DIRECTIONAL SENSITIVITY OF MICROPHONIC POTENTIALS FROM THE PERCH EAR

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INTRODUCTION

The sacculus and lagena (pars inferior) form the part of the ear generally believed to be involved in hearing in teleosts (Dijkgraaf, 1950, 1952). Shearing movements of the sensory hairs are mediated by the existence of heavy otoliths in close contact with the maculae. Due to their larger density the otoliths will lag behind the motion of the hair cells when the fish is vibrated in a sound field. The unaided otolith/haircell system is therefore sensitive to the kinetic part of the sound (Chapman & Sand, 1974). However, fish possessing a gas-filled swimbladder may utilize this organ as a pressure/displacement transformer, and such fish will respond to sound pressure, although the hair cells are still sensitive to particle motion. Evidence for an accessory role of the swimbladder in fish hearing generally, even in groups with no specialized association between the bladder and the ear, has recently been provided (Chapman & Hawkins, 1973; Sand & Enger, 1973; Chapman & Sand, 1974). The aspects of fish hearing which will be considered in this paper are peripheral frequency analysis, directional hearing and the ability of re-radiated swimbladder pulsations to stimulate different parts of the ear.

Although lacking an analogue to the cochlea, fish are able to discriminate between frequencies (see Tavolga, 1971). However, the mechanism for this pitch discrimination is still unknown. Synchronization between sound frequency and action potentials in the auditory neurones might provide the necessary information, and recordings from the auditory nerve in different groups of fish have showed nerve discharges to be phase-locked to the sinusoidal stimuli (Lowenstein & Roberts, 1951; Enger, 1963; Furukawa & Ishii, 1967). Enger (1963) and Furukawa & Ishii (1967) also reported differences in frequency sensitivity between nerve fibres, however, and even if the tuning curves of the individual fibres were rather broad, the possibility of a peripheral frequency analysis in fish was demonstrated. This idea was strengthened by Andersen & Enger (1968) who by recording saccular microphonic potentials from the sculpin (*Cottus scorpius*) found differences in frequency sensitivity between the anterior and posterior part of the sensory macula. A peripheral frequency analysis might either be due to differences in physiological properties of various hair cells, or, as in the cochlea, may be due to mechanical frequency analysis. This last possibility was considered by van Bergeijk (1967), who suggested that travelling waves with frequency-dependent maxima were generated along the macula during sound stimulation. On the other hand, in the present paper the notion is put forward that mechanical frequency analysis in fish might be based upon a frequency-dependent pattern of otolith movements.

It has been suggested that an auditory function of the swimbladder is contradictory to directional hearing in fish. Most fish have a single swimbladder, and since a single pressure receptor is insufficient to locate a sound source, fish would be unable to detect the direction of a pressure wave (van Bergeijk, 1964). Earlier behavioural studies supported this view (Reinhardt, 1935; von Frisch & Dijkgraaf, 1935), but these investigations were conducted in small tanks or at extremely shallow depth in the field. The acoustical conditions were therefore very complicated and difficult to predict (Parvulescu, 1964, 1967). However, directional responses could be elicited if the distance to the sound source was less than a few cm, but in these cases detection by cutaneous receptors was suggested. On the other hand, recent studies by Olsen (1969a, b) on herring (*Clupea harengus*) and cod (*Gadus morhua*) and by Schuijf, Baretta & Wildshutt (1972) on wrasse (*Labrus bergylta*) were undertaken under free-field conditions in the sea, and clearly demonstrated that these species (which possess a swimbladder) can distinguish between different sound-source directions at a range of several metres. The herring was reported to detect differences in sound direction of less than 45° for frequencies from 20 to 6000 Hz, whereas the corresponding figure for wrasse at 115 Hz (the only frequency tested) was less than 70°, and possibly as good as 10°. Chapman (1973), working on the cod, found that the masking of tone signals by white noise was reduced when the tone and noise were transmitted from different directions, thus confirming that even fish with a swimbladder may possess well-developed directional discrimination.

Sound pressure is a scalar quantity, while particle motion is a vector. Reception of the direction of a pressure wave therefore requires comparison of arrival time, intensity or phase between at least two pressure detectors. However, for a plane sound wave the particle displacement will be normal to the wave front, and a single kinetic vector detector is therefore sufficient to determine the sound direction. Each hair cell has an axis of maximal sensitivity (Flock & Wersäll, 1962), and groups of hair cells with different orientation will thus constitute such a detector. By comparing the output from each group, the direction of the sound source could be determined directly. However, the amplified particle motions re-radiated from the swimbladder might mask the incident sound at the two ears, but this masking effect could be reduced by an arrangement of the haircells which made these insensitive to radial displacements emanating from the swimbladder. The maculae of the pars inferior are situated in an approximately vertical plane, and the orientation pattern of the saccular hair cells in the burbot (*Lota lota*) (Wersäll, Flock & Lundquist, 1965) and the goldfish (*Carassius auratus*) (Hama, 1969) indicates maximal sensitivity for vertical displacements of the otolith. On the other hand, complex movements of the otoliths might be expected when a fish is vibrated linearly in a sound field, and the directional sensitivity of different parts of the fish ear can not easily be predicted from morphological data.

Enger *et al.* (1973) therefore recorded microphonic potentials from the sacculus in the haddock (*Melanogrammus aeglefinus*) during horizontal vibration of the fish in air. The fish were mounted on a rotatable vibrating table to obtain well-defined directional stimuli. The microphonic potential amplitude turned out to be a function of the vibration angle, and maximal amplitude was most often obtained for vibration directions parallel to the long axis of the fish. The hair cells contributing to this response were thus maximally sensitive to displacement in nearly the same direction as sound-
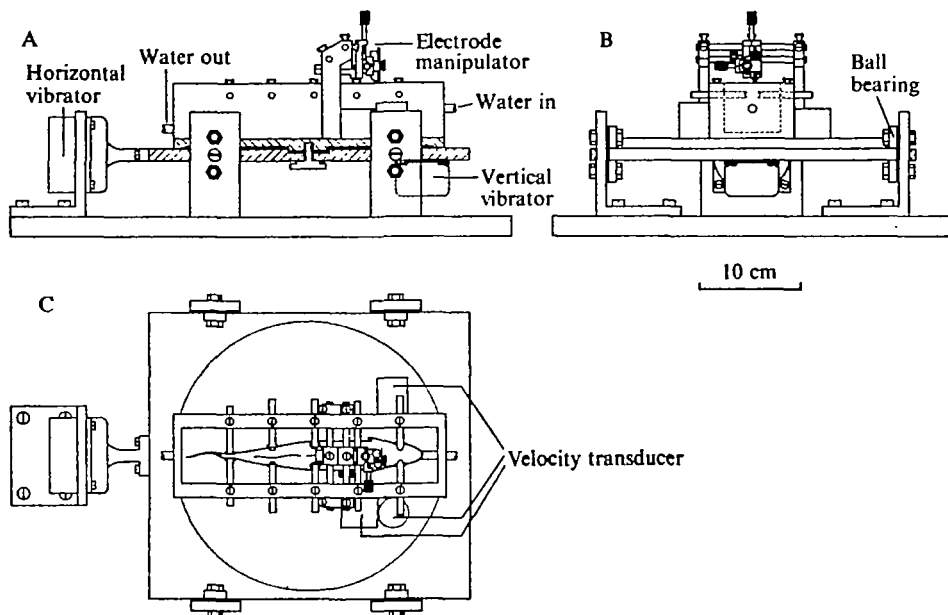
Text-fig. 1. Drawing of the vibration table; lateral (A), frontal (B) and top side (C) view.
See text for details.

induced swimbladder pulsations would produce, and this result therefore supports the theory of an auditory function of the swimbladder. However, in some cases the optimal sensitivity was obtained for other vibration directions, and the authors concluded that a possible explanation for the ability of fish to detect the direction of a sound source could be that different hair cells have different axes of maximal sensitivity to vibration.

