ACOUSTIC STIMULATION OF THE EAR OF THE GOLDFISH (CARASSIUS AURATUS)

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SUMMARY

Microphonic potentials were recorded from the ears of the goldfish during acoustic stimulation in a situation where sound pressure and particle displacement could be varied. Microphonic potentials from fishes with the swim bladder intact were proportional to sound pressure. After removal of the swim bladder, sound pressure sensitivity declined by 20–35 dB and the response was generated in proportion to particle displacement. The ear's sensitivity to direct vibration of the head increases at between —3 and —6 dB/octave between 70 and 1500 Hz and is not affected by the removal of the swim bladder. It is concluded that the peripheral auditory system of the goldfish may function as a pressure detector or as a displacement detector, depending upon the impedance of the applied signal.

INTRODUCTION

The auditory portions of the inner ears of fishes are unique among vertebrates in that the simple hair cell macula is overlain by a solid calcareous otolith. Hair cell stimulation is presumed to occur as a result of relative shearing movements between the hair cell body and its ciliary hairs, which appear to be in contact with the overlying otolith (Hama, 1969).

The otolithic organs of most animals can be viewed as inertial devices such that movement transmitted within the body tissues is taken up by the dense otolith with an amplitude and phase which differs from that of the surrounding fluid and tissues. This view of auditory reception in fishes is attractive since under water sound energy is readily transmitted through the fish's body because of the close impedance match between water and tissue (Alexander, 1966; van Bergeijk, 1967, and others). In fact, it appears unavoidable that relative movement would occur as the result of the large difference in density between the otolith and adjacent tissues. In general, however, the

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Peripheral auditory apparatus of fishes is comprised of more than the otolithic organs themselves. Most teleost species have an abdominal swim bladder or other gas-filled cavity which in many species is brought to close mechanical contact with the ear (see description in Lowenstein, 1971; Popper and Fay, 1973; Tavolga, 1971). In all members of the superorder Ostariophysi modified portions of the first several vertebrae form an ossicular chain (the Weberian ossicles) between the swim bladder and the fluid systems of the ear. These ossicles have been shown to contribute to the behavioral auditory sensitivity of this group (Poggendorf, 1952).

Wever (1969, 1971) has suggested that the swim bladder and its connexion to the ear via the Weberian ossicles may function in hearing in a way analogous to the round window in the air-filled middle ear cavity of tetrapods. The compressible gas in the bladder may thus provide a pressure release system capable of enhancing the small relative movements set up inertially between the otolith and the hair cells.

An alternative view of the functioning of the ostariophysine ear has been proposed by von Frisch (1938), van Bergeijk (1967) and others. They have emphasized that the swim bladder, as any gas bubble in water, will expand and contract in response to pressure variations at a greater amplitude than will homogeneous tissue. This amplified movement is communicated to the fluids of the ear via the Weberian ossicles where it engages the otoliths through fluid drag and results in relative movement between the cilia and the hair cell body.

In order to test whether the teleost peripheral auditory system is displacement-sensitive according to Wever's view or pressure-sensitive as argued by van Bergeijk, a stimulus field is necessary in which displacement and pressure levels can be independently varied. Clearly, such a manipulation cannot be made using a plane progressive wave in the free field. Two methods which are potentially useful in this respect are manipulations of the near-field effect (van Bergeijk, 1964; Harris & van Bergeijk, 1962), and the manipulation of standing wave patterns (Cahn, Siler & Wodinsky, 1969).

Harris & van Bergeijk (1962) showed that the lateral-line system of the killifish (*Fundulus heteroclitus*) is a displacement detector since the microphonic response from a single receptor organ declined with distance from the sound source in direct proportion to the calculated near-field displacement amplitude. The manipulation of sound source distance has been used in several behavioural (Chapman & Hawkins, 1973; Chapman & Sand, 1974; Enger, 1967) and electrophysiological (Enger & Anderson, 1967) studies of teleost sound detection. These experiments generally show that low frequency auditory sensitivity (measured in sound pressure units) increases within the near-field as the distance between the fish and sound source decreases, showing that the auditory system of the species studied responded to stimulus variables other than sound pressure. For several reasons, however, these near-field experiments do not resolve the question of how the inner ear is stimulated. For example, a demonstration that auditory sensitivity ceases to be related to sound source distance when these distances become large, cannot be taken to suggest that the receptor system involved responds to sound pressure since pressure and displacement attenuate equally with distance in the far-field (Siler, 1969). Similarly, a simple change in the pressure sensitivity of the animal as the sound source is moved closer cannot be proof that the ear responds directly to particle movements. In beha...
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Experiments of this type, receptor organs other than those stimulated in the far-field may become involved. In similar electrophysiological experiments (Enger, 1967; Enger & Anderson, 1967) the possibility that the swim bladder itself may respond to particle displacement was not ruled out. Finally, it must be noted that the amplitude and extent of the near-field varies with the type of sound source used and the frequency of the signal. Experiments which show that pressure sensitivity is independent of sound source distance (for example, Enger & Anderson, 1967) are convincing demonstrations that the system under study is pressure-sensitive only when the existence of the near-field is verified within the range of distances used.

