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DIRECTIONAL SENSITIVITY OF SACCULAR MICROPHONIC POTENTIALS IN THE HADDOCK

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INTRODUCTION

The function of the swimbladder in fish hearing is well established for the ostariophysine species, which possess a chain of ossicles connecting the swimbladder mechanically to the perilymph of the labyrinth (cf. von Frisch, 1936; Poggendorf, 1952). However, the auditory function of the swimbladder in fish lacking a mechanical connexion between the swimbladder and the ear is less clear. Van Bergeijk (1964) and Alexander (1966), from hypothetical considerations, have pointed out the possible widespread use of the swimbladder in sound reception. They suggested that the swimbladder might function as a pressure/displacement transformer, even in the absence of a mechanical linkage, acting as a secondary sound-source re-radiating near-field particle displacements which stimulate the auditory receptors. However, experimental evidence supporting this theory has been sparse. Several authors have compared the hearing of species with and without swimbladders under the same acoustic conditions and concluded that the swimbladder is involved in sound reception (Enger & Andersen, 1967; Iversen, 1969; Chapman & Sand, 1973). Recently a more direct approach has been employed by Sand & Enger (1973), who showed that in cod kept at 6 m depth the saccular microphonic potentials were drastically reduced when the swimbladder was emptied by sucking the gas out of it through a hypodermic syringe.

The advantage of utilizing the swimbladder pulsations in hearing is a lowering of the auditory threshold. A concomitant disadvantage, however, is a possible loss of ability to determine the direction of the sound source (van Bergeijk, 1964). This follows from the assumption that the re-radiated sound from the swimbladder will mask any differences in arrival time, phase and intensity of the incident sound at the two ears. Earlier behavioural studies supported this view by indicating that fish are unable to determine the direction of a sound source except at close range, where the lateral line organs probably are involved (von Frisch & Dijkgraf, 1935). Sharks are known to have directional hearing at long range (Nelson & Gruber, 1963; Myrberg, Banner & Richard, 1969), but recent investigations by Olsen (1969) on herring and by Schuijf, Baretta & Wildschut (1972) on wrasse (*Labrus berggylta*), undertaken under much better acoustic conditions than earlier studies, have shown that fish with swimbladders can also distinguish between different sound-source directions. Herring were reported to be able to determine direction within at least 45°, probably

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better, for frequencies from 20 to 6000 Hz, whereas the wrasse at 115 Hz (the only frequency tested) showed a directional resolution better than 70° and possibly as good as 10°.

This experimental evidence thus conflicts with any theory which supposes that directional hearing is impaired in fish possessing a swimbladder. However, as stressed by Schuijf *et al.* (1972), the excitation of the maculae in the pars inferior by shearing forces makes this receptor inherently directionally sensitive. It is therefore possible that stimulation of the maculae by re-radiated sound from the swimbladder is avoided by a proper orientation of the hair-cells, i.e. some or all of the hair-cells could be so arranged that they are insensitive to radial displacements emanating from the swimbladder. Wersäll, Flock & Lundquist (1965) have examined the orientation of the hair-cells in the sacculus of a gadoid fish, the burbot (*Lota lota*). From the figure they present it would appear that the hair-cells are arranged in two opposing directions, giving maximal sensitivity to vertical or near-vertical displacement of the otolith. However, the macula has a complex curved shape, which suggests that taken as a whole it is not exclusively sensitive to any single displacement direction. The directional response of the otolith organ is determined not only by the orientation of the hair-cells but also by the nature of the otolith suspension. The manner in which the otolith is suspended or mounted may restrict its motion to particular directions. Little information is available on this question, however.

Clearly, experimental evidence is needed to establish whether fish with an unspecialized swimbladder are sensitive to displacements radiated from the bladder. Furthermore, it must be established whether separate groups of hair-cells are sensitive to other vibration directions, thus providing the fish with directional sensitivity. To clarify these questions we have therefore measured saccular microphonic potentials in the haddock (*Melanogrammus aeglefinus*) as a function of the direction of vibration. To obtain well-defined directional stimuli the experiments were performed by mounting the fish on a rotatable vibration table.

