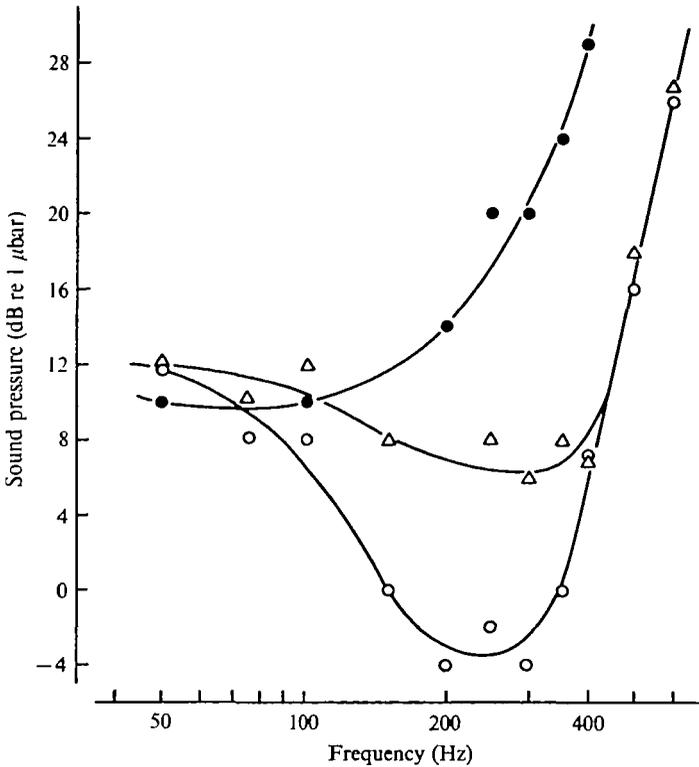


Fig. 4. Relative audiograms showing the sound pressure necessary to evoke microphonic potentials just above the noise level as a function of frequency. Values for three different swimbladder volumes are included. Same fish and symbols as in Fig. 3. Note that for all frequencies above about 100 Hz the existence of gas in the swimbladder has a positive effect on the microphonic potentials.

these potentials are independent of the gas content at lower frequencies. The difference in microphonic potentials generated with 18 and 10 ml swimbladder volumes was negligible above 400 Hz, while for frequencies between 100 and 400 Hz the relative auditory thresholds were lower for the greatest swimbladder volume. At 300 Hz the sound pressure necessary to evoke the threshold microphonic potential was about 34 dB higher for the empty bladder compared to the full bladder of 18 ml. The corresponding value for the bladder volume of 10 ml was about 15 dB. With gas in the bladder, the relative auditory thresholds were lowest at 200–300 Hz. No such minimum values were detected with an empty bladder.

Relative audiograms, of the type shown in Fig. 4, are presented for two other cod in Fig. 5. Because of high electric noise level in these cases, the absolute values of the sound pressures necessary to evoke microphonic potentials of 1.5 dB above the noise level are much higher than for the example given in Fig. 4. However, emptying the bladder has the same effect on the saccular microphonic potentials. It is seen that this treatment increased the relative auditory thresholds in the two fish with about 20 and 15 dB at 300 Hz.