More satisfactory studies of the relative contributions of sound pressure and particle displacement in stimulation of the teleost ear are possible using a standing wave. In a properly generated standing wave, areas where the ratio between pressure and displacement are large alternate with areas in which this ratio is reversed at one-quarter wave-length intervals. It is thus possible to create conditions of either high or low pressure to displacement ratios in the location of a fish providing it is small relative to the wavelengths involved. Using a long waterfilled tube with underwater speakers at either end, Cahn et al. (1969) made behavioural measurements of the auditory sensitivity of two species of grunt at several points within the standing wave. They found that the fish appeared to be displacement sensitive at 100 and 200 Hz, while only pressure sensitive at 400 Hz and above. Although it is not possible to distinguish between inner ear and lateral-line function in these behavioural experiments, the authors interpreted the low frequency displacement sensitivity as being due to lateral-line stimulation.

In order to study the adequate stimulus for the ostariophysine ear, we have applied a modification of the standing wave tube used by Cahn et al. (1969) which is better suited for electrophysiological investigations. Since the frequency range within which relatively uncomplicated standing waves could be generated extended no further than 250−315 Hz, an additional and complementary technique involving direct vibratory stimulation of the head was used. In this way, our analysis was extended throughout the entire frequency range of hearing for the goldfish.

METHODS AND MATERIALS

The experiments involved measuring the amplitude of microphonic potentials from the inner ear of goldfish (Carassius auratus) in response to pure tone standing waves which were varied in sound level, frequency, and location, and to direct vibration of the head. Measurements were also made with animals whose swim bladders had been removed to determine the contributions of this structure to hearing under different acoustic conditions.

A. Standing wave tube

Standing waves were generated in a Poly-vinyl-chloride (PVC) tube (150 cm long; 33 cm inner diameter; 0.5 cm wall thickness) (Fig. 1). The tube was cut and hinged at the centre perpendicular to its long axis to allow access to the inside. Short steel pipes (7.6 cm diameter), attached to the outside of the tube just below the hinges, rested on supports (see Fig. 1). A plastic water-filled bag hung from an oval wooden ring in the
centre of the tube (Fig. 1). The ring was supported by two steel pipes (2.54 cm diameter) which passed through the tube-support pipes and were independently supported outside the tube.

Standing waves were produced by an enclosed loudspeaker (12.32 cm diameter) at both ends of the tube. The standing wave was manipulated by changing the phase and amplitude of the signal to one speaker relative to the other using the electronic control system shown in Fig. 1. The sinusoidal output of a function generator (Ex
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Model 7056) was divided into two channels, one for each speaker. Channel 1 went to an attenuator (Hewlett Packard model 350D), to channel 1 of a stereo power amplifier (Dyna Stereo model 120) and then to one of the speakers. Channel 2 went from the generator to a phase shifter (Keithley model 821) which produced an output signal shifted from 0° to 220° relative to the input signal. The signal was then attenuated and amplified. A double-pole switch was used so that the phase of the channel 2 speaker could be reversed by 180° relative to the other speaker.

In order to achieve a pressure maximum or minimum at the location of the fish in the water bag, a pair of hydrophones (Clevite CH-17T) was placed on either side of the fish (Fig. 1) and a pressure null (minimum) was set up at the hydrophones. The output of the hydrophones was monitored on an oscilloscope and voltmeter and the phase and amplitude of speaker 2 were shifted relative to speaker 1, thereby moving the standing wave until the best pressure minimum was obtained. Once the null was achieved, the 0-180° switch could be reversed to move the standing wave one-quarter wavelength, thereby producing a pressure maximum at the hydrophones.