MATERIALS AND METHODS

Eight haddock (*Melanogrammus aeglefinus*), about 30 cm long, were used in the experiments. The procedure employed to detect and record the saccular microphonics was essentially that described by Enger & Andersen (1967). The fish was anaesthetized in a solution of MS–222 (Sandoz, Basle). During the operation, and later during the experiments, artificial respiration was provided by running sea water across the gills. The anaesthesia was maintained by intermittently switching from fresh sea water to sea water containing MS–222. The skull was exposed and two recording electrodes, made of 0·30 mm diameter stainless-steel wires diamel-insulated except at the tips, were implanted through small holes in the skull. One electrode was placed with the tip as close as possible to the sensory epithelium of one of the sacculi. Post-mortem dissection was performed to determine the approximate electrode position. The other electrode was placed symmetrically and contralaterally to the first one but was shorter, with its tip only having contact with the extra-cranial fluid. Thereby microphonic potentials were essentially picked up from the longest electrode, but differential recording from the two electrodes minimized in-phase signals, such as

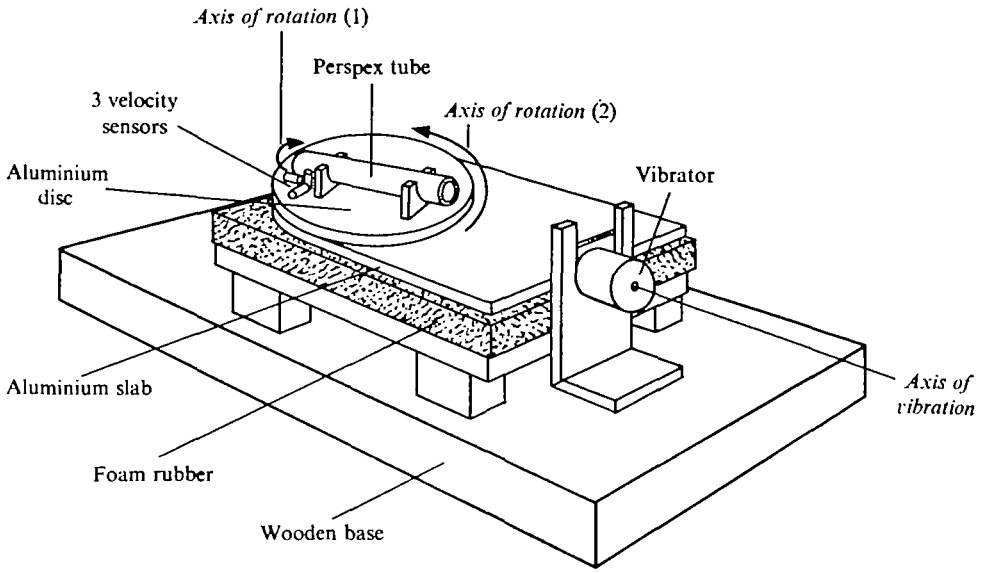

Fig. 1. Vibration table. Perspex tube with fish is rotatable around its long axis and in the horizontal plane. Further explanation in text.

those produced by gill movements. The electrodes were bent at right angles with the proximal part of the electrode resting on the skull, where it was secured by dental cement. The thin flexible electrode leads were attached to the skin by sutures at two points, just behind the incision. A short piece of stainless-steel wire inserted into the back muscles served as the ground electrode.

With the electrodes implanted, the fish was placed in a perspex tube 30 cm long and 6 cm in diameter. A pair of stainless-steel bars clamped the fish skull firmly, just above the eyes. The tube was then placed on the vibration table.

Stimulating and recording equipment. The vibration table, shown schematically in Fig. 1, consisted of a $38 \times 28 \times 1{\cdot}3$ cm slab of aluminium resting upon a foam-rubber bed. The slab was driven back and forth by an electro-magnetic vibrator (Derritron, VP 2 MM) fed by amplified signals (Derritron, 20 W power amplifier) from a sine-wave oscillator (Brüel & Kjær, 1022). The fish tube was attached to a disc of aluminium 1·3 cm thick and 28 cm in diameter. The tube could be rotated in two axes, namely (1) around its long axis and (2) in the horizontal plane around the vertical axis by turning the disc. (Numbers refer to Fig. 1.) The position of the tube relative to the table could be readily adjusted, but it was firmly tightened with screws before each measurement. In describing the orientation of the fish we will refer to two angles —the angle of tilt (rotation about axis 1) and the angle of aximuth (rotation about axis 2). The movement of the table was measured by three velocity transducers (SM–2, Sensor, Netherlands) attached to the disc in three mutually perpendicular directions, close to the head of the fish. Two of the transducers were sensitive in a horizontal direction along the long axis of the fish, and at right angles to this axis, whereas one was sensitive in the vertical direction. The motion of the table was thus completely described. Signals from the transducers were fed into separate low-noise amplifiers (Brookdeal, 450) for amplification and measurement. Vibration velocity values