The recording technique used by Enger *et al.* (1973) was rather crude, and the exact recording loci were not determined. The electrodes were coarse, and the activity of a large number of hair cells, probably including cells from both ears, was recorded simultaneously. Furthermore, the stimuli were restricted to horizontal vibrations only, whereas the fact that the natural surroundings of most fish is a three-dimensional medium makes it interesting to measure the sensitivity to vertical vibrations as well.

The present investigation was thus undertaken to record microphonic potentials from different parts of the pars inferior in perch (*Perca fluviatilis*) as a function of vibrations in the horizontal and vertical plane. The stimulation technique used was developed from that described by Enger *et al.* (1973). The perch was chosen as experimental animal because of its rigid skull and skeleton, which facilitated the vibration of the fish, and because its limited auditory frequency range (Wolff, 1967) nearly coincided with the working range of the vibrating table.

## MATERIALS AND METHODS

Fish

Twenty-one perch (*Perca fluviatilis*) ranging in length from 16 to 24 cm were used in the experiments. The fish were anaesthetized in a solution of MS 222 (Sandoz Ltd.,
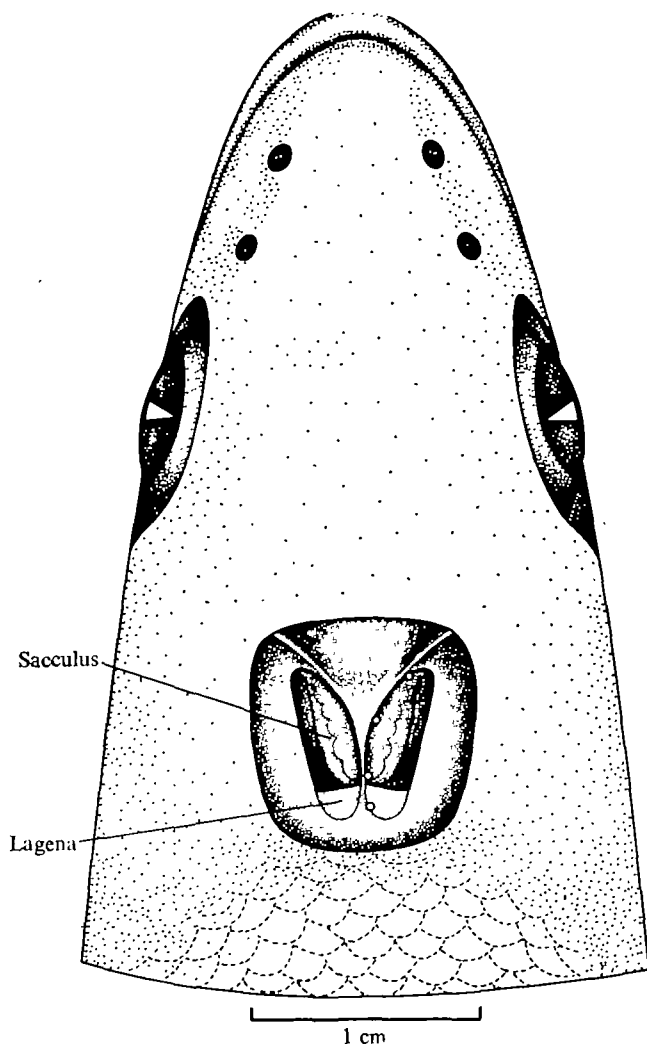
Text-fig. 2. Sketch of the head of a 20 cm long perch with the dorsal part of the skull opened and the brain removed. Recordings were obtained from four electrode positions for each ear, as indicated by black and white dots for the right ear.

Basel), and thereafter clamped by five pairs of steel rods in a special Perspex holder. The first pair had a V-shaped incision which firmly held the skull just above the eyes. The structure of the fish holder is shown in Text-fig. 1.

The dorsal part of the skull was opened and the brain was gently removed, exposing the sacculus and the lagena (Text-fig. 2). The pars inferior in the perch is positioned in a groove in the skull floor, but while the sacculus is only covered with the saccular membrane, a thin bony shield protects the lagena. The electrode easily passed through the saccular membrane, but before the electrode could be positioned in the lagena a small hole in the bony shield had to be drilled with a fine needle. The electrode was placed as medially as possible, with the tip just inside the sacculus or lagena. As indicated in Text-fig. 2, recordings from the sacculus were made with anterior, mid
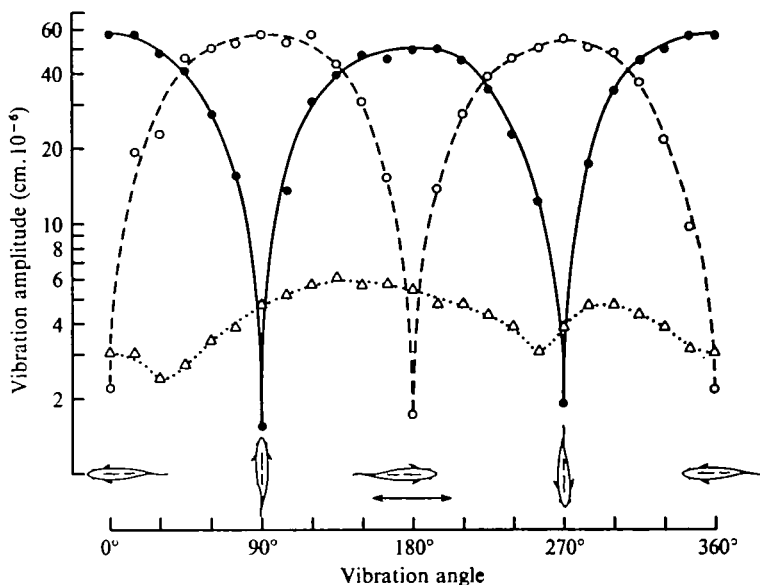
Text-fig. 3. Vibration amplitude of the fish holder during horizontal vibrations of 70 Hz as a function of the angle between the long axis of the fish and the vibration direction. The measurements were obtained using three transducers. Two were sensitive in the horizontal plane, parallel to (●) and at right angles to (○) the long axis of the fish, while one was sensitive to vertical vibrations (△).

and posterior electrode positions, whereas only a mid position was used when recording from the lagena.