Adjustments of phase and amplitude ratios were made for each frequency and speaker position to produce pressure minima. These settings were highly repeatable from day to day and did not change with differences of water level in the plastic bag.

**Experimental procedure**

Experiments were performed using 21 standard goldfish (10-12 cm in standard length) obtained from commercial sources or as 'wild' stocks caught in Nuuanu reservoir on the island of Oahu, Hawaii. There were no differences in the results from the commercially obtained and wild animals. The animals were maintained in an open freshwater system until used in the experiments.

Animals were anaesthetized in a 1:6000 solution of Tricane methanesulphonate (Sigma Chemical Co.). After the animal's respiratory movements had stopped, they were placed in a simple holder (the same as shown in Fig. 2) and dilute anaesthetic was passed through the gills throughout the surgical procedure, using a gravity feed system.

The fish holder (Fig. 2) consisted of an oval Plexiglass ring from which two steel rods projected horizontally. Inverted V-shaped plastic holders were attached to the top rod. A steel pin was pushed through a small hole in one arm of the V and through the fish body to the other arm, thus holding the animal in place during the experiment. The respirator tube was attached to the lower steel rod and the animal's mouth was loosely tied to the tube.

In order to implant the electrode, a 1.0 cm diameter opening was made in the cranium immediately posterior to the eyes. The brain was exposed and that portion overlying the base of the cranial cavity posterior to the sacculi was aspirated away, exposing the suture between the occipito-temporal bones. This suture directly overlies the unpaired sinus (sinus impar), a medial bony canal containing endolymphatic fluid which forms a direct connexion between the sacculi of both ears (via the transverse canal) and Weberian ossicles. A glass-insulated tungsten wire (1 cm long and 100 μm tip diameter) was manually pushed through the suture, thereby making electrical contact with the endolymph. The cranial cavity was then filled with mineral oil and the hole in the skull was covered with melted paraffin which rapidly solidified around
the electrode. The electrical connexion to the electrode was made by fine teflon-insulated silver wire which was sutured to the animal's skin just posterior to the opening in the skull.

In experiments on the swim bladder, the scales in the vicinity of the 6th and 7th lateral line scales were removed and an incision made in the dorsal-ventral direction. The incision was spread apart with forceps and the swim bladder exposed in order to determine its exact position. The incision was then allowed to close and the cranium prepared as described above.

After the electrode was placed, the animal was lowered into the plastic bag and attached to a closed freshwater respirator system driven by a pump outside the soundproof room. The fish holder was then attached to a crossbar resting on an oval ring supporting the water bag. A second crossbar supported the hydrophones which were placed on either side of the animal just behind the opercles and within 0.5 cm of the fish. The plastic bag was filled with water until the holder was fully submerged. Respirator tubes and all electric cables entered the PVC tube through the small diameter pipes supporting the water bag.

Initially, measurements were made to determine the microphonic response amplitude with a standard intensity, 400 Hz signal. This measurement was periodically repeated during all experiments in order to make sure the animal remained in good condition. Experiments were terminated if the response to this standard signal declined by 6 dB or more. Most animals remained in acceptable condition for several hours.

In those experiments where the swim bladder was removed, initial measurements of the microphonic response in various standing wave positions were made befo
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Moving the swim bladder. The animals were then removed from the water (respiration was continued) and small forceps were inserted into the lateral incision. It was then possible to pull out the whole swim bladder with little or no other damage to the fish. Generally, the anterior chamber was punctured by the forceps (a hiss was usually heard when this happened) and the posterior chamber was pulled out intact. If a complete swim bladder deflation or removal had not obviously occurred, a dissection was performed at the end of the experiment to verify its absence. After the swim bladder was removed, the animal was replaced in the water bag and water was injected into the abdominal cavity with a hypodermic needle in order to remove any air bubbles which could have remained.

Microphonic recording

Microphonic potentials were recorded using the system shown in Fig. 1. The output of the electrode was pre-amplified in the soundproof room by 40 dB (Ortec model 4660 AC pre-amplifier with a band pass from 10 to 4000 Hz). The pre-amplifier output was brought out of the room and amplified by an additional 50 dB (Keithly model 823 pre-amplifier). The signal was then filtered (Briel & Kjaer 2143 1/3rd octave filter set) and the output of the filter was measured using a wave analyser (Hewlett-Packard model 3590-A) within a 10 Hz band.

