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Vibration Measurements of the Perch Saccular Otolith

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Summary. 1. The vertical movement of different parts of the perch saccular otolith was measured with a laser vibrometer during horizontal vibration of the fish back and forth along its long axis. Data were obtained at four different frequencies within the audible range of the fish. Vibration at these frequencies caused very little vertical movement of the skull.

2. No vertical oscillations of the otolith were detected at 20 Hz, whereas both ends of the otolith showed vertical vibrations at 40, 90 and 220 Hz. An area of minimum vertical movement appeared around the midpoint of the otolith at these frequencies, thus indicating the existence of a horizontal axis of rotation.

3. It is argued that the stimulation technique is a reasonable approximation to underwater sound exposure. The measurements thus support the idea of a coarse, peripheral frequency analysis in fish based on a frequency dependent pattern of sound induced otolith movements.

Introduction

The sacculus and the lagena are the parts of the teleost ear which are mainly involved in hearing. These structures consist of an endolymph-filled sac containing a heavy calcareous otolith in close contact with the sensory epithelium. The otoliths have a specific gravity of about 2.9 (de Vries, 1950). Due to their greater density the otoliths lag behind the motion of the sensory epithelia when the fish is vibrated in a sound field, thus creating shearing movements of the sensory hairs.

Fish lack an obvious mechanical frequency analyzer comparable to the mammalian cochlea. Behavioural studies have, nevertheless, shown fish to have a well developed ability of frequency discrimination (see Sand and Enger, 1974). Detection of only 3–4 % frequency difference has been reported for ostariophysine species. In comparison, humans are able to discriminate between tones differing about 0.2 % in frequency (within the optimal frequency range).

The mechanism for frequency discrimination in fish is not known, but there are two hypothetical possibilities: 1) Central analysis exploiting the synchronization between sound frequency and nerve impulse discharge, and 2) peripheral analysis based upon sensory units having different frequency sensitivities. Recordings from auditory neurones in fish show a clear phase locking between the afferent spikes and the sound stimuli, particularly at lower frequencies (Lowenstein and Roberts, 1951; Enger, 1963; Furukawa and Ishii, 1967). In addition, the tuning curves of single auditory nerve fibers may cover different frequency ranges (Enger, 1963; Furukawa and Ishii, 1967). The tuning of these fibers is considerably broader than those of mammals possessing a cochlea (Evans and Wilson, 1973). The data are, on the other hand, in agreement with the idea that fish may have a coarse, peripheral analyzer in addition to a central one.

Differences in the frequency sensitivity of the receptor cells may be caused by variation in size, sensory hair structure, stiffness etc. (Stylis, 1971). Such mechanisms have not been supported experimentally in either vertebrates or invertebrates. Another hypothesis has been introduced by van Bergeijk (1967), who suggested that sound stimulation might cause frequency dependent travelling waves along the fish maculae, thus creating a spatial distribution of stimulation maxima. The mechanism would be rather similar to the mechanism of frequency analysis in the cochlea. Other mechanisms based on the mechanical properties of the peripheral auditory system might, however, be responsible for a spatial distribution of stimulation maxima (Sand, 1974a):

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Most teleosts have a gas filled swimbladder, and this organ may have an auditory function by acting as a pressure-movement transformer (Sand and Enger, 1973). The effective driving force acting on the inner ear in a fish exposed to pressure waves will thus be oscillations of the body originating from and travelling radial to the swimbladder, irrespective of the direction of the incident sound pressure wave. The otoliths are situated almost at the same horizontal level as the swimbladder. The otoliths should therefore oscillate in the horizontal plane when the fish is exposed to sound pressure waves. The movements of the otoliths may, however, also have a component in the vertical plane, since the asymmetrical shape and suspension of the otolith may cause rotational movements. Furthermore, a propeller effect may create torques acting on the stone due to the relative movements between the asymmetrical otolith and the surrounding liquid. A horizontal driving force could thus partly be translated into vertical otolith movements. Such a translation would be of physiological significance, since parts of the sound sensitive maculae of teleosts are situated in a vertical plane with a dorso-ventral orientation of the hair cells' sensitivity axis (Wersäll et al., 1965; Hama, 1969; Dale, 1976; Jørgensen, 1976; Popper, 1976; Enger, 1977). If the pattern of otolith movement is frequency dependent, then the part of the macula which is maximally stimulated by the otolith may change with frequency.

