

MODES OF STIMULATION OF THE TELEOST EAR

By RICHARD R. FAY*

*Laboratory of Sensory Sciences, University of Hawaii,
Honolulu, Hawaii 96822*

AND ARTHUR N. POPPER

*Department of Zoology, and Laboratory of Sensory Sciences
University of Hawaii, Honolulu, Hawaii 96822*

(Received 27 August 1974)

SUMMARY

Microphonic potentials were recorded from the inner ears of a catfish (*Ictalurus punctatus*) and an African mouthbreeder (*Tilapia macrocephala*) in response to underwater sound stimulation and direct vibration of the head. The shape of the vibratory isopotential functions of frequency was similar in both species up to 600 Hz. Above 600 Hz, the sensitivity of *Ictalurus* continued to increase to 4000 Hz while the sensitivity of *Tilapia* declined.

Deflation of the swim bladder did not affect the response to vibration in either species, the response of *Tilapia* to the underwater sound stimulus being minimal and unaffected by removal of the swim bladder.

Ictalurus was pressure-sensitive to above 4000 Hz, there being a significant decline in the response with deflation of the swimbladder.

INTRODUCTION

Recent experiments (Fay & Popper, 1974) have described at least two distinct pathways by which sound reaches the inner ears of the goldfish (*Carassius auratus*). Sound pressure fluctuations cause expansion and contraction of the swimbladder, this motion being thought to be transmitted to the fluids and otoliths of the inner ear (cf. van Bergeijk, 1967; Popper & Fay, 1973). In this case, pressure sensitivity is partially determined by the efficiency of transmission between the swimbladder and the inner ear. The degree of acoustic coupling is quite variable between species, the elements ranging from intervening non-specialized amorphous structures (e.g. muscle, bone, fat, etc.) to the specialized Weberian ossicle system of the Ostariophysi which directly couples the swimbladder to the inner ear via a series of movable bones.

The second pathway to the inner ear is provided by general tissue conduction in which the particle movement associated with any acoustic phenomenon is taken up by the dense otolith at an amplitude and phase which differs from that of the surrounding bone and other tissue (a mechanism analogous to an accelerometer).

In both the pressure sensitive and accelerometer-type of system for sound detection

* Present address: Department of Otolaryngology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C., U.S.A. 27103.

the stimulus results in a relative shearing motion across the hair-cell cilia in the inner ear. However, the magnitude of labyrinthine stimulation via the swimbladder is proportional to sound pressure while the magnitude of stimulation produced by the accelerometer principle is proportional to displacement amplitude. In recent experiments with *Carassius*, an ostariophysine, we have found that the predominant detection system in the normal animal is pressure-sensitive (Fay & Popper, 1974). After the swimbladder has been removed, however, the predominant system is displacement-sensitive.

The predominance of the pressure system in the intact goldfish may be related to the close coupling between the swimbladder and the inner ear. For this reason the present study of potential acoustic pathways to the ear was carried out in a non-ostariophysine fish, *Tilapia macrocephala*, a species with no known specialized acoustic coupling system. In addition, we have also obtained data for a catfish, *Ictalurus punctatus*, a representative of a different suborder of Ostariophysi from the goldfish. *Ictalurus* is of special interest since its hearing bandwidth exceeds that of the goldfish at the high frequencies (above 1500 Hz) (Poggendorf, 1952; Weiss, Strother & Hartig, 1969). The basis for this difference is considered in this paper.

METHODS AND MATERIALS

The animals used in the experiments were 10 commercially bred catfish (*Ictalurus punctatus*), each of about 20 cm in length (obtained from Fish Farms Hawaii, Kihei, Maui) and 10 African mouthbreeders (*Tilapia macrocephala*) of about 16 cm in standard length (collected from the Wahiawa Reservoir, Oahu, Hawaii). All of the animals were housed in a large commercial pool which was continuously aerated and filtered.