Microphonic potentials from the fish ear are complex waveforms consisting of two major frequency components. In addition to the fundamental component which is the same frequency as the tonal stimulus, a large second harmonic component is characteristically also found (e.g. Enger & Anderson, 1967; Fay, 1973; Furukawa & Ishii, 1967a, and others). This component has been shown to be due to the presence of nonlinear and oppositely oriented hair cell populations in the fish sacculus (Furukawa & Ishii, 1967b; Hama, 1969) and possibly the lagena (Saito, 1973). Since this component is relatively free of possible mechanical and electrical artifacts which would tend to appear at the same frequency as the tonal stimulus, all responses reported in this paper were measured only at the 2nd harmonic frequency.

Calibration

Pressure measurements were made using a pair of Clevite CH-17T hydrophones suspended from a heavy lead bar in the general position of the fish during experiments. A displacement detector was suspended from the same bar directly between the two hydrophones. The displacement detector consisted of a 0.32 cm diameter photoprobe from a Fotonic Sensor (Mechanical Technology Inc. model KD-45-A). The photoprobe consisted of a set of fibre optics half of which carried light from the Fotonic Sensor and half of which returned reflected light to the sensor. Light from the photoprobe was reflected off a narrow strip of thin (1 μm) silvered mylar stretched across but not touching the face of the photoprobe. In this configuration, with no restrictions on either side of the mylar, the photoprobe measures the amplitude of relative movement between the fibre optic tube and the mylar reflector. Measurements of the displacement changes with the Fotonic Sensor indicated that there was significant spatial separation between pressure and particle displacement fields up to, but not above 10 Hz (Fig. 4B).
Sound pressure measurements were also made during all experiments using hydrophones placed on either side of the animal. Except within a frequency region between 800 and 1000 Hz, the phase setting needed to produce pressure nulls rarely differed between the two hydrophones by more than 8° and the amplitude difference at a pressure maximum rarely exceeded 3 dB. In order to confirm that similar levels would be found in the location of the fish, a third hydrophone was substituted for the fish and its response compared to that of the two outer hydrophones. Again, except between 800 and 1000 Hz, the amplitude and phase differences between the three hydrophones rarely exceeded ±8° and ±3 dB (Fig. 4b).

B. Vibratory stimulation

Since the standing wave tube was not suitable for discrete particle displacement stimulation of the fish above 315 Hz, the responses of additional animals were measured using a vibratory stimulator (Ling Dynamic Systems type 203) firmly coupled to the head of the fish (Fig. 2). The experiments were conducted in the sound-proof room and all aspects of the animal preparation, stimulation and response measurements were similar to those for the experiments in the standing wave tube except as explicitly noted. The fish were prepared for recording, transferred to a water-filled plastic aquarium (25 cm on a side) and attached to the respiration system. The fish holder was suspended from a metal bar across the top of the aquarium, with the fish submerged 1 cm beneath the water surface.

Experiments and calibration

Responses were measured from seven animals with intact swim bladders. The swim bladders were removed from several animals after the initial thresholds were obtained, and the vibrator was uncoupled from the head in several others in order to determine whether the recorded response was due to the displacements of the head, or to the sound pressures produced by the moving coil.

The acceleration of the vibrator was monitored continuously throughout all experiments by a small accelerometer (Endevco model 2264A) which was attached to the vibrator coil as shown in Fig. 2. The linearity and frequency response of the accelerometer was calibrated using the Fotonic Sensor which had a flat frequency response extending between DC and 20 kHz. The accelerometer and Fotonic Sensor were both calibrated absolutely using a calibrated dissecting microscope and strobe between 50 and 400 Hz. All systems were shown to be operating linearly at the amplitudes used in calibration and in stimulation of the animal.

RESULTS

Effects of standing wave location

Measurements were made with intact animals to determine the phase difference set up between the two loudspeakers which was needed to achieve pressure nulls at the two hydrophones. The phase angle necessary to null the microphonic response from the fish was then determined. Responses typical of both the hydrophones and the fish at 160 Hz are shown in Fig. 3. In this case, the phase shifter was swept twice to cover a 0–360° range with a slow-speed motor, and the output of the wave analy
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Fig. 3. Recordings made of the amplitude of the microphonic response from a typical animal at 160 Hz with the swim bladder present and with the swim bladder removed. The recordings were made while the phase angle of one speaker was swept relative to the other. The response of the normal animal decreased as the sound pressure decreased (pressure null and displacement maximum) at 90° relative phase. The same animal without the swim bladder had a minimum response at the displacement null. Note: The response from the fish without the swim bladder was 30 dB lower than for the animal when the swim bladder was present so the overall stimulus level was raised by this amount in order that the two curves could be compared in shape.