During the experiments water was run over the gills. Respiration and blood circulation of course ceased after the brain had been removed but the water flow kept the preparation cool. Microphonic potentials could be recorded for several hours without any marked decrease in amplitude.

Stimulation

A drawing of the vibration table is shown in Text-fig. 1. The table was mounted on a 50 × 40 × 2 cm slab of iron, and consisted of a 32 × 29 × 1·2 cm plate of aluminium which rested on adjustable (excentric) ball bearings. The fish holder was attached to a rotatable disc of aluminium, 1·0 cm thick and 26 cm in diameter, which was centred on the table. The disc was readily locked in any position, relative to the table. The table was vibrated either by a horizontal (Derritron, VP 2MM) or a vertical (Echonic, WA-3020) vibrator. During horizontal vibrations the angle between the fish and the direction of vibration was varied, whereas the position of the fish was fixed when it was vibrated vertically. The head of the fish was then just above the vertical vibrator, and the upper set of ball bearings was loosened. The vibrators were fed by amplified signals (20 W power amplifier, locally built) from a sine-wave oscillator (Heathkit, IG-82).

The movement of the table was measured by three velocity transducers (SM-2, Sensor, Netherlands) attached to the disc in three perpendicular directions, close to the head of the fish. One of the transducers was sensitive in the vertical direction,

whereas the other two were sensitive in the horizontal plane, along the long and short axes of the fish, respectively. The motion of the table was thus completely described. The vibration amplitude never exceeded 2×10^{-4} cm.

Signals from the transducers were fed into separate amplifiers (locally built) and voltmeters (Brüel & Kjær, 2409) for amplification and measurement. Text-fig. 3 presents a set of vibration measurements at 70 Hz during horizontal vibration and rotation of a fish. The vibration velocity values (rms) are here, and throughout the paper, recalculated to displacement values. The ratio between the horizontal vibration component parallel to the driving force and the horizontal component normal to this was about 45, thus giving nearly linear horizontal movements. The ratio between the horizontal and vertical vibration was about 10.

The table showed rather similar behaviour up to about 200 Hz, above which frequency the ratio between horizontal and vertical vibrations dropped drastically. Below 30 Hz the vibrations became markedly distorted, and 30–250 Hz was thus the practical frequency range for the experiments involving rotation of the fish in the horizontal plane.

During vertical vibration of the table the ratio between vertical and horizontal vibrations was close to 10, but distortions here occurred below 100 Hz. Only 200 and 100 Hz were therefore tested in this type of experiments.
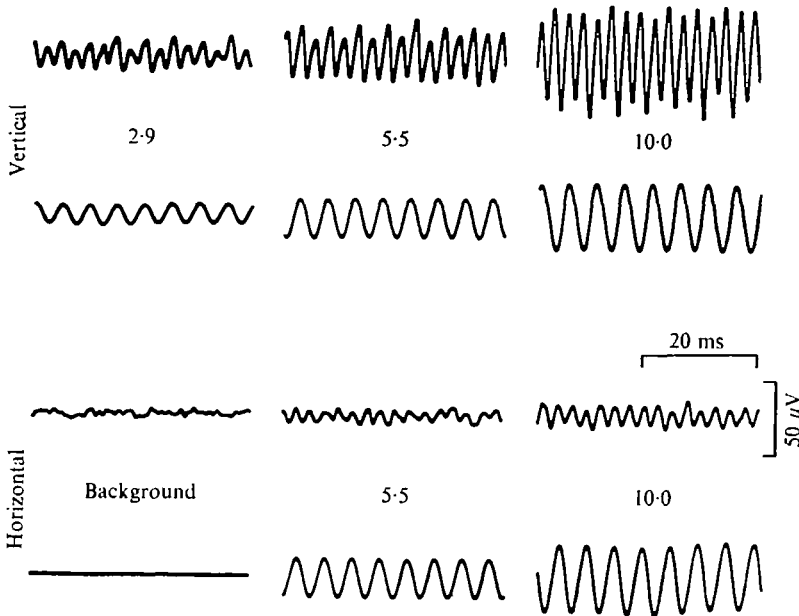
Recording of microphonic potentials

The electrode manipulator (Narishige, C-1) was fixed to the rotatable disc by a rigid bridge suspension (Text-fig. 1). Due to the low mass and the rigid suspension of the manipulator, the relative movement between the fish and electrode was probably very slight. The electrodes were NaCl-filled glass pipettes with tip diameters of 10–15 μ. Microphonic potentials from the fish were amplified by a pre-amplifier (Tektronix, 122), displayed on an oscilloscope (Tektronix, RM 565) for photographing (Grass Kymograph Camera, C 4) and measured with a vacuum tube voltmeter (Brüel & Kjær, 2409). Microphonic potentials in this paper are given in dB re 1 μV rms.

RESULTS

The microphonic potentials recorded from the pars inferior during vibration of the fish were twice the frequency of vibration, but the two peaks corresponding to one vibration cycle could be of different amplitude. To facilitate comparison between different recordings, the microphonic potentials were therefore measured by an integrating a.c. voltmeter. The amplitude of the potentials was a function of both the stimulus strength and the direction of vibration. Two types of experiments were conducted, namely measurement of relative vertical and horizontal sensitivity of the microphonic potentials for the different electrode positions, and recording of micro-phonic potentials evoked by horizontal vibration during horizontal rotation of the fish.

Ratio between vertical and horizontal sensitivity of the
microphonic potentials

Text-fig. 4 shows an example of the microphonic potentials recorded from the lagena for vertical and horizontal vibrations of different intensities at 200 Hz. The
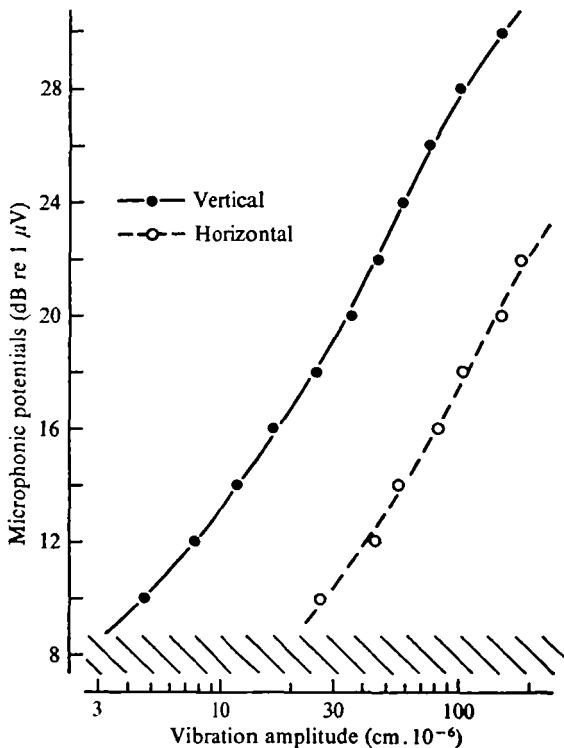
Text-fig. 4. Microphonic potentials (upper trace of each recording) obtained from the lagena during vertical and horizontal vibrations of 200 Hz (lower trace of each recording). The electrode position was the same throughout the series, and the vibration amplitudes (cm × 10⁻⁵) are indicated on the figure. Note that vertical vibrations were the most efficient in evoking microphonic potentials.

electrode position was the same for the two vibration modes. During the horizontal vibration the angle between the direction of vibration and the fish was chosen to be optimal for evoking microphonic potentials. It is seen that a much higher stimulus intensity was necessary for horizontal than for vertical vibrations to generate the same amplitude of the microphonic potentials.