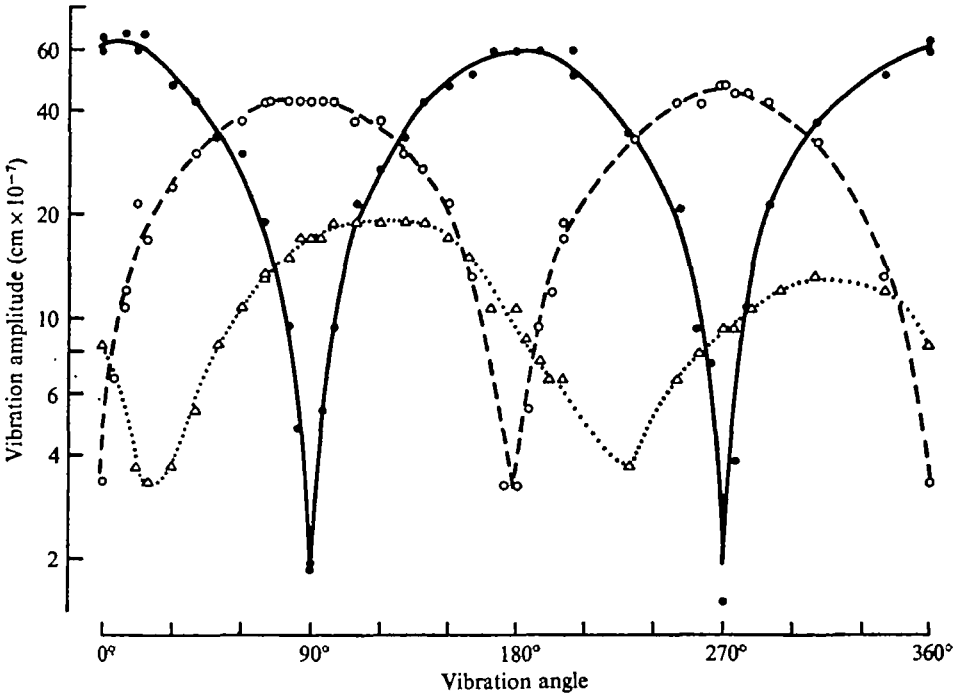

Fig. 2. Vibration amplitude at 200 Hz of the rotatable, experimental table, versus azimuth angle of the fish holder, measured with three transducers. Two are placed in the horizontal plane, parallel to the long axis of the fish (●), and at the right angles to the long axis of the fish (○), and one placed in the vertical axis (△).

(rms) were recalculated to displacement values. One series of measurements, as the fish was rotated stepwise from 0° to 360°, is given in Fig. 2 for 200 Hz. It can be seen that the two horizontal components were 90° out of phase, as expected, and that the ratio between the horizontal vibration component parallel to the driving force and the horizontal component perpendicular to this direction was as high as 30. The horizontal movement of the disc was thus essentially linear. The vertical vibrations varied with the angular position of the fish holder and in some positions were unfortunately rather high. The maximum amplitude was about five times the minimum, reaching values of about one-third of the horizontal vibration amplitude. The table responded in much the same way to the other frequencies tested.

Microphonic potentials from the fish were amplified by a pre-amplifier (Tektronix 122) and displayed on a storage oscilloscope (Tektronix 564). The signal was also fed into a logarithmic level recorder (Brüel & Kjær, 2305) for measurement, after being amplified, and in some cases filtered through a frequency analyser (Brüel & Kjær, 2107). Microphonic potentials are in this paper given in dB re 1μVrms.