To conserve time, the complete phase sweep was not made in all experiments. Instead, measurements were made of the relative phase angles between the two speakers corresponding to a response minimum for both the hydrophones and the fish. Data obtained from at least four animals at each frequency are shown in Fig. 4A in which the lines connect median values. The phase angle shown in the figure is the difference between the phase angle between the two speakers necessary to null the response from the two side hydrophones (represented as 0° in Fig. 4) and that neces-
Fig. 4. The response of the fish and the calibration systems in the standing wave tube. In both (a) and (b), \(0^\circ\) is a reference for all other measures and represents the difference in phase between speakers 1 and 2 necessary to produce a pressure null at the side-hydrophones.

The curves in A show the speaker phase angles, relative to the pressure null (\(0^\circ\)), necessary to null the microphonic response in normal animals (dashed lines) and swim bladderless animals (solid lines).

The curves in B show the speaker phase angles, relative to the pressure null (\(0^\circ\)) necessary to produce a displacement null at the Fotonic Sensor (solid lines) and a pressure null at the center hydrophone (dashed lines). Notice that the curve for the normal animals is similar to that for the center hydrophone, except below 100 Hz, and that the curve for the swim bladderless animals is similar to that for the Fotonic Sensor throughout the frequency range tested.

Note: All relative phase measurements fall between \(0^\circ\) and \(180^\circ\) in this figure since the data were recorded as the smallest deviation from a pressure null (\(0^\circ\)) without regard to the sign of the difference.

sary to null the microphonic response. Above 100 Hz (and except between 800 and 1000 Hz) the phase angle for the microphonic null is essentially the same as that for the side hydrophone null. Below 100 Hz, the microphonic null occurred in closer relation to the displacement null as measured by the Fotonic Sensor (Fig. 4b). Figure 4B also shows measurements of the difference between the phase angles necessary to null the response from the two side hydrophones, and those necessary to null the response from a third hydrophone which replaced the fish in the restrainer during calibration. It is clear that the hydrophone differences parallel the phase angle differences between the microphonic null and the side-hydrophone null between 800 and 1000 Hz, but not below 100 Hz.

The microphonic null points for the swim bladderless animals are similar to those from the normal fish below 100 Hz, indicating that in both cases the goldfish ear responded in proportion to particle displacement amplitude. In contrast to the normal animals, however, the operated fish continued to show microphonic null points...
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Fig. 5. \(1 \mu V\) (RMS) iso-sensitivity functions for (A), normal fish, and (B), fish with deflated swim bladders both measured in a pressure maximum within the standing-wave tube. Each point represents the mean of seven animals with the brackets indicating \(\pm\) one standard deviation. (C) predicted far-field pressure sensitivity of swim bladderless animals based upon vibration sensitivity of experimental animals (calculated from curve (a) in Fig. 6).

which correlated well with the null points of the displacement-sensitive calibrating device (Fig. 4B) up to about 250 Hz. This type of response is more fully illustrated in Fig. 3, showing that the removal of the swim bladder in one animal shifts the point of minimum response from a pressure null to a pressure maximum (and thus a displacement null at 160 Hz). Above 250 Hz, however, the null points from the swim bladderless fish fall close to zero. Since the displacement nulls, as measured by the Fotonic Sensor, also fall to zero in the same frequency range, it is clear that the standing wave tube had ceased to function effectively, and that the relative contributions of pressure and displacement to the microphonic response cannot be analysed above 250 Hz. Note that the curve for the swim bladderless animals falls sharply from large relative phase values at frequencies above 200 Hz, while the Fotonic Sensor curve begins to fall at 315 Hz. This difference between the fish's response and that of the Fotonic Sensor is most probably due to the fact that the fish is significantly larger than the transducer element of the calibrating device. Since the fish's response may reflect vibration of any part of the body, an exact correlation between the fish displacement null and that of the calibrating device should not be expected.