In Text-fig. 5 these potentials are presented as a function of the vibration amplitude, and the displacement amplitude necessary to evoke microphonic potentials just above the electric background noise is taken as a measure of the microphonic sensitivity for the particular kind of vibration. The higher these threshold values are, the lower is the microphonic sensitivity. It is seen that for the case presented in Text-fig. 5, the microphonic sensitivity was about 5·8 times greater for vertical than for horizontal vibrations. Measurements of this type were performed on 5 lagenae for 100 and 200 Hz, and the average ratio between the vertical and horizontal microphonic sensitivity was 2·9 (S.D. = 0·8) and 5·6 (S.D. = 1·9), respectively.

In contrast to this high microphonic sensitivity to vertical vibrations, which was obtained when recording from the lagena, the anterior part of the sacculus gave a different picture. This is seen from Text-figs. 4 and 6, which are based on directly comparable data from two different fish. Text-fig. 6 shows recordings of microphonic potentials for a fixed electrode position from the anterior part of the sacculus, and the microphonic sensitivity to vertical and horizontal vibrations was clearly rather similar. Text-fig. 7 gives the microphonic potentials evoked by the two different vibration modes as a function of the vibration amplitude, in a comparable way as in the example
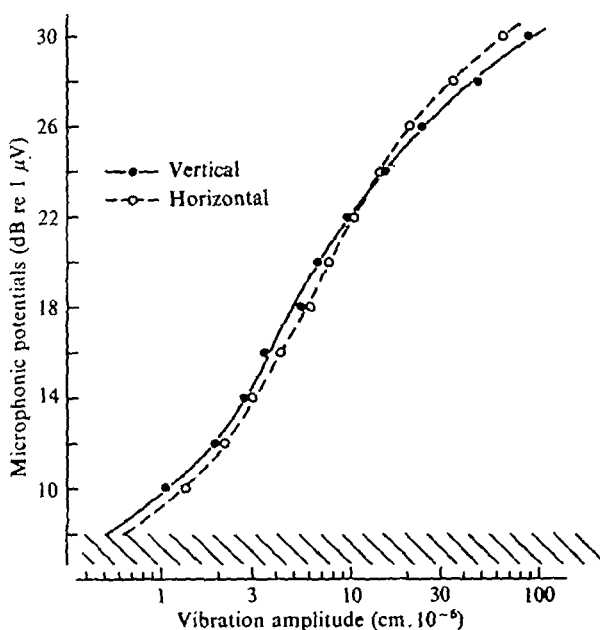
Text-fig. 5. Microphonic potentials from the lagena as a function of vibration amplitude. Recording locus is the same as in Text-fig. 4. Level of electric background noise is indicated by hatched area. Note that the horizontal vibration amplitude required to evoke microphonic potentials just above the electric background noise was 5·8 times greater than the corresponding vertical vibration amplitude.

from the lagena shown in Text-fig. 5. It is seen that the microphonic sensitivity was nearly identical for horizontal and vertical vibrations. Similar series of measurements were obtained from nine sacculi for 100 and 200 Hz, and the mean ratio between the microphonic sensitivity to vertical and horizontal vibrations was 1·2 (S.D. = 0·3) and 1·1 (S.D. = 0·4), respectively.
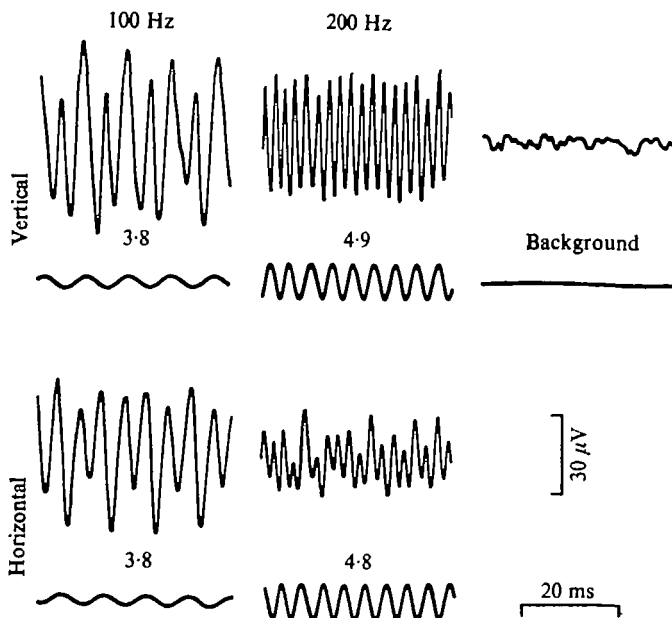
Recordings of microphonic potentials were also obtained from mid and posterior positions of the sacculus, and ratios between vertical and horizontal microphonic sensitivity were measured following the same procedure outlined above. From eight mid positions the mean value for 100 and 200 Hz was 1·5 (S.D. = 0·5) and 1·8 (S.D. = 0·7), respectively, whereas the corresponding values from eight posterior positions were 1·6 (S.D. = 0·4) and 2·7 (S.D. = 0·9). For the electrode positions which showed more pronounced vertical than horizontal sensitivity this difference in sensitivity decreased when the frequency was changed from 200 to 100 Hz. This phenomenon is shown in Text-fig. 8, where the microphonic potentials evoked in response to horizontal and vertical vibrations of 100 and 200 Hz are compared. The electrode position was the same for all recordings, and the potentials were picked up from the posterior part of the sacculus. It is evident that whereas for the same intensity horizontal vibrations evoked potentials which were but slightly less than the response to vertical
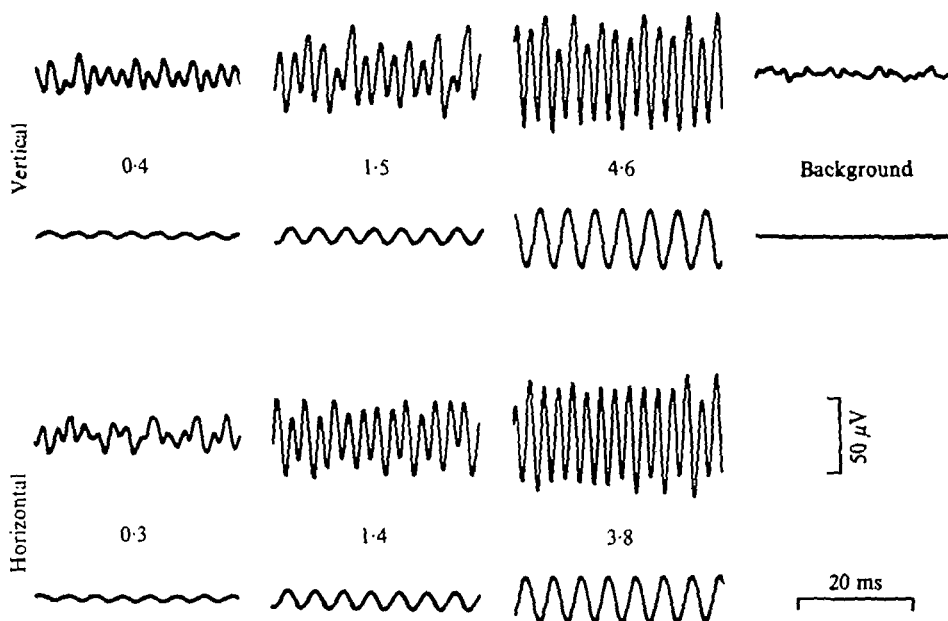
Text-fig. 6. Microphonic potentials recorded from the anterior part of the sacculus during vertical and horizontal vibrations of 200 Hz. The recordings are directly comparable to those in Text-fig. 4. Note that the efficiency of horizontal and vertical vibrations in evoking microphonic potentials was about the same.