RESULTS

Examples of the saccular microphonic potentials recorded from a haddock during vibration of constant amplitude at a freqnuecy of 100 Hz are shown in Fig. 3. The frequency of these potentials is twice the frequency of vibration, as is commonly

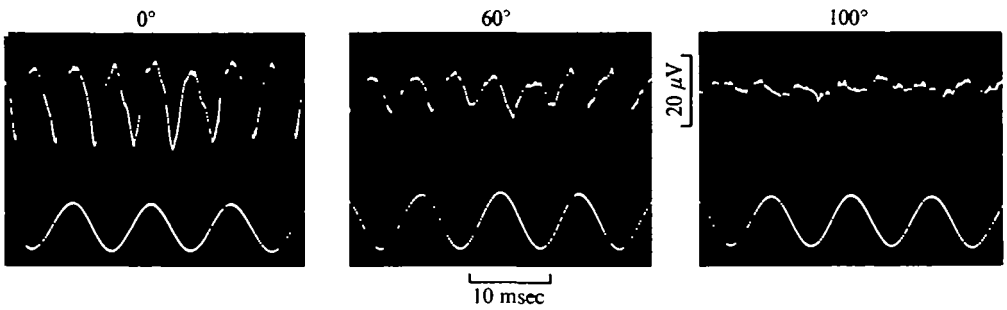

Fig. 3. Saccular microphonic potentials (upper trace) recorded during 100 Hz vibration of $1{\cdot}2 \times 10^{-5}$ cm amplitude (lower trace) at three different angles of azimuth.

found when recording from the fish sacculus during sound stimulation (Zotterman, 1943; Enger & Andersen, 1967). The amplitude of the potentials was a function both of the stimulus strength and of the direction of vibration. In this and most other cases the fish was subjected to vibration in the normal upright position (zero tilt). However, some experiments were performed on fish which were tilted about the long axis, as mentioned below.

In Fig. 3 the maximum microphonic amplitude was recorded at an azimuth angle of 0° (i.e. when the fish was subjected to vibration along its axis). A minimum was recorded at an azimuth angle of 100° (i.e. when the fish was subjected to vibration from the side). The microphonic potential amplitudes recorded from two different fish at 100 and 200 Hz, respectively, are plotted versus the angle of azimuth in Fig. 4A and B. The amplitude minima were rather sharp at 90° and 270° in A, and at 100° and 280° in B. In most other fish tested corresponding minimum values were obtained at azimuth angles in the range 85–105° and 260–280°. Maximum amplitudes in these cases were recorded over a wide sector of 60–70°, centred around 0° and 180°.

A comparison between Figs. 2 and 3 confirms that the microphonic potential amplitude reached a maximum when the horizontal vibration component along the long axis of the fish was maximal (i.e. at azimuth angles 0–180°). At the angle of minimum microphonic response the horizontal vibration component transverse to the long axis was maximal and the vertical vibration component close to maximal. Therefore, it must be concluded that the sensory cells which produced the recorded responses were relatively insensitive to vibration in these directions.

The above description applied to most recordings, but one of the notable exceptions is presented in Fig. 5. This figure shows that the maximum microphonic potential amplitude was obtained from this particular fish when it was subjected to vibration in a direction at right angles to the long axis (i.e. at azimuth angles of 90–270°). This result indicates that the sensory cells contributing to the recorded potentials at this electrode position are mainly sensitive to transverse vibration of the fish. However, in this particular case the amplitude of the vertical vibration component was relatively large (though not at a maximum) with the fish in this position. We therefore cannot eliminate the possibility that a sensitivity to vertical vibrations might have contributed to the maximal response.

In Figs. 3 and 4 the amplitude difference in microphonic potential between maximum and minimum was 9–13 dB. In other recordings this difference could be much