Sound pressure iso-sensitivity functions

Pressure sensitivity was measured using microphonic responses in animals with and without swim bladders. The sound field was set up as a pressure maximum at both side hydrophones and the sound pressure level was attenuated in order to obtain a \(1.0 \mu V\) (RMS) microphonic response. The iso-sensitivity functions are shown graphically in Fig. 5 (curves A and B) as means and standard deviations for the seven animals in each treatment group. The thresholds for both groups are similar below
Fig. 6. (a) 1 μV (RMS) iso-sensitivity function for fish with direct vibratory stimulation of the head. Points represent mean of seven animals with bracket indicating ± one standard deviation. (b) Displacement amplitude accompanying the sound pressure levels (in the far-field) which produce a 1 μV microphonic response in normal animals in the standing-wave tube (curve a, Fig. 5).

100 Hz, but above this frequency the fish with the intact swim bladders were clearly about 20–35 dB more sensitive than the fish with the swim bladders removed. Although there was considerable variability for the fish with the swim bladders removed, it should be noted that the peak at 160 Hz and the minimum value at 750 Hz for the swim bladderless animals were consistent for every animal tested. This frequency effect was determined to be independent of the tube length through control experiments in which the speaker enclosures were moved.

**Vibration amplitude sensitivity**

Vibration sensitivity was measured using microphonic responses from intact and swim bladderless animals. The displacement level (in cm) needed for a criterion response of 1 μvolt (RMS) was determined and the mean responses and standard deviations for 7 test animals are shown in curve a of Fig. 6. The poorest sensitivity for all animals occurred at 50 Hz with a steady increase in sensitivity at about −3 to −6 dB per octave from 80 to 1600 Hz. Beyond 1500 Hz the sensitivity of the animals began to decline.

Responses were determined both with and without the swim bladder present in the first three animals tested and no measurable differences were found in the frequency range shown.

Controls were run with the fish disengaged from the stimulator and also with the coil attached to the head at slightly different points. The variation in threshold w...
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changes in the position of the holder was 2 or 3 dB, while complete disengagement caused a flat 30-40 dB drop in the response.

DISCUSSION

The experiments reported here were designed to analyse the mechanisms and pathways of sound conduction to the goldfish ear under a variety of stimulus conditions both with the swim bladder intact and eliminated. Although neither of the two stimulation methods were in themselves totally suitable to answer the questions we have posed, the combined data from both methods give a good indication about the nature of stimulation of the inner ear and the role of the swim bladder in sound detection for the goldfish.

Experiments with shifts of pressure and particle velocity nodes in the standing wave tube indicate that the response from the ear of the intact goldfish is produced in direct proportion to the sound pressure level at frequencies above about 80-100 Hz while below this frequency range the response appears to be proportional to the displacement amplitude (Fig. 4A). This is indicated by the fact that the minimum response, at least up to 250 Hz, was obtained when the fish was in a pressure minimum while a significantly larger response was obtained when the fish was in what was determined to be a displacement minimum. However, when the swim bladder was removed, the response of the fish from 50 to 250 Hz reached a minimum value along with the response of the displacement transducer (compare the solid lines of Figs. 4A and B). In both the intact and operated animals, the microphonic potential nulls closely followed the displacement transducer nulls from 50 to 80 Hz indicating that at these frequencies and amplitudes, the inner ear is responding directly to water particle motion. Above 315 Hz the results are more equivocal since the pressure and displacement maxima tended to occur together, thus eliminating the spatial separation necessary for our analyses. However, as will be shown below, there is good reason to suggest that the animals with swim bladder present were responding to the pressure portion of the signal while the animals without the swim bladder were responding to displacement energy throughout the frequency range studied.

Iso-sensitivity functions were also determined in the standing wave tube for each animal and the function for the intact animals has a similar shape to behavioural
pressure audiograms for the goldfish (e.g. Fay, 1969; Jacobs & Tavolga, 1967; Popper, 1971) and the carp, *Cyprinus carpio* (Popper, 1972) and to the iso-sensitivity saccular potential functions for the carp (Fay, 1974). The iso-sensitivity functions for the goldfish with the swim bladder removed showed a 20–35 dB loss relative to normal animals above 100 Hz (see Fig. 5, curve B). If we make the assumption that the fish without the swim bladder is a displacement detector, then the sound pressure iso-sensitivity functions for these animals are essentially meaningless measures since displacement varies somewhat unpredictably relative to pressure in our test situation. This is highlighted by the fact that within the frequency range where displacement could be varied relative to pressure (50–315 Hz), the sound pressure iso-sensitivity values could be lowered by as much as 40 dB simply by changing the standing wave’s position relative to the fish. This is, in effect, what tended to occur in the 400–800 Hz range where maximum particle movement occurred at nearly the same points as maximum sound pressure.