Text-fig. 7. Microphonic potentials from the anterior part of sacculus as a function of vibration amplitude. Recording locus is the same as in Text-fig. 6. Note that the horizontal vibration amplitude required to evoke microphonic potentials just above the electric background noise was only 1·2 times greater than the corresponding vertical vibration amplitude.

Text-fig. 8. Recordings of microphonic potentials (upper trace of each recording) from the posterior part of the sacculus in response to vertical and horizontal vibrations (lower trace of each recording) of 100 and 200 Hz. The vibration amplitudes (cm × 10⁻⁸) are indicated on the figure. The recordings are from the same electrode position. Note that the relative effect of horizontal and vertical vibrations is frequency dependent.
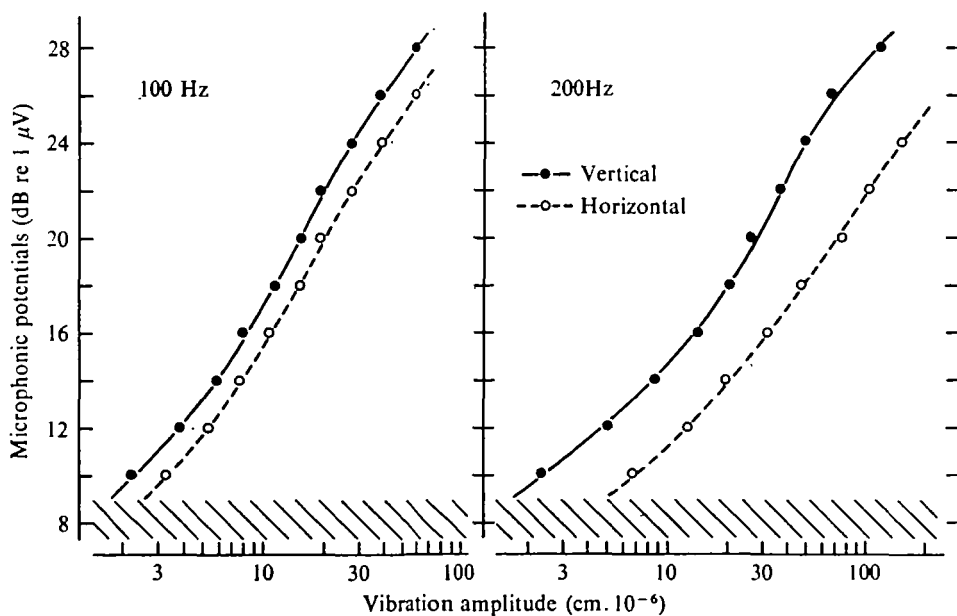

Text-fig. 9. Microphonic potentials evoked by vertical and horizontal vibrations of 100 and 200 Hz as a function of vibration amplitude. Hatched area indicates level of electric background noise. Electrode position was the same as for Text-fig. 8. Note that vertical vibrations are relatively more efficient than horizontal vibrations in evoking microphonic potentials at 200 than at 100 Hz.
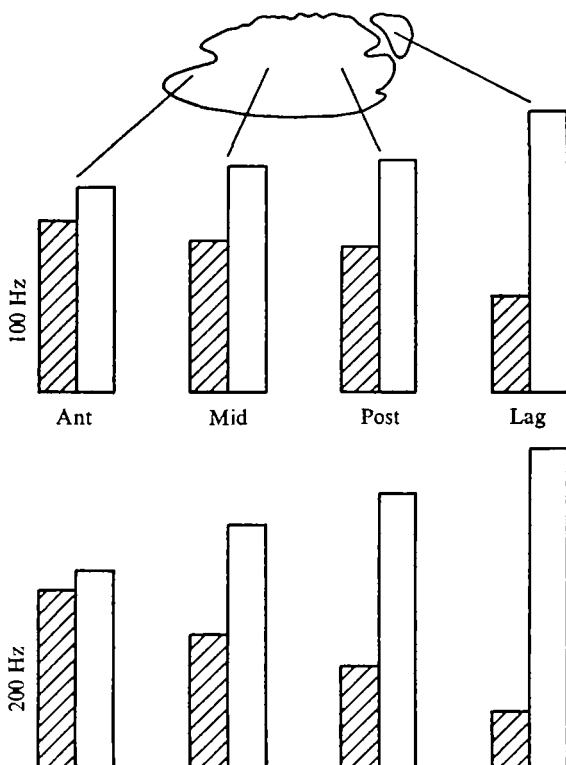
Text-fig. 10. Comparison of the mean ratios between the microphonic sensitivity to horizontal (hatched columns) and vertical (open columns) vibrations for the anterior (Ant) mid (Mid) and posterior (Post) parts of the sacculus, and for the lagena (Lag). The ratios are indicated by the relative heights of the columns within each pair. The figure does not give any information about differences in absolute sensitivity between the different parts of the ear.

vibrations, this difference in response grew considerably when the frequency was increased by one octave. Text-fig. 9 presents the microphonic potentials as a function of vibration amplitude for the two frequencies and stimulation modes. It is seen that for 100 Hz the ratio between horizontal and vertical vibration amplitudes giving a microphonic response just above the level of electrical background noise was about 1·5, whereas this ratio increased to 2·9 at 200 Hz.