In preparation for recording microphonic potentials, the animals were anaesthetized in a 1:3000 solution of tricane methanesulfonate (Sigma Chemical Co.) and were then immobilized with an intramuscular injection of Flaxedil at 1 $\mu\text{g/g}$ of body weight. The animals were respired with aerated tap water during the surgical procedures and throughout the experiments. The animal was placed in an elastic restrainer and a section of the dorsal cranium removed from just over the cerebellum. The brain stem was cut, and the medulla and portions of the vagus lobes were aspirated away so as to expose the floor of the cranium. In *Ictalurus* a 1 cm long glass-insulated tungsten wire (50 μm tip diameter) was pushed through the midline suture of the occipitotemporal bones. This suture overlies the unpaired sinus, and endolymphatic connexion between the anterior end of the Weberian ossicles and the sacculus of both ears. The cranial cavity was then filled with mineral oil and the hole in the skull covered with melted paraffin which, when hardened, provided additional support for the electrode. Electrode implantation in *Tilapia* differed only in that the electrode was inserted into the saccular lumen through a small hole drilled in the shelf of bone directly overlying the posterior portion of the right sacculus. Since the two ears are not connected in *Tilapia*, as they are in *Ictalurus*, the saccular potential recordings from *Tilapia* only represent a single ear while those from *Ictalurus* represent the summated response from two ears.

The stimuli were continuous pure tones from 50 to 5000 Hz generated by standard electroacoustic equipment. All experiments were conducted in a double-walled sound-proof acoustic chamber.

To stimulate animals with underwater sound they were submerged in a water-filled PVC cylinder 25 cm in diameter and 20 cm high and filled to a height of 16 cm. The bottom of the cylinder was constructed of 0.5 cm thick Rho C rubber which was supported by a plastic grating. A 20 cm diameter loudspeaker was suspended 25 cm below the water tank and faced upwards into an air-filled extension of the PVC cylinder. This extension formed an airtight cavity bounded above by the Rho C rubber floor of the fish chamber and below by the speaker diaphragm.

To provide vibratory stimulation the restrainer was raised from the water and the fish's head rigidly attached to the coil of a 6 lb (2.7 kg) Ling shaker (Ling-Tempco-Vought) using pins which were set into the animal's skull (Fay & Popper, 1974). During both stimulation modes, the fish were essentially freed from the elastic restrainer which was then used only to maintain general body position and provide a limited amount of support.

The saccular potentials were amplified by 40 dB inside the sound proof room (Ortec model 4660 a.c. preamplifier) with a band-pass from 10 to 10000 Hz. The output of the preamplifier was measured by a wave analyser with 10 Hz filter bandwidth (Hewlett-Packard model 3590-A). In all experiments the isopotential functions of frequency were determined by measuring the stimulus levels which produced a 1 μ V (RMS) response from the inner ear. For *Ictalurus* the amplitude of the sinusoidal component equal in frequency to the stimulus tone was measured since this was the largest spectral component of the recorded response. In measurements on *Tilapia* however, the largest response component was measured at twice the frequency of the stimulus (2nd harmonic) (see Fay, 1974, for a discussion of the spectral components of the grossly recorded microphonic response from the fish ear).

Several methods were evaluated for removal or deflation of the swimbladder. In *Ictalurus*, a small incision was made in the body wall on both sides of the animal at the level of the lateral-line canal and about $\frac{1}{2}$ cm behind the operculum. This exposed the swimbladder which could then be punctured and filled with water. Considerable care was taken to insure that all air bubbles were removed from the cavity. With *Tilapia*, two hypodermic needles were inserted into the swimbladder of the intact animals and coloured water slowly injected through one of the needles while air escaped through the second needle. To ensure that the swimbladder had been completely filled with water, the animal was frozen after the experiment and quickly dissected to determine whether water had filled the swimbladder chamber.

A typical experimental series for both species was as follows. A flap of bone was removed from the dorsal cranium and the animal was placed in the test tank, surgery being continued in order to implant the electrode. The fish was submerged and sound pressure isopotential determinations made at 10–15 frequency points using the loudspeaker for stimulation. The animal was then raised from the water and connected to the shaker for the vibratory isopotential measures. The animal was then again submerged and retested at several frequencies using the air loudspeaker, to ensure that there had been no deterioration in the response during the shaker experiments. The swimbladder was then filled with water, isopotential recordings being made in such experimental animals using the air loudspeaker and the vibrator. Data from animals in which the saccular response declined by more than 6 dB during retests were discarded. Since the removal of the swim bladder did not affect the response to vibration, the final

retest of the response to vibratory stimulation served as a control for general deterioration of the animal. In most cases the saccular response remained at its initially recorded levels throughout the experiments.

Calibration. Sound pressure levels were measured with a small hydrophone (Clevite model CH-17T) which was suspended in the position normally occupied by the fish's swimbladder. The sound pressure levels necessary to produce the $1 \mu\text{V}$ saccular responses were then calibrated and expressed in dB re: 1 dyne/cm^2 ($1 \mu\text{bar}$) using the calibration supplied with the hydrophone.