The results from experiments with direct vibratory stimulation of the fish’s head show that the goldfish ear responds directly to displacement under certain conditions, and that the swim bladder is not involved in the response. In addition, we have shown that the iso-sensitivity functions (Fig. 6, curve A) are not pressure functions since uncoupling the fish’s head from the shaker resulted in a 30–40 dB increase in response. The isosensitivity function for this direct vibration of the head is generally a linear function between 70 and 1500 Hz with a slope of between −3 and −6 dB/octave. If one calculates the displacement amplitude existing in the far-field for sound pressure levels producing a 1 μV response within the standing wave pressure maxima using the far-field formula provided by Harris (1964), it is clear that the resultant vibration amplitude is 45–70 dB below those found for direct measures of vibratory sensitivity of the inner ear (Curve B, Fig. 6). This difference, plotted in Fig. 7, can be considered to approximate to the gain in displacement amplitude due to the impedance transformer characteristics of the goldfish’s peripheral auditory system (swim bladder, Weberian ossicles, fluid systems of the ear). This function is similar in form to the calculated gain in displacement amplitude provided by the swim bladder of the cod, as derived recently by Chapman & Hawkins (1973), for far-field conditions. However, Chapman & Hawkins’ calculations show a maximum gain of about 30 dB occurring at 400–600 Hz, while our data appear to suggest an additional gain of about 40 dB at all frequencies. This 40 dB difference is most likely due to such factors as the shallower depth (lower ambient pressure) at which the present measurements were made, and any additional gain provided by the Weberian ossicles of the goldfish (Poggendorf, 1952). In addition, it is likely that relative movement between the otolith and hair cells is less efficiently produced by the method of skull vibration than it is through the normal pathways involving the sinus impar and its coupling to the Weberian ossicles.

The overall differences in behavioural sensitivity between the cod (Chapman & Hawkins, 1973) and the goldfish (as summarized by Popper & Fay, 1973) are about 20–25 dB at the lower frequencies, and grow quite large at frequencies above 400 Hz due to the goldfish’s significantly wider bandwidth. Chapman & Hawkins’ (1973) calculation of the displacement sensitivity of the cod’s otolithic ear shows a very restricted frequency range, too, in contrast to the wide vibratory frequency range of the goldfish.
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The goldfish ear (curve A, Fig. 6) which was determined under conditions where the Weberian ossicles and the swim bladder were shown not to be involved. We tentatively conclude, then, that the overall sensitivity difference between cod and goldfish (or between non-ostariophysines and ostariophysines in general) is due to an additional displacement amplification of the Weberian ossicles, while the wider bandwidth of the goldfish is most likely to be a function of its relatively small saccular otolith (see also Popper, 1972).

The vibratory iso-sensitivity function of Fig. 6 is of additional value since it allows the estimation of the displacement amplitude of the goldfish head at behavioural threshold. For example, the sound pressure iso-sensitivity function of Fig. 5 falls above the behavioural sensitivity of the goldfish (as summarized by Popper and Fay, 1973) by 35-45 dB at frequencies below 1100 Hz. This difference is quite comparable to the differences found to exist between behavioural sensitivity and cochlear potential iso-sensitivity functions in other animals (1 μV, RMS, recorded at the round window) as summarized by Wever (1959). It should be noted, too, that the characteristic decline in behavioural sensitivity relative to the microphonic response at the higher frequencies occurs also for the goldfish at frequencies above 1000 Hz or so. At any rate, by subtracting this 35-45 dB difference from the vibratory iso-sensitivity function of Fig. 6, we find that the head would have to be moving at between 2 and 50 Å (peak) in order for sound to be detected behaviourally. This range of values compares well with the calculated displacement sensitivity of lateral-line receptor organs as determined by Kuiper (1956). These values, too, are within the range of displacement amplitudes of the ear drum at man’s absolute threshold (Békésy & Rosenblith, 1951).