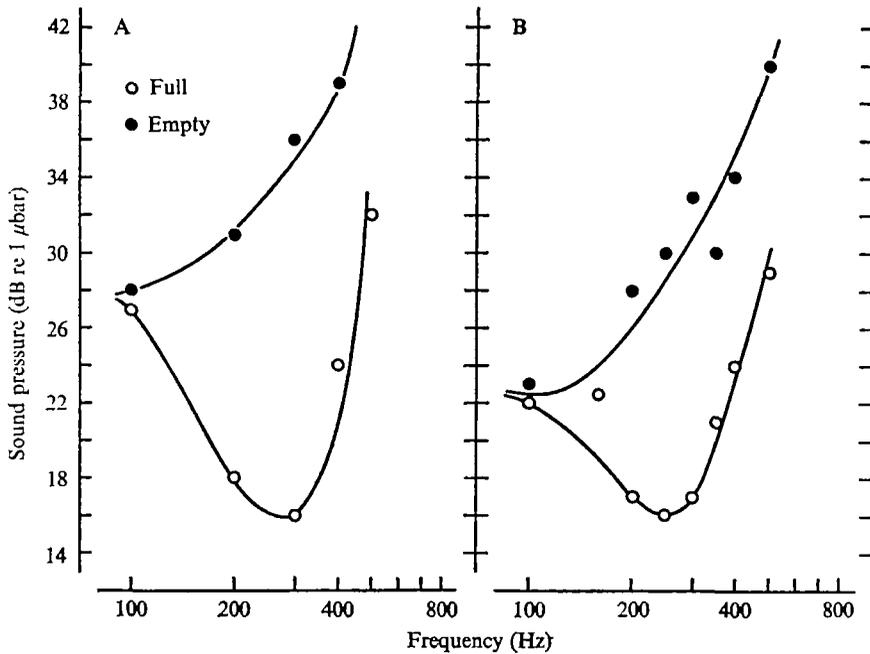


Fig. 5. Relative audiograms for two cods, including values for two different swimbladder volumes.

#### DISCUSSION

The summed extracellular current from several hair cells, due to the receptor potentials, contributes to the microphonic potentials recorded with our extracellular macroelectrodes (Flock, 1971). The microphonic potentials are thus ultimately connected with the excitation of auditory nerve fibres. Comparison of microphonic potentials at different swimbladder states therefore gives good indication of a possible connexion between hearing ability and swimbladder state.

As pointed out by Chapman & Hawkins (1973) and Sand & Hawkins (1973), the swimbladder will cease to be beneficial for hearing below a certain frequency (which is a function of several parameters and will thus vary under natural conditions). This is in good agreement with our results, which showed that the microphonic potentials are unrelated to swimbladder state at the lower frequencies tested. However, the data presented in this paper show clearly that the existence of gas in the cod swimbladder has a positive effect on the saccular microphonic potentials, and thus on hearing, for all frequencies from about 100 Hz to the upper frequency limit of hearing. A result of this increase in auditory sensitivity for the higher frequencies is that the upper frequency cut-off is extended above the value for an empty bladder. From Fig. 4 it is seen that this extension is about three-quarters of an octave in our experiments. The maximum auditory sensitivity was found in the frequency range 200–300 Hz with gas in the bladder, and the relative auditory thresholds were for these frequencies 15–24 dB below those for an empty bladder. However, this positive effect of the swimbladder on hearing will decrease with growing hydrostatic pressure