The mean ratios between the microphonic sensitivity to vertical and horizontal vibrations at 100 and 200 Hz from different parts of the pars inferior are summarized in Text-fig. 10. The lagena is predominantly sensitive to vertical vibrations, whereas the anterior part of the sacculus is equally sensitive to vertical and horizontal vibrations. The mid and posterior part of the sacculus give intermediate values. A gradient is seen along the sacculus, in that the more posterior positions show a tendency towards greater relative vertical sensitivity. Furthermore, it is clear that the microphonic potentials recorded from both the lagena and the posterior part of the sacculus are relatively more sensitive to vertical vibrations at 200 than at 100 Hz.
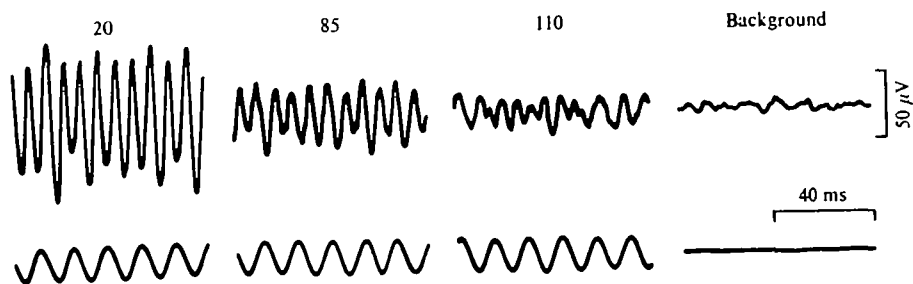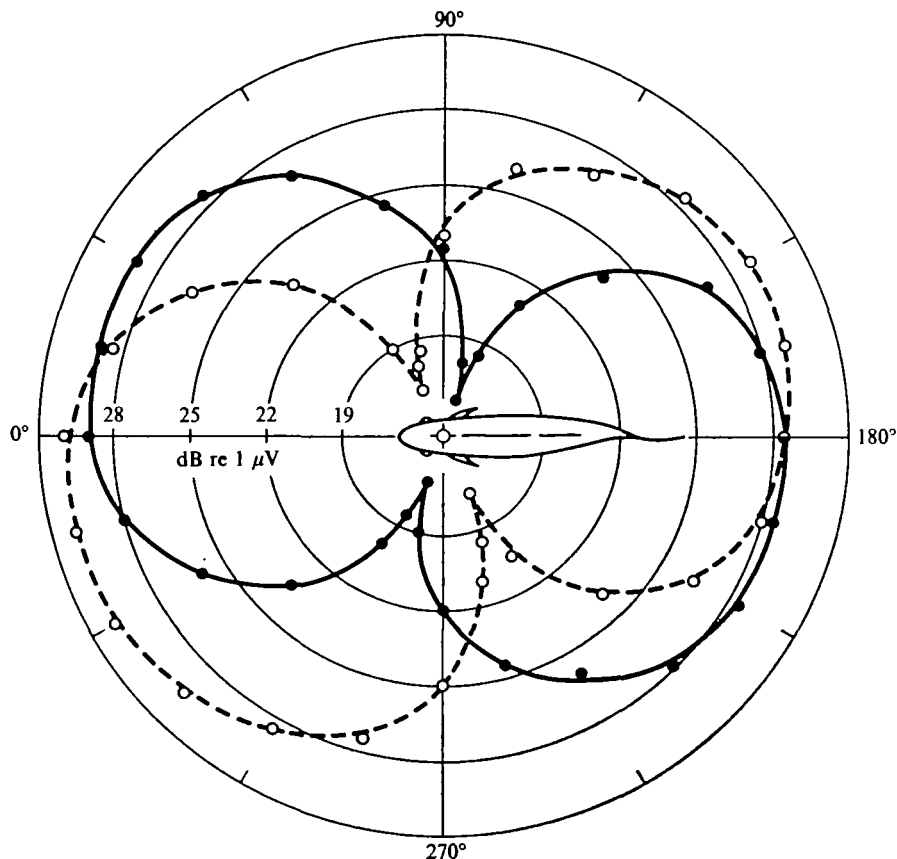
Text-fig. 11. Microphonic potentials (upper traces) recorded from the anterior part of the sacculus on the right side during horizontal vibrations of 70 Hz (lower traces). The vibration amplitude was 3.0×10^{-5} cm. The angle between the long axis of the fish and the vibration direction is indicated on each recording. Note that the potentials were a function of the vibration direction.

Directional sensitivity of the microphonic potentials evoked by horizontal vibrations

For horizontal vibrations of constant amplitude the microphonic potentials were a function of the angle between the fish and the vibration direction. Text-fig. 11 presents a series of recordings from the anterior part of a sacculus from the right side during 70 Hz vibration. It is seen that variation of the vibration angle has a marked effect on the microphonic potentials. In this particular case maximal potentials were obtained at vibration angles around 20° and 200°, whereas the potentials reached minimum values at 110° and 290°. The maximal microphonic potentials were about 12 dB above the minimum values. Similar recordings from the left sacculus gave a corresponding picture, but the optimal angles were here 335° and 155°, whereas minimal potentials were evoked at angles of 65° and 245°. Text-fig. 12 presents the recordings from this fish in a polar coordinate diagram, which gives the amplitude of the microphonic potentials as a function of the horizontal vibration angle. As expected from Text-fig. 3, maximal microphonic potentials occurred at angles within rather wide sectors, and a sharp decline of the potentials was seen towards the angles giving minimum microphonic responses. The angle between the axis of optimal microphonic sensitivity for the right and left otoliths was 45°, which approximates to the angle between the two otoliths.

From the point of view of directional hearing it might have been beneficial for the fish if other parts of the macula showed a different axis of optimal horizontal sensitivity. However, this seemed not to be the case. Recordings of the type described above were made for numerous frequencies at several positions along the sacculus, but the results were roughly the same as shown in Text-fig. 12. Measurements were then concentrated about three positions, and from the right sacculus four, six and four series of recordings were made from the anterior, mid and posterior positions, respectively. The mean angles of the axis of optimal microphonic sensitivity for the three positions were 18·8° (S.D. = 4·8), 20·0° (S.D. = 8·9) and 17·5 (S.D. = 8·6), and the differences between these are not significant (F-test, $P < 0.01$). Four series of recordings from each of the anterior, mid and posterior parts of the left sacculus were obtained, and the corresponding mean optimal angles were 337·5° (S.D. = 8·6), 338·7° (S.D. = 6·3) and 340° (S.D. = 7·0). This again gives no significant differences between the three positions (F-test, $P < 0.01$). To test whether different vibration frequencies

Text-fig. 12. Polar diagram giving microphonic potentials recorded from the right (●) and left (○) sacculus as a function of the horizontal vibration angle. Same fish and recording conditions as in Text-fig. 11. Note that maximal microphonic responses are evoked by vibration directions deviating about 20° from the long axis of the fish.

caused differences in the optimal direction of vibration, the angular deviation of these directions from the long axis of the fish were arranged in three groups according to the stimulation frequency. Data from all the recording loci in both ears were pooled together, and eight, eleven and seven series of measurements were obtained for the frequency groups < 70 Hz, 70–110 Hz and > 110 Hz. The respective mean deviations were 21·2 (S.D. = 7·4), 20·0 (S.D. = 8·1) and 18·6 (S.D. = 5·6). Thus, no significant differences between these groups were detected (F-test, $P < 0.01$). If all the recordings from the right sacculus are combined the mean optimal horizontal vibration angle is 18·9° (S.D. = 7·4), whereas the corresponding mean value for the left sacculus is 338·7° (S.D. = 6·7). The mean angle between the axis of optimal microphonic sensitivity of the left and right sacculus was thus 40·2°. The radiograms presented in Pl. 1, fig. 1, show the position of the sacculi in a living perch. The sacculi have a curved shape, and it is therefore impossible to determine a definite angle between them. However, it is evident from the figure that the mean angle between the two axes of optimal horizontal microphonic sensitivity of the right and left sacculus is very close to the average angle between the two otoliths.