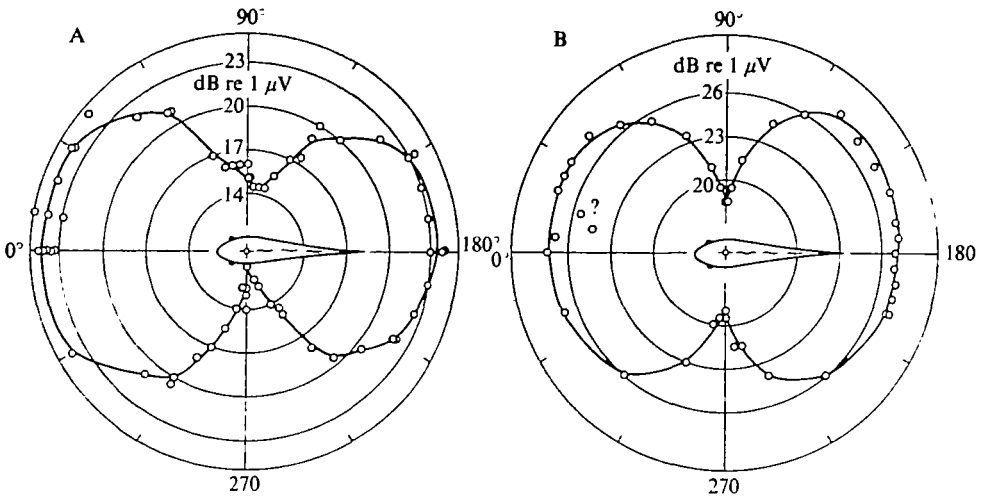

Fig. 4. Polar diagrams, showing rms-values of saccular microphonic potential at different angles of azimuth. Note that maximal response is obtained to vibration along the long axis of the fish (azimuth of 0 and 180° angles). A. Vibration frequency 100 Hz, amplitude $1{\cdot}2 \times 10^{-5}$ cm. B. Vibration frequency 200 Hz, amplitude 6×10^{-6} cm (another fish).

less (4–6 dB), and the variation pattern did not seem to follow any of the vibration components given in Fig. 1. One reason for this might be that we were simultaneously recording from cells of different directional sensitivity. Alternatively the cells may have been mainly sensitive to the vertical vibration component (which varied much less than the horizontal components).

We must therefore stress that though our data indicate that in the majority of fish examined the parts of the sensory macula examined showed greatest sensitivity to vibration along the long axis of the animal, in other preparations greatest sensitivity was shown to vibration in other directions.

In some cases saccular microphonic potentials were also recorded while the fish was tilted stepwise around its long axis. If this was done at azimuth angles giving maximal microphonic potentials, the microphonic potentials decreased in amplitude when the fish was rotated from its normal upright position towards a position with the dorsal side down. Furthermore, if the fish was quickly returned to the upright position, several seconds elapsed before the microphonic potential amplitude regained its previous maximal amplitude. This indicates that the position of the otoliths changes only slowly when the fish is tilted, and that the movement of the otolith must therefore be opposed by relatively strong visco-elastic forces.

DISCUSSION

In the fish labyrinth a clear distinction between auditory and equilibrium functions with respect to anatomical localization is difficult to make (cf. Lowenstein, 1971). In particular, the lagena can have either function in different species while the sacculus has a predominantly auditory and the utriculus an equilibrium function. A partial overlapping in functions of the three otolith organs is possible. The present study was undertaken to determine whether the saccular sensory epitheliam has any particu-

ar axis of highest sensitivity to vibrational stimuli. This has a bearing on two aspects of fish hearing, namely (1) directional hearing, i.e. the ability of the fish to determine the direction of the sound source, and (2) the role of the swimbladder in the hearing process. The latter aspect becomes clear when bearing in mind that the swimbladder may act as a pressure/displacement transformer and thus produce displacements which stimulate the ear in a direction along the long axis of the fish.

At first sight our method of subjecting the fish to vibration in order to simulate auditory stimulation may seem unusual and unnatural, since it only produces particle displacement on the experimental table and not sound pressure. However, we believe that this method is justified since it now seems to be well established that particle displacement, and not sound pressure, is the adequate stimulus for the auditory receptors. To use pure vibrational stimuli is advantageous in that any effects arising from the swimbladder are eliminated. In addition, the vibration direction can be readily determined and thus yield information about the possible sensitivity axes of the sensory cells. The vibration amplitude of the vibration table reached a maximum of $1{\cdot}2 \times 10^{-5}$ cm at 100 Hz. In comparison, threshold values for particle displacement in dab (Chapman & Sand, 1973) are around 3×10^{-9} cm in the optimal frequency range, thus giving a difference of 72 dB. An auditory stimulus intensity of 72 dB above threshold cannot be considered abnormally high, and artifacts caused by overdriving the hearing system probably did not play a role in our experiments.