The present investigation was carried out to test this hypothesis. The vertical movement of different parts of the perch saccular otolith was measured during horizontal vibrations of the fish along its long axis. The measurements indicate the existence of a horizontal axis of rotation of the otolith and provide data for comparison at different frequencies. The measurements were performed using a laser vibrometer, which employs the reflected laser light to determine the vibration velocity of the reflecting surface (Michelsen and Larsen, 1978).

Materials and Methods

Preparation

The measurements were performed on six perch (*Perca fluviatilis*), ranging in length from 18 to 20 cm. The handling and operation of the fish has been described previously (Sand, 1974a). A fish was fixed in a holder, the skull was opened and the brain removed. The two sacculi lying in grooves in the skull floor were then exposed. The grooves are covered with the saccular membrane, which was removed in order to obtain laser reflections from the otolith surface. The reflections from the otoliths were too diffuse to give reliable measurements. Presumably, the somewhat translucent nature of the otoliths caused the light to be reflected not only from the surface, but also from inner parts of the otoliths. Fortunately, the otoliths are so heavy that we could add a small amount of white paint to their surface without a marked change in their weight. The liquid level of the sacculus was therefore temporarily lowered by means of a small suction pipette, to allow the upper rim of the saccular otolith to be painted with white latex paint. After about 15 min the liquid was allowed to rise to its original level. It is not known whether this rise was caused by endolymph drained from other parts of the inner ear, or if the refilling liquid had a different source. White spots were also painted along the skull floor between the two sacculi, in order to facilitate vibration measurements of the skull itself.

The weight of the saccular otoliths used for our measurements ranged from 15 to 19 mg, and the paint added 0.5–0.7 % to these weights. The length of the otoliths was arbitrarily divided into 10 regions, which were numbered 0 to 9. The extreme anterior and posterior positions (named 0 and 9, respectively) were covered by the upper edge of the saccular groove in the skull floor. The vertical motion was measured at the eight remaining, evenly spaced points along the upper rim of the otolith.

Vibration

The vibrating table used for our measurements is a modified version of the type previously described (Sand, 1974a). The fish holder is attached to a $30 \times 16 \times 1.2$ cm aluminum plate, which is vibrated horizontally by a coil vibrator driven by amplified signals from an oscillator (Fig. 1). The fish is clamped in air, so that no interference from water vibrations occurs. The table is freely suspended from steel brackets by steel wires (3 cm long, 0.3 mm diameter) attached at each of the four corners of the table. The movement of the table is monitored by three velocity transducers, positioned on the table in three orthogonal directions. The laser vibrometer was used to measure the vertical vibrations of the skull. At many frequencies the horizontal vibration of the fish caused vigorous vertical movements of the skull. At other frequencies, which were selected for the measurements of otolith movement, vertical movements of the skull could hardly be detected. The frequencies used (20, 40, 90 and 220 Hz) cover most of the audible frequency range in perch, which has an upper auditory frequency cut-off at about 300 Hz (Sand, 1974b). The velocity of the sinusoidal horizontal vibrations was kept constant at 630 μm/s (peak-peak) at all frequencies. This is within the physiological range.