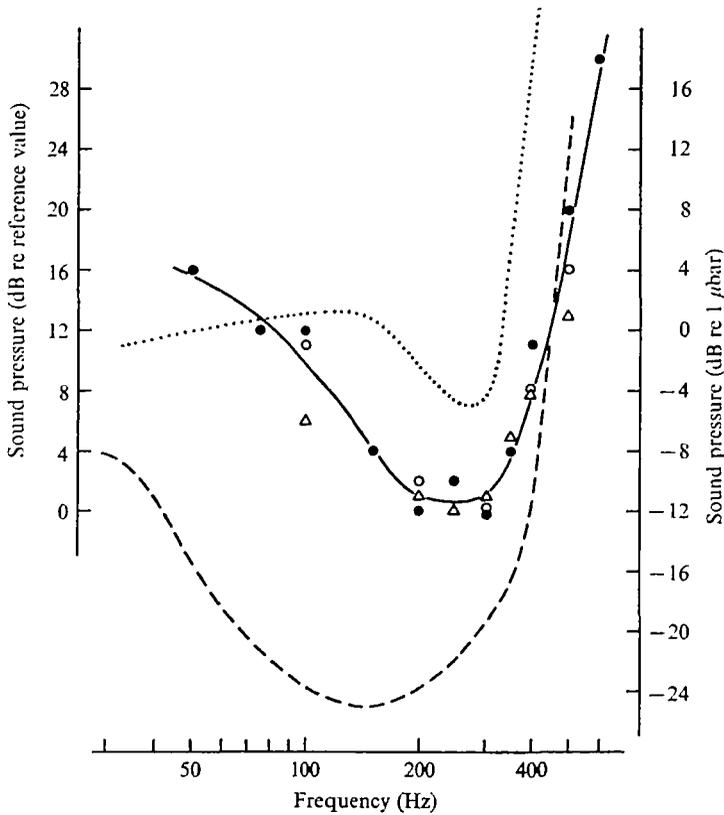


Fig. 6. Average relative audiogram for full swimbladders compared to two behavioural audiograms for cod (dotted line, Buerkle, 1967; broken line, Chapman & Hawkins, 1973). Thresholds are presented as absolute sound pressure in the behavioural audiograms (right ordinate), whereas in the relative audiogram absolute values are converted to dB re value at the most sensitive frequency in each fish (left ordinate). The symbols (●), (○) and (Δ) indicate the cods presented in Fig. 4, 5A and B, respectively.

and increase with swimbladder volume, and thus with fish size (see Chapman & Sand, 1973).

The effect swimbladder gas in the cod has on the relative auditory thresholds is in good agreement both with the decrease in auditory thresholds and the extension of the audible frequency range which Chapman & Sand (1973) observed in flat-fish after introducing an artificial swimbladder. We conclude that the swimbladder in cod, and probably in all fish possessing a swimbladder, acts as a pressure/displacement transformer, and thus increases the hearing sensitivity for all frequencies from a lower limit to the upper frequency cut-off for hearing.

It is interesting to compare the shape of our relative audiograms with the audiograms for cod obtained by other workers using a conditioning technique. This is done in Fig. 6, where the microphonic data obtained with full bladders are compared with the audiograms reported by Buerkle (1967) and Chapman & Hawkins (1973). The absolute values of the relative auditory thresholds are converted to dB referred to the lowest relative threshold measured in each fish. It is evident from the figure that the

Upper frequency cut-off corresponds fairly well in the two types of audiograms, thus indicating that the upper limit of the audible frequencies may be determined by inadequacy of the peripheral auditory apparatus. The differences between the two behavioural audiograms, both in regard to shape and absolute threshold values, are mainly due to the high level of background noise in Buerkle's experiments. The thresholds reported by him were thus heavily masked, and the audiogram below 140 Hz was related to the background aquarium noise. Reports on other fish (see for instance Offutt (1968) for data on goldfish) have also shown that different techniques and experimental conditions may result in different audiograms for the same species. The general shape of the microphonic and behavioural audiograms for cod given in Fig. 6 are therefore in reasonably good agreement. Enger & Andersen (1967), on the other hand, recorded saccular microphonic potentials in cod at frequencies up to 1000 Hz, which is about one octave above the upper frequency cut-off in this species. However, these authors worked at shallow depths, where the relation between particle displacement and sound pressure is higher than for free-field conditions (Banner, 1968). This may explain the difference between their results and ours.

#### SUMMARY

1. Saccular microphonic potentials in cod (*Gadus morhua*) were recorded by means of implanted electrodes during sound stimulation with different swimbladder volumes. To obtain acceptable acoustic conditions, the experiments were conducted at 6 m depth in the sea.
2. Swimbladder volume had no effect on the microphonic potentials at 100 Hz, whereas its effect was marked at higher frequencies. The sound pressure necessary to evoke microphonic potentials just above the electric background noise was about 20 dB higher for the empty bladder, compared to the full bladder at 300 Hz.
3. The microphonic potentials are ultimately linked to excitation of eighth-nerve fibres, and it is concluded that the existence of gas in the cod swimbladder has a positive effect on hearing sensitivity for all frequencies from a lower transient frequency to the upper frequency limit of hearing. Gas in the swimbladder will furthermore extend the audible frequency range.
4. The upper frequency limit for the saccular microphonic potentials is in good agreement with the upper audible frequency limit determined from behavioural audiograms for cod, thus indicating that this frequency cut-off may be due to inadequacy of the peripheral auditory apparatus.

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