Calibration of the displacement amplitude in the vibration experiments were made using a Fotonic Sensor (Mechanical Technology Inc. model KD-45A) with a flat frequency response extending to above 10000 Hz. This instrument consists of a 0.32 cm diameter fibre optic light guide which is placed perpendicular to a reflective moving surface. One-half of the fibres carry light from a source in the Fotonic Sensor housing, the remaining fibres carrying reflected light back to a photocell. The response of the photocell is proportional to the distance between the end of the light guide and the reflective surface. Measurements were made of the fish's head and the shaker coil both in loaded and unloaded conditions (with and without the fish attached to the coil) and the amplitude of vibration was essentially the same in both cases. The measurements were referred to peak-to-peak amplitude values measured visually using a dissecting microscope and strobe to observe movements of the coil (see also Fay & Popper, 1974). During the vibration experiments, the displacement amplitude of the shaker coil was continuously monitored using a ceramic phonograph cartridge which was also calibrated with the Fotonic Sensor and, ultimately, with visual observations of a moving object.

In several cases the amplitude of the fish's head movement was measured (in the vertical direction) in response to acoustic stimulation produced by the loudspeaker. These values were then compared to the amplitude of expected particle displacement accompanying a plane wave in a far-field given equal sound pressure levels. This calibration showed that the displacement amplitude existing in the cylindrical water tank at 50 Hz was approximately 55 dB greater than would be expected in a free field plane wave. However, this difference declined with increasing frequency at almost exactly 6 dB per octave. These relatively high displacement values are to be expected in a soft-walled water chamber bounded by air (Parvulescu, 1964). Implications for the present data are treated in the discussion.

RESULTS

Intact *Ictalurus* responded to stimulation from 50 Hz (the lower limits of reliability of our acoustic system) to over 4000 Hz with maximum sensitivity between 315 and 3000 Hz (Fig. 1a). Behavioural data (Poggendorf, 1952; Weiss *et al.* 1969) indicates that *Ictalurus* is likely to show some response to sounds above 4000 Hz; although experimental artifacts at higher frequencies prevented reliable measurements. Experiments with deflated swimbladders were also performed on the same animals and in all cases there was a considerable loss of sensitivity at all frequencies above 100 Hz with losses generally 30 dB or greater above 200 Hz. These losses closely resemble those associated with removal of the swim bladder in the goldfish (see Fay & Popper, 1974). There was

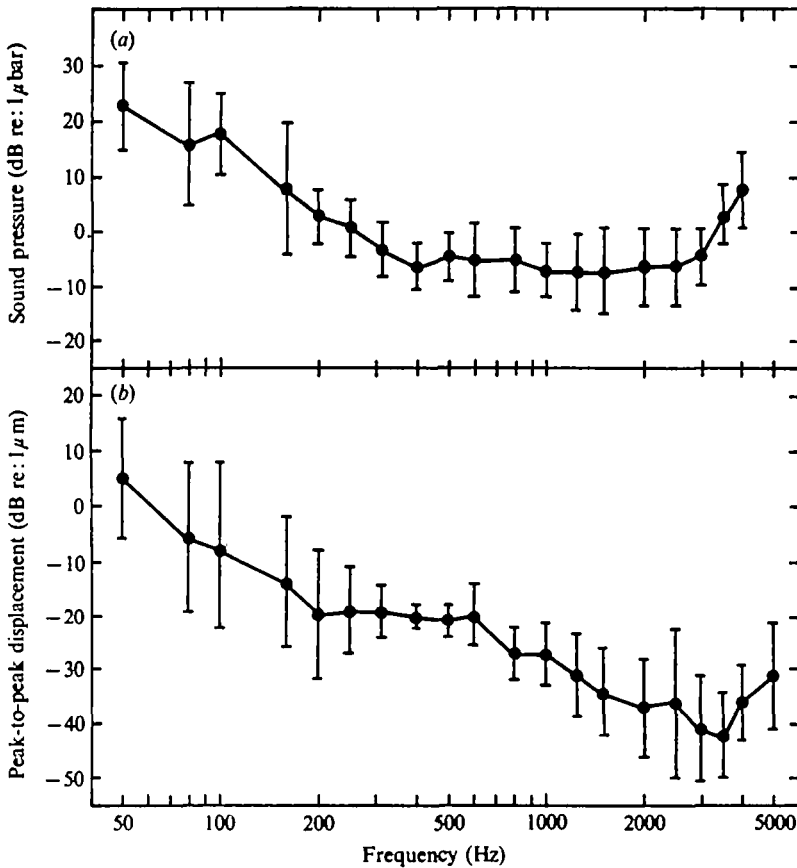


Fig. 1. $1\mu\text{V}$ (RMS) isopotential functions of the fundamental frequency component recorded from the unpaired sinus of *Ictalurus punctatus*. The line connects the mean stimulus levels for 10 animals, and the vertical bars indicate the size of + or - 1 standard deviation. (a) Underwater sound stimulation. (b) Vibration of the head.