Laser Measurements

The use of laser vibrometry for measuring the vibration velocities in hearing organs is discussed in detail elsewhere (Michelsen and Larsen, 1978). The laser vibrometer utilizes the principle of optical heterodyne detection for measuring the Doppler shift of light scattered from the surface of moving objects (Buchhave, 1975). The laser beam is divided into a measuring beam and a reference beam by a beam-splitter (Fig. 1). The measuring beam is focused on the object (here a spot of 30 μm diameter on the surface of the otolith, or, as a control, on the skull). The reflected light passes through the same lens system onto a pair of photo-sensitive diodes. The reference beam is given a 40 MHz frequency shift in a Bragg cell and is passed onto the diodes. The interaction of the two beams produces a beat frequency of 40 MHz plus the Doppler shift caused by movements of the object. A frequency tracker is used for demodulating the FM-signal, thus providing an analog signal proportional to the instantaneous velocity of the object. The apparatus can be used to detect vibrations from a fraction of a Hz to above 100 kHz.

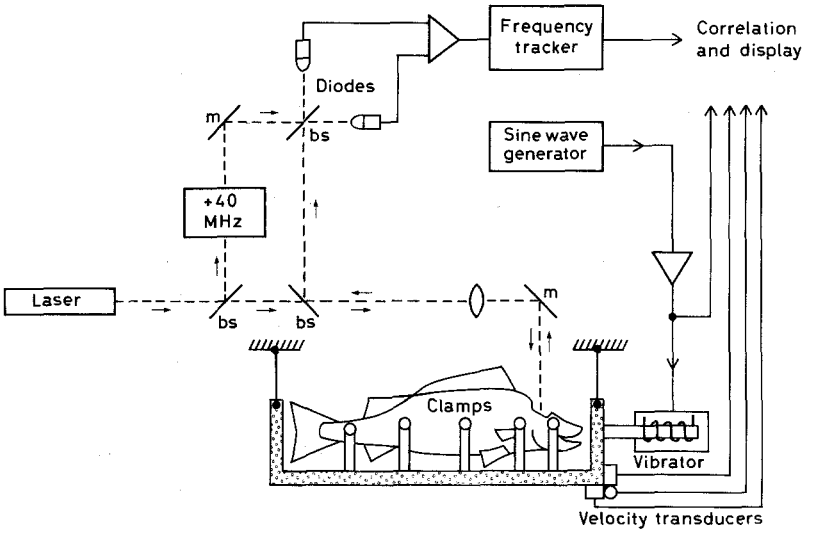

Fig. 1. Block diagram of the experimental set-up. The fish is subjected to controlled, *horizontal* vibration. The *vertical* vibration of the skull and otolith is measured by means of a laser beam. Reflected laser light is sent to light-sensitive diodes, which also receive light from a reference beam (further explanation in the text). *m*, mirror; *bs*, beam splitter

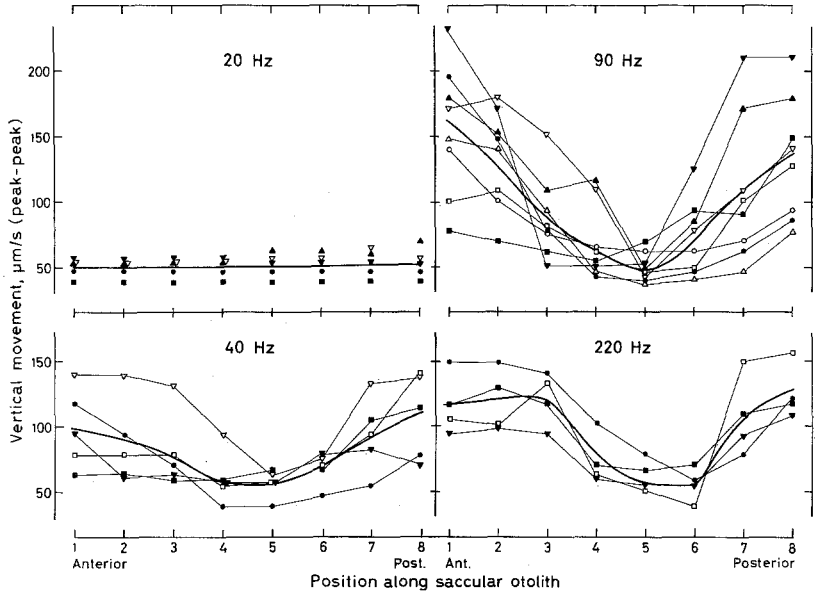

Fig. 2. *Vertical* otolith vibration as a function of position along the saccular otolith. Each symbol represents one otolith. The smooth curves in thick line are drawn through the mean values at the different positions. The vibration frequency is indicated for each set of data. The fish was subjected to a *horizontal*, sinusoidal vibration of 630 µm/s (peak-peak) velocity. The vertical "background" movement of the skull was 30–60 µm/s (peak-peak)