As shown in the previous section, the lagena was predominantly sensitive to vertical vibrations. However, horizontal vibrations also evoked microphonic potentials, and these showed axes of optimal and minimal microphonic sensitivity in a similar way as shown for the sacculus. Only three complete series of measurements of this type were obtained from the lagena, and none of these showed an axis of optimal horizontal microphonic sensitivity which deviated more from the long axis of the fish than did the corresponding values from the sacculus.

DISCUSSION

A fish in water is acoustically nearly transparent, and in a sound field it will therefore vibrate with the same phase and amplitude as the surrounding medium. Vibration of the fish in air will thus provide good simulation of the effect caused by water-borne sound. However, since the vibration stimuli have no pressure component, the pressure/displacement transforming ability of the swimbladder will not be activated, and by changing the angle between the fish and the direction of vibration, well defined directional stimuli are readily obtained.

Auditory effect of vibrations radiating from the swimbladder

Due to the high compressibility of gas compared to water, the surface of a gas-filled swimbladder will undergo much larger displacements when exposed to sound than would the water particles in the absence of the bladder. In an unspecialized fish like the perch, which has no mechanical linkage between the swimbladder and the ear, these amplified displacements will reach the ear through the intervening body tissues. The swimbladder-induced vibrations stimulating the ear will therefore have a radial direction to the bladder. For all the recording loci the sacculus showed maximal horizontal sensitivity when it was vibrated approximately parallel to its own long axis. This axis has an orientation which ensure nearly optimal horizontal stimulation of the saccular macula by vibrations radiating from the swimbladder (Text-fig. 12 and Pl. 1, fig. 1).

In most of their haddocks Enger et al. (1973) found optimal horizontal sensitivity of the saccular microphonic potentials when vibrating the fish parallel to its long axis, while the present study showed optimal sensitivity at vibrations deviating about 20° from the long axis of the fish. However, due to their coarse recording technique, Enger et al. probably picked up potentials from both right and left sacculi simultaneously, and this may explain the minor difference between their results and the present results. On the other hand, Enger et al. also presented data from a fish which showed maximal saccular microphonic potentials when it was vibrated horizontally at right angle to its long axis, which is in direct contrast to the results from perch. The authors were rather cautious in interpreting these data, and suggested that confusion might have occurred by recording from haircells which were sensitive to vertical vibrations, which in this particular case were relatively large. In the light of the present study this explanation seems reasonable, and it is concluded that the directional sensitivity of the sacculus to horizontal vibrations facilitates reception of the sound-induced swimbladder pulsations.

In perch the anterior part of the sacculus was equally sensitive to vertical and horizontal vibrations, while responses from the posterior part were most easily evoked by vertical vibrations. If the orientation pattern of the saccular hair cells in burbot and goldfish is valid for perch, i.e. the hair cells are vertically oriented, the observed ability of horizontal vibrations to evoke saccular microphonic potentials needs an explanation. The movements of the otoliths are not necessarily linear when the fish is exposed to sound. Torques acting on the stone during the forced vibrations may arise from the unsymmetrical shape of the otolith in combination with its suspension and the surrounding liquid. The result will be rotational or rocking movements of the otolith, and a horizontal driving force may thus be translated into vertical otolith movements.

The present study showed that the lagena, and to a lesser degree the posterior part of the sacculus, were mainly sensitive to vertical vibrations, while the vibrations radiating from the swimbladder will have a nearly horizontal direction at the ear. For 200 Hz the lagena was measured to be 5·6 times, or 15 dB, more sensitive to vertical than to horizontal vibrations. However, since it was impossible to generate pure horizontal vibrations, this ratio may well be underestimated. At 100 Hz the measured ratio was 2·9 or 9 dB. The auditory gain due to the swimbladder effect is probably not more than 20 dB at 200 Hz, and the effect will decrease towards lower frequencies (Sand & Enger, 1973; Chapman & Sand, 1974). It is thus not unreasonable to suppose that the lagena is about equally affected by the incident and the re-radiated sound if the sound direction is vertical to the fish. This is in contrast to the anterior part of the sacullar macula, which seems to be mainly stimulated by swimbladder vibrations, whereas the incident sound has relatively little effect.

Mechanical frequency analysis

According to the hypothesis described above, linear driving forces may be translated into complicated rotational movements of the otoliths during vibration of the fish. For the lagena and the posterior part of the sacculus the ratio between vertical and horizontal sensitivity was noted to be frequency dependent. Since the sensitivity axes of the hair cells are independent of frequency, this observation is interpreted as indication of a frequency-dependent pattern of otolith movements during sound stimulation. From this it follows that the part of the macula which is maximally stimulated by the otolith may change with frequency, which constitutes a possible basis for peripheral frequency analysis in fish. The evidence for this theory is sparse and unsatisfactory, but techniques are today available which may be used to measure the vibration pattern of otoliths directly (see for instance Michelsen, 1971). It is suggested that future work should employ such techniques to explore the present notion of a frequency-dependent pattern of otolith movements.

In fish possessing a swimbladder the otolith system will be exposed to forced oscillations radiating from the swimbladder. For most audible frequencies these oscillations will greatly exceed the amplitude of the displacements due to the incident sound. Changes in the pattern of movements of the otoliths will therefore be due to alterations of frequency, while a change of the direction of the incident sound will have comparatively little effect. However, for species without a swimbladder the

direction of the forced otolith vibrations is not fixed, and it is difficult to imagine how the mechanism for mechanical frequency analysis outlined above may be operating.

Directional hearing

Enger *et al.* (1973) suggested that different hair cells in the ear may have different axes of maximal sensitivity to vibration, and that this would provide a possible neurological explanation for the ability of fish to detect the direction to a sound source. However, they only studied the effect of horizontal vibrations, and their data did not conclusively support their theory. For fish possessing a swimbladder the main problem would be possible masking of the incident sound by the amplified vibrations re-radiating from the bladder. Since the lagena is mainly sensitive to vertical vibrations, the swimbladder will influence this part of the ear much less than the sacculus. Although the effect of vertical sound on the sacculus is heavily masked by re-radiated swimbladder pulsations, especially at the anterior end, the incident sound may be efficient in stimulating the lagena directly. By comparing the nervous output from the lagena and the anterior part of the sacculus, the fish should then be able to get directional information about the vertical position of the sound source. Since no evidence exists to confirm that such a mechanism is operating in practice, the theory presented above must be considered tentative. The same must be stressed for the development of the theory below, where a possible mechanism for directional sensitivity in the horizontal plane is suggested.