no loss in sensitivity following removal of the swimbladder, from 50 to 100 Hz, the loss between 100 and 200 Hz increasing to 30 dB at 200 Hz.

Isopotential functions determined for head vibrations are shown in Fig. 1 b. Again, the animals responded from 50 to 5000 Hz with maximum sensitivity occurring at 3500 Hz. Deflation of the swimbladders caused no observable loss in displacement sensitivity at any frequency in most of the animals studied.

Tilapia responded to underwater sound stimulation from 50 to 900 Hz (Fig. 2 a). The maximum sensitivity was at 100 Hz the sensitivity then declining monotonically with increasing frequency. Elimination of the swimbladder caused no change in sensitivity at any frequency. Maximum sensitivity for *Tilapia* was +16 dB (re: $1\mu\text{bar}$) as compared to a maximum sensitivity of -8 dB (re: $1\mu\text{bar}$) for *Ictalurus*.

The results from direct head vibration are shown in Fig. 2 b. *Tilapia* responded to stimulation from 50 to 2000 Hz with maximum sensitivity from 400 to 600 Hz. Removal of the swimbladder resulted in no changes in the isopotential values.

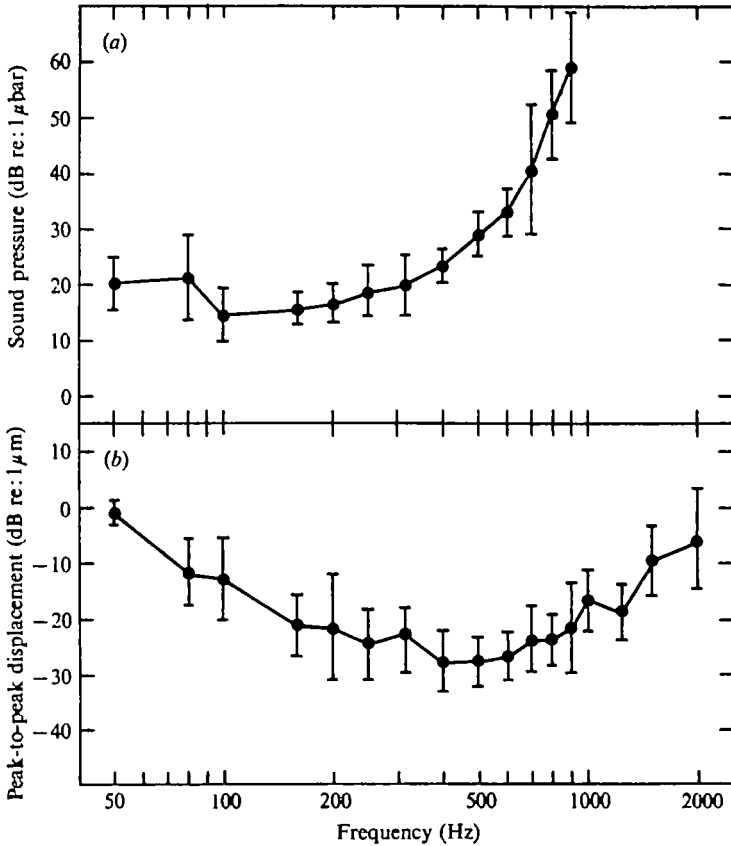


Fig. 2. $1 \mu\text{V}$ (RMS) isopotential functions of the 2nd harmonic component recorded from the right sacculus of *Tilapia macrocephala*. The line connects the mean stimulus levels for 7 animals and the vertical bars indicate the size of $+1$ or -1 standard deviation. (a) Underwater sound stimulation. (b) Vibration of the head.