Results

Figure 2 shows the patterns of vertical otolith movements revealed by our measurements. Each symbol represents data from one saccular otolith. The vertical vibration of the skull was between 30 and 60 µm/s at the frequencies investigated (as mentioned, the horizontal vibration was 630 µm/s). At 20 Hz no parts of the otolith showed vertical movements significantly above the "background movement" of the skull. At 40 Hz, however, both ends of the otolith displayed vertical vibrations, and an area of minimum vertical movement appeared around the midpoint of the otolith. The values were here not significantly larger than the "background" vibrations. The highest values measured at the ends of the otolith were about 22% of the forced, horizontal vibrations.

The vibration pattern at 90 Hz was similar to that described for 40 Hz, but the differences between the areas were accentuated. An area of minimum vertical movements equal to the "background" vibration was evident around the midpoint of the otolith, whereas the vertical oscillation close to the otolith ends could reach values up to 37% of the forced, horizontal vibration.

The patterns of vertical movements at 40 and

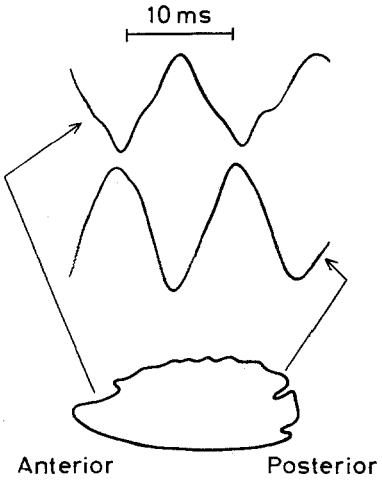

Fig. 3. *Vertical* vibrations of the two opposite ends of the saccular otolith. The fish was subjected to *horizontal* vibrations at 90 Hz. Note that the two ends of the otolith are nearly 180° out of phase

90 Hz suggest the existence of a horizontal rotation axis in the area of minimum vertical movements, and approximately perpendicular to the length axis of the otolith. The vertical movements of the two parts of the otolith on each side of such a rotation axis would be expected to be about 180° out of phase. Figure 3 compares the phase of the vertical vibrations of the two otolith ends at 90 Hz. It is clear that the two ends were moving nearly 180° out of phase. The average phase difference between the two otolith halves was $155 \pm 20°$ (s.d.) at 40 Hz and $168 \pm 14°$ (s.d.) at 90 Hz.

At 220 Hz an area of minimum vertical vibration was still evident, and the vertical velocity of the otolith ends could be up to 24 % of the forced, horizontal vibration velocity. The average phase difference between the two otolith ends was $134 \pm 15°$ (s.d.), but the phase of the vertical movements changed gradually along each of the two otolith halves. The otolith movements at 220 Hz thus seem to be rather complex, possibly involving rotation around more than one axis. But our technique did not permit a full description of this vibration pattern.

The rather large scatter of the points at the three highest frequencies reflects a wide range in absolute values between different otoliths. On the other hand, each otolith followed the basic pattern described above, and the position of the area of minimum vertical vibration at a given frequency was approximately the same for all otoliths. Minimum vertical vibration occurred between positions 4 and 5 at 40 Hz, around 5 at 90 Hz and between 5 and 6 at 220 Hz. The area of minimum vertical motion thus seems to be shifted slightly towards a more posterior position for increasing frequencies, although our data are too sparse to determine if this trend is significant.