In conclusion, it has been well established that the otolithic ear of the goldfish may function both as a displacement (or acceleration) sensitive device or as a pressure sensitive system (making use of the swim bladder and Weberian ossicles) depending upon the ratio of particle movement to pressure of the applied stimulus. In ‘natural’ situations for the goldfish, the most likely variable producing these different ratios would be distance from the sources of sound and the resulting magnitude of near-field effects.

However, using the formula provided by Harris (1964) relating sound pressure to particle movement in the near- and far-fields with a monopole source (equation (1)), we have calculated that the goldfish would have to be within 0-02 cm and 2 cm of the sound source, at 1250 and 50 Hz, respectively, before the response due to the vibration of the head would exceed that produced from sound pressures impinging upon the swim bladder.

\[
D_0 = \frac{p_0}{\omega pc} \left( 1 + \frac{1}{(kr)^2} \right)^{1/2}
\]

(1)

Where

- \(D_0\) = displacement (cm),
- \(p_0\) = sound pressure (dynes/cm²),
- \(\rho c\) = acoustic impedance of water (1.5 x 10⁵),
- \(\omega = 2\pi\) frequency (Hz),
- \(r\) = distance from source (cm),
- \(k = 2\pi/\lambda\) (wavelength).
These distances are exceedingly small compared to the actual extent of the near-field produced by monopole sources. It is thus clear that near-field effects are negligible in determining the response of the goldfish ear, and that in almost any acoustic field (except direct vibratory stimulation of the head), sound pressure is an adequate measure of the degree of auditory stimulation. This conclusion may not apply, of course, in behavioural experiments where stimulation of the lateral line system may contribute to the response.

Although our data lead to several conclusions about the function of the goldfish inner ear as a whole, it is not clear from which otolithic organ (or organs) the potentials were generated. There is evidence that the responses recorded from the sinus impar of normal animals are generated from the sacculi at frequencies above about 100 Hz (Fay, 1974). However, at lower frequencies, and under conditions where the swim bladder is removed or vibration is applied directly to the head, the lagenae may be significantly involved in the response. In any case, further experimentation in this area, using different recording techniques, is called for.

Finally, some estimate should be made of the relevance and practicality of the standing wave manipulation in relation to the kinds of questions approached here. The standing wave tube's greatest promise lies in the possibility of spatially separating the pressure and displacement maxima and minima and in the ease with which the wave can be manipulated in a situation ideal for electrophysiological investigations. However, the tube ceases to function effectively above a certain frequency range. This is probably due to the combination of geometrically complex air-water interfaces with the tube's dimensions relative to the wavelengths involved. This problem might be solved using a smaller tube, or one that is completely water-filled, such as described by Cahn et al. (1969). It is likely, too, that the effective impedance of the water in the soft-walled bag is somewhat less than that of water in a free-field, thus increasing particle movement relative to pressure. This is suggested in a comparison between the data from the fishes without the swim bladder (curve B, Fig. 5), and the calculated sound pressure levels accompanying the displacement threshold values determined in the head vibration experiment (calculated sound pressure levels are shown in curve C of Fig. 5). Theoretically, these calculated values are the threshold that should have been obtained if our animals without swim bladders were direct displacement detectors in a true far-field. However, the actual values obtained were about 30 to 35 dB lower than the calculated values. Consequently, we conclude that the swim bladderless fish appeared to be more sensitive in our tube than they would be in a true far-field situation where particle displacement amplitudes would be considerably lower, given equal sound pressures. This is not an unreasonable hypothesis since observations of other investigators also indicate that in a soft-walled air bounded water tank, the wave impedance approaches that of air (Cahn et al. 1969; Parvulescu, 1964). However, in spite of the low impedance signal present in our standing-wave tube, the normal fish remain pressure sensitive organisms and in an actual free-field situation this displacement independence would probably extend far below 100 Hz.

Finally, the problem of displacement calibration remains. While we were able to make relative measures of displacement amplitude using the Fotonic Sensor, an absolute calibration of the device could not be made for its use underwater. In addition, the direction of the particle movement, which is an important aspect of the stimulating effect (Enger et al. 1973) could not be measured in all directions in this
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Experiment since it was impossible to rotate the transducer within the small water bag. A small, very sensitive and submersible accelerometer would be preferable, assuming that its coupling to the water medium would not change significantly with the proximity of air–water interfaces.

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REFERENCES