DISCUSSION

The results of these experiments with *Tilapia* and *Ictalurus* may be compared with those obtained in similar experiments with the goldfish, *Carassius auratus* (Figs. 3 and 4). In contrast to the wide differences in pressure sensitivity (Fig. 3) the data for vibrational stimulation (Fig. 4) indicate that from 50 to about 600 Hz the three species show remarkably similar frequency response functions. It is not certain whether these curves represent the response of the sacculus alone or the combined response of the sacculus, lagena and, possibly, the utriculus. Nevertheless, the bandwidth differences in sound pressure sensitivity are clearly reflected in the species differences evident in response to vibration. Since removal of the swimbladder had no effect on the vibration response of the species tested, it is suggested that the frequency range of hearing is determined by the mechanical properties of the inner ear and not necessarily by differences in the frequency response of the system coupling the swimbladder with the ear. The swimbladder itself is known to have a relatively flat frequency response, and a bandwidth which exceeds the frequency range of pressure sensitivity measured behaviourally (Chapman & Hawkins, 1973; Popper, 1974; Sand & Hawkins, 1973).

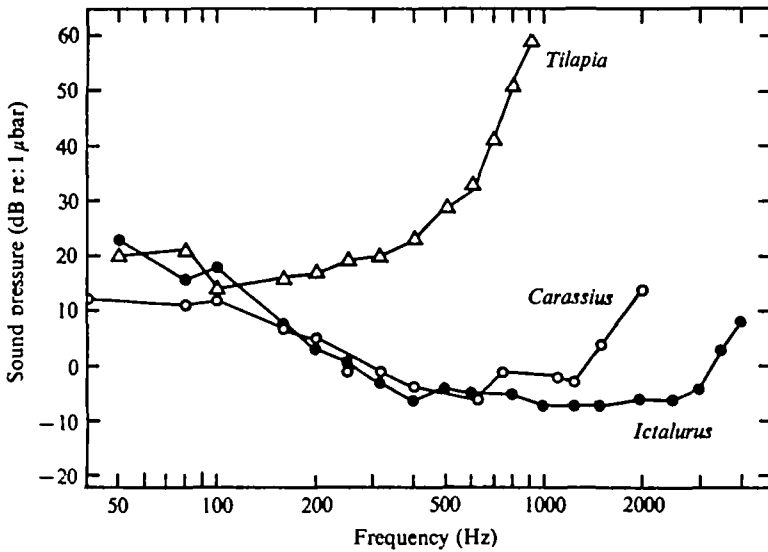


Fig. 3. Sound pressure isopotential functions for *Tilapia* and *Ictalurus* compared with the function for *Carassius*, taken from Fay & Popper (1974). In each case, the mean RMS sound pressure levels which produce a 1 μ V (RMS) response are plotted.

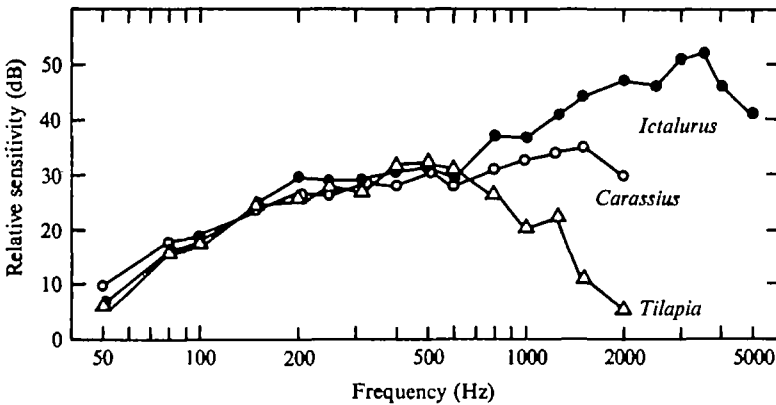


Fig. 4. Vibratory amplitude isopotential functions for *Tilapia* and *Ictalurus* compared with that for *Carassius* (Fay & Popper, 1974). In order to illustrate best the frequency response of the three systems, the isopotential values are plotted in terms of relative sensitivity with an arbitrary reference. The curve for *Ictalurus* was elevated by 12 dB, and the curve for *Tilapia* was elevated by 6 dB relative to that for *Carassius* in order to facilitate the comparison.