For fish lacking a swimbladder, and with a similar directional sensitivity of the saccular microphonic potentials as shown in Text-fig. 12, directional information about the horizontal position of the sound source could be obtained by comparing the nervous output from the two ears. For instance, if the horizontal direction of the sound source is at an angle of 70° to the long axis of the fish, the microphonic potentials evoked in the right sacculus would exceed those in the left by approximately 8 dB. In sharks, which have no swimbladder, orientation to low-frequency sounds over long distances in their natural environment has been demonstrated (Myrberg *et al.* 1972; Nelson & Johnson, 1972). However, the amplified swimbladder displacements will affect the two ears equally, and since the bladder is a pressure/displacement transformer, the auditory gain it provides will be independent of the direction to the sound source. The microphonic sensitivity to horizontal vibrations was nearly maximal for radial directions to the swimbladder for all parts of the pars inferior. It is therefore difficult to imagine how the horizontal component of the incident sound may avoid being masked by the vibrations re-radiating from the swimbladder. However, the swimbladder effect will not cause a total elimination of the difference in response to horizontal vibrations between the two ears, and it may be that the fish is able to utilize these minor differences. Furthermore, the auditory effect of the swimbladder will decrease towards lower frequencies, and this may improve directional hearing. A fish possessing a swimbladder seems nevertheless to be better suited for detecting the vertical than the horizontal position of a sound source, which is in direct contrast to the situation in man (von Békésy, 1960). On the other hand, no information is available on the ability of fish to discriminate between different sound directions in the vertical plane. In any case, the fish will only get directional information from the incident sound, and not from the amplified vibrations

emanating from the swimbladder. As pointed out by Enger *et al.* (1973), this implies that the threshold for detection of the direction of a sound should be higher than for simply detecting the sound itself.

This hypothesis for directional hearing in fish is oversimplified. For instance, how the fish may avoid confusion due to directional ambiguity is not discussed at all. On the other hand, it is demonstrated that different parts of the maculae in the pars inferior have different axes of optimal sensitivity, and it is concluded that these differences in sensitivity may be utilized in directional hearing. Such a model would be very simple for fish lacking a swimbladder, but even in fish which take auditory advantage of a swimbladder this model provides a possible explanation of the ability of these fish to detect the direction of a sound source.

The behavioural data on directional hearing in fish is too sparse to support the model presented above. It is therefore suggested that future behaviouristic experiments should be conducted to test the following assumptions. For a fish possessing a swimbladder the threshold for simply detecting a sound should be lower than the threshold for detecting the sound direction. It should also be easier to detect direction at low frequencies, where the effects of the swimbladder are less. Furthermore, the capacity of a fish to determine the vertical direction of a sound source should be at least as good as the ability to detect the horizontal direction.

In regard to the directional sensitivity in the horizontal plane, it is possible, on the basis of the data given in this paper, to make a tentative estimate of the power of angle separation. If each of the number 8-shaped microphonic response curves presented in Text-fig. 12 is simplified to consist of two touching circles of diameter a, the difference in response between the two ears is easily calculated. The angle between the long axes of the two 8-shaped figures is 2α, and the angle between the long axis of the fish and the direction of vibration is ϕ. Within the sectors $270° + \alpha < \phi < (90° - \alpha)$ and $90° + \alpha < \phi < (270° - \alpha)$ the difference in response is given by:

$$F_1 = \alpha \cos(\phi - \alpha) - a \cos(\phi + \alpha) = 2a \sin \alpha \sin \phi. \quad (1)$$

Within the sectors $90° - \alpha < \phi < 90° + \alpha$ and $270° - \alpha < \phi < 270° + \alpha$ the difference is given by:

$$F_2 = \alpha \cos(\phi - \alpha) + a \cos(\phi + \alpha) = 2a \cos \alpha \cos \phi. \quad (2)$$

It is reasonable to propose that the power of angle separation is optimal for the sectors where a small change of vibration angle gives maximal change of the difference in response between the ears; or, in other words, for the vibration angles where the numerical value of the derivative of F is maximal.

$$\frac{dF_1}{d\phi} = 2a \sin \alpha \cos \phi, \quad (3)$$

$$\frac{dF_2}{d\phi} = -2a \cos \alpha \sin \phi. \quad (4)$$

For perch the value of α was about 20°, which gives:

$$\frac{dF_1}{d\phi} \approx 0·68a \cos \phi, \quad (5)$$

$$\frac{dF_2}{d\phi} \approx -1·98a \sin \phi. \quad (6)$$

It is seen that the derivative of F has maximum values for $0°$, $180°$ and $90°$, $270°$. Furthermore, because the numerical value of $dF_2/d\phi$ at its maxima is greater than the maximal $dF_1/d\phi$ value, this should imply that for horizontal sound waves the power of angle separation in perch is optimal for sound directions side on to the fish.

SUMMARY

1. Microphonic potentials were recorded from the lagena and from different parts of the sacculus in the perch during horizontal and vertical vibration of the fish in air. This stimulation technique gives a good simulation of sound stimulation in water.

2. The lagena was predominantly sensitive to vertical vibrations, whereas the anterior part of the sacculus was equally sensitive to vertical and horizontal vibrations. A gradient is seen along the sacculus, in that the more posterior positions show a tendency towards greater relative vertical sensitivity. By comparing the nervous output from the lagena and from the sacculus the fish might thus get information about the vertical direction of the sound source.

3. The amplitude of the saccular microphonic potentials evoked by horizontal vibrations was a function of the vibration direction. Maximal responses were obtained when this direction deviated about $20°$ from the long axis of the fish, which is approximately parallel to the long axis of the sacculi. The difference in response between the two ears might be utilized to give directional information about the horizontal position of the sound source.

4. Sound-induced pulsations radiating from the swimbladder will be efficient in evoking saccular microphonic potentials. This causes masking of the difference in response between the two ears, but directional information may still be obtained. It is proposed that the power of angle separation in the horizontal plane should be optimal for sound waves side on to the fish, and that a fish possessing a swimbladder may be able to detect the sound direction with higher accuracy in the vertical than in the horizontal plane.

5. The relative effect in evoking microphonic potentials of vertical compared to horizontal vibrations was frequency dependent, and it is concluded that the pattern of otolith movements during sound stimulation may also change with frequency. This phenomenon constitutes a possible basis for peripheral frequency analysis in fish.

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EXPLANATION OF PLATE

Fig. 1. Radiographs of a 20 cm perch, dorsal (A) and lateral (B) view. Note the position of the sacculi (*sa*) and the swim-bladder (*sb*). The angle between the broken rules is 40°, which is close to the angle between the sacculi.