Discussion

The present investigation was aimed at testing two related hypothesis, namely: 1) Horizontal vibration of a fish may induce both horizontal and vertical otolith oscillations. 2) The pattern of vertical otolith movements during forced, horizontal vibrations is frequency dependent. Both ideas were clearly confirmed by our measurements. At 40, 90 and 220 Hz, which were the three highest frequencies tested, horizontal vibrations were evidently translated into vertical otolith movements, whereas no such induction of vertical otolith movements was detected at 20 Hz. However, the measured change in vibration pattern with frequency was gradual and undramatic, and the position of the suggested horizontal rotation axis did barely move throughout the frequency range tested.

It is difficult to achieve well defined underwater sound stimulation in experimental tanks, since reflections at the boundaries tend to create standing waves. Workers in aquatic bio-acoustics therefore commonly perform their experiments at considerable depth in the sea. In the present investigation we vibrated the fish in air. The otolith organ in fish without a swimbladder detect the kinetic sound component, rather than sound pressure (Chapman and Sand, 1974). Teleosts possessing a swimbladder may also respond to sound pressure, but in this case the bladder may act as a pressure-movement transformer. The ears of the perch are positioned close to the median plane at about the same level as the swimbladder (Sand, 1974a). Horizontal vibrations along the long axis of a fish in air are, therefore, a reasonable approximation to the forced vibrations from the swimbladder during underwater sound stimulation.

To measure the otolith vibrations, we had to disrupt the inner ear structure severely. Other smaller sources of error might be changes in viscosity of the liquid surrounding the otolith or very light changes in the otolith's centre of gravity. The vibration patterns revealed by our measurements may therefore be a distorted version of otolith vibrations in the intact fish. Nevertheless, we believe that qualitatively the same vibration patterns as those described here occur in the natural situation.

One possible source of error is apparent vertical movement measured during the horizontal vibration because of surface curvature within the otolith area covered by the laser beam. The magnitude of such erroneous velocity signals would, however, be independent of the frequency of vibration, if the horizontal vibration velocity is kept constant. Since only little vertical displacement was detected at 20 Hz, we conclude that the vertical movements detected at

higher frequencies by our laser vibrometer were genuine vertical vibrations.

Enger (1963), recording from 1. order auditory neurons in the non-ostariophysine sculpin *(Cottus scorpius)*, found only three main types of tuning curves. One group of fibres had an optimal frequency range of 100–200 Hz, while in the two other types the sensitivity decreased with increasing frequency. These two groups were distinguished by differences in the slope of the "tuning curves". The peripheral frequency analysis indicated by these neurophysiological data is very coarse indeed, and it might well be accounted for by a mechanical mechanism similar to that suggested here.

The sensory epithelia of the perch sacculus and lagena are situated in vertical planes. The orientation of the sensitivity axis of the hair cells is dorso-ventral in the lagena and in the posterior part of the sacculus, but rostro-caudal in the anterior part of the sacculus (Enger, 1977). Thus, the sensory epithelium of the anterior part of the sacculus will be most sensitive to horizontal otolith movements, while the posterior part is mainly sensitive to vertical movements. Stimulation of 1. order fibres from the posterior part therefore requires translation of horizontal vibrations into vertical otolith movements, and our data hence suggest that these fibres show an optimal frequency range. The decrease in sensitivity towards lower frequencies might then be due to the decreased vertical vibration component of the otolith, while a general loss in hair cell sensitivity might be the most important factor determining the upper frequency cut-off.

The notion that different areas of the sensory macula are sharply tuned to different frequencies must, on the other hand, be rejected. The present measurements only support a very coarse frequency tuning of the hair cells and 1. order auditory fibers, which is in agreement with the neurophysiological data.

Although our experimental techniques do not permit us to draw conclusions regarding the vibrational response in the intact animal, the results do suggest that a frequency dependent pattern of otolith movement during sound exposure might explain the observed peripheral frequency analysis in teleosts. The rather poor peripheral frequency analysis in fish indicates that central mechanisms are relatively more important for frequency discrimination in fish than in higher vertebrates.

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