The available evidence indicated that the sound pressure isopotential function for *Tilapia*, and the values for *Carassius* and *Ictalurus* below 160 Hz are probably not valid indications of true pressure sensitivity in the far-field (see Popper & Fay, 1973, for a discussion of the far-field vs. near-field construction). Removal of the swim-bladder caused a reduction in the saccular response only at frequencies above 160 Hz for *Carassius* and *Ictalurus*, and caused no reduction at all for *Tilapia*. Furthermore, the recalculation of isopotential values for *Tilapia* (Fig. 2), in terms of displacement amplitude rather than in terms of sound pressure level, yields a function identical to that determined by direct vibration of the head. In short, under all the conditions of

stimulation used in these experiments, the ear of *Tilapia* always responded to the vibrational component. The implication here is that true far-field pressure sensitivity, if it exists at all for *Tilapia*, would be significantly poorer than the values plotted in Figs. 2 and 3. The same argument also applies to the low frequency portions of the curve for intact *Carassius* and *Ictalurus* and for both species without swimbladders.

These comparative electrophysiological data suggest, then, that the large sensitivity differences found in 'sound pressure thresholds' determined behaviourally in wide range of species (cf. Popper & Fay, 1973) may not reflect simple quantitative differences in sensitivity. Instead they may reflect the predominance of one or other distinctly different modes of sound conduction to the sacculus (i.e. bone conduction and swimbladder stimulation). This consideration also applies to studies of swimbladder and Weberian ossicle function in which thresholds expressed in terms of sound pressure level are measured before and after some interference with the peripheral system. As in the present experiment, the operation may produce a qualitative change in the mode of best stimulation rather than simply reduce pressure sensitivity. The high particle displacement activity accompanying the acoustic stimulation condition in the present experiment prevents us from extending these conclusions to a true far-field situation. Thus, it is probably unwise to categorize *Tilapia* or *Ictalurus* as being either 'displacement-' or 'pressure-sensitive'. For example it is clear that as an ostariophysine moves closer to an underwater sound source a frequency dependent distance is reached within the near-field where stimulation of the ear via the pressure-sensitive swimbladder is exceeded by the stimulation due to direct displacement activity. For species with inferior pressure sensitivity these distances within the near-field would be large. For species with sufficiently poor pressure sensitivity the response of the ear would be controlled by displacement amplitude even outside the limits of the near-field. It is not yet certain whether or not *Tilapia* falls into this latter category.

There are three basic considerations which are relevant to these observations. First, and most obviously, a behavioural or electrophysical measurement of sound pressure sensitivity for any particular species only has meaning when the sound pressure component of the impinging stimulus is shown to determine the response. Secondly, the general auditory sensitivity of most, if not all species, may be characterized by specifying a range of distances between the fish and the sound source (assuming a certain frequency range of hearing) within which the response to vibration would predominate. It is now clear, for example, that these distances are quite large for *Tilapia* compared with those for the Ostariophysi. For non-ostariophysine species such as holocentrids thought to have better sound pressure sensitivity than *Tilapia* (Tavolga, 1974; Tavolga & Wodinsky, 1963) these distances are assumed to be intermediate.

Finally, the varieties of acoustic information available to a fish would most probably depend on the degree of pressure stimulation relative to the degree of direct displacement stimulation in any given situation. For example, van Bergeijk (1964) has argued that the binaural cues necessary for auditory localization are absent in the Ostariophysi when the swimbladder reception mode predominates, since both sacculi are stimulated equally via the unpaired sinus. On the other hand, there are at least some conditions under which binaural cues may be preserved during direct vibratory stimulation (Enger *et al.* 1973). At frequencies above 600 Hz, where *Tilapia* could be termed effectively 'deaf', *Carassius* and *Ictalurus* hear relatively well, although infor-

mation necessary for binaural localization is probably not preserved. At intermediate frequencies (200–600 Hz) all three species show a similar sensitivity to particle displacement and, thus, fulfill some of the requirements for binaural localization. However, the vibratory sensitivity of *Tilapia* is still relatively good so that binaural cues arriving via the direct vibratory route would not be overshadowed by stimulation possibly arriving via the swim bladder. At extremely low frequencies (below 160 Hz) predictions of this kind are not possible since actual sound pressure sensitivity has not been successfully measured in any of the three species. It is a reasonable assumption, however, that the relative contribution of the direct displacement stimulation, complete with possible binaural cues, should increase for all species at the lower frequencies.

This work was supported by Public Health Service Grants NS-09374 and NS-06890 from the National Institute of Neurological Diseases and Stroke. We would like to thank Dr Kenneth Kato of Fish Farms Hawaii for supplying the catfish and Mr Phillip Lobell for identifying the species of *Tilapia*.

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