The position of the electrode in all the experiments performed was found to be in the middle and anterior half of the saccular sensory epithelium, but no detailed and systematic mapping of the recording loci was performed. Generally, it must be assumed that the greatest contribution to the amplitude of the microphonic potentials comes from the cells closest to the electrode. From this rather safe assumption it is quite clear that a certain proportion of saccular sensory cells have their axes of highest sensitivity to vibratory stimuli orientated along the long axis of the fish. It seems therefore justified to conclude that a definite prerequisite for the participation of the swimbladder in fish hearing, namely that the otolith/hair cell system is sensitive to vibrations issuing radially from the swimbladder, is fulfilled.

There is an apparent discrepancy between this finding and the morphological indication of a predominantly dorso-ventral sensitivity axis in the macula (Wersäll *et al.* 1965). This difference must be sought either in a species difference or, more likely, in that the suspension of the otoliths causes complicated otolith movements when the fish is subjected to vibration. However, the vibration pattern of the otoliths in response to auditory or vibrational stimuli is not known. We suggest that this is a field which is definitely worth further study.

Van Bergeijk (1964) pointed out that the participation of the swimbladder in hearing might obscure the detection of the direction to a sound source, but he considered that only the displacements re-radiated in the near field of the swimbladder were adequate to stimulate the hair cells. However, the individual hair cells are inherently directionally sensitive, and it is possible that they might extract directional data directly from the particle displacements caused by the incident sound. Though the present study has indicated that many saccular sensory cells are maximally sensitive to vibrations in the direction from the swimbladder, it was also evident that other sensory cells are maximally sensitive to vibrations in other directions. One example of a presumably

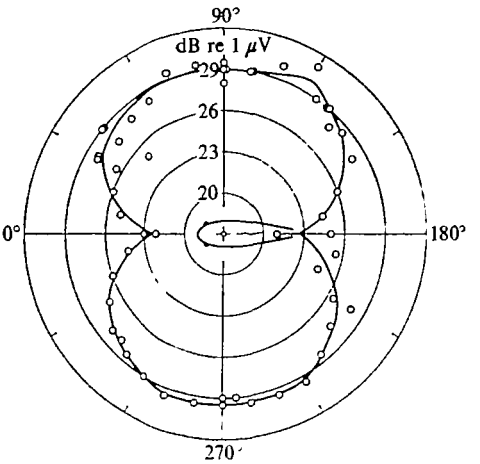

Fig. 5. As for Fig. 4, but note that maximal response is obtained to vibration at right angles to the long axis of the fish (azimuth angles of 90 and 270°). Vibration frequency 175 Hz, amplitude $6{\cdot}9 \times 10^{-6}$ cm.

transversal sensitivity maximum in the horizontal plane has been presented in Fig. 5, and other recordings indicate sensitivity axes in other directions. Although further studies are required for a complete description of the response patterns of the sensory cells, the results presented provide the first step towards a neurological understanding of the behaviouristically observed ability of fish to detect the direction of the sound source (Olsen, 1969; Schuijf *et al.* 1972).

We suggest that one possible way in which a fish could detect the direction of a sound source is by possessing different groups of hair cells in the ear, having different axes of maximal sensitivity. While some of these cells may be sensitive to particle displacements re-radiated from the swimbladder, other cells are rather insensitive to displacements in this direction. The latter cells may thus detect the particle displacements of the incident sound, without being affected by the amplified displacements from the swimbladder. If such a mechanism is operating, one would expect to find a considerably higher threshold for detection of the direction of a sound source than for simply detecting the sound itself.

SUMMARY

1. Microphonic potentials from the sacculus in the haddock have been recorded by implanted electrodes during horizontal vibration of the fish in air. This gives a good simulation of sound stimulation in water.

2. The microphonic potential amplitude was a function of the vibration angle, and from most recording loci maximal amplitudes were obtained for vibration directions parallel to the long axis of the fish. The sensory cells contributing to this response are therefore most sensitive to displacements in the same direction as sound-induced swimbladder pulsations would produce. This result thus supports the theory of an accessory role of the swimbladder in sound reception.

3. Highest sensitivity to vibration directions other than parallel to the long axis

f the fish has been obtained from other recording loci. One example of highest sensitivity to a vibration direction at right angle to the long axis of the fish is presented.

4. The findings that different sensory cells appear to have different axes of maximal sensitivity to vibration provides one possible neurological explanation for the ability of fish to detect the direction to a sound source.

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