J. exp. Biol. (1982), **98**, 49–66
With 8 figures
Printed in Great Britain

BIOPHYSICS OF UNDERWATER HEARING IN ANURAN AMPHIBIANS

BY THOMAS E. HETHERINGTON[1] AND R. ERIC LOMBARD[2]

[1,2]*Department of Anatomy, The University of Chicago, 1025 East 57th Street, Chicago, Illinois 60637 (USA)*

(*Received 22 October 1981*)

SUMMARY

A standing wave tube apparatus was used to determine the biophysical basis of underwater hearing sensitivity in 3 species of *Rana* and in *Xenopus laevis*. A speaker inside the base of a vertical, water-filled 3 m steel pipe produced standing waves. Pressure and particle motion were measured with a hydrophone and geophone respectively and were spatially 90° out of phase along the length of the tube. Microphonic responses were recorded from the inner ear of frogs lowered through pressure and particle motion maxima and minima. The air-filled lungs of whole frogs produced distortions of the sound field. Preparations of heads with only an air-filled middle ear produced little distortion and showed clear pressure tracking at sound intensities 10–20 dB above hearing thresholds from 200–3000 Hz. Filling the middle ear with water decreased or abolished microphonic responses. Severing the stapes reduced responses except at certain frequencies below about 1000 Hz which varied with body size and likely represent resonant frequencies of the middle ear cavity. We conclude that the frog species examined respond to underwater sound pressure from about 200–3000 Hz with the middle ear cavity responsible for pressure transduction.

INTRODUCTION

Many species of frogs are either predominantly aquatic or spend much time in water, and certain forms, such as *Rana aurora* (Licht, 1969) and species of *Xenopus* (Passmore & Carruthers, 1979) are known to produce mating calls underwater. Lombard *et al.* (1981) determined underwater hearing threshold curves for *Rana catesbeiana* based on midbrain recordings and discovered sensitivity similar to aerial hearing sensitivity when sound amplitude is expressed as sound intensity (Watts m^2). Studies of comparative aerial and aquatic hearing abilities in mammals have not found comparable dual sensitivities. In man, for example, hearing sensitivity is markedly lower underwater (Smith, 1969), while in pinnipeds possessing middle ears apparently modified for underwater hearing, auditory capabilities are significantly lower in air (Terhune & Ronald, 1972). The ear of *Rana* and other frogs may therefore represent a unique structure adapted for sensitivity in both air and water.

Hearing underwater may involve very different mechanisms than hearing in air. Due to the close matching of the impedances of vertebrate flesh and water, sound

waves are easily transmitted through the body of a submerged organism. Based on studies of hearing in fishes, two modes of acoustic mechanoreception, each based on a different parameter of sound waves, have been proposed (Wever, 1971). In the inertial mode, the particle displacement energy associated with sound waves causes differential movement of hair cells and an overlying structure, such as an otolith, to vibrate at differing amplitude and phase, producing deformation of the hair cell cilia. In the pressure mode, pressure changes associated with sound waves produce movements in the walls of a gas-filled cavity which in turn are transmitted as displacements to the inner ear. Such a gas bubble may amplify the particle displacement component of a sound stimulus since it is more compressible than surrounding tissue and undergoes greater volume change in response to pressure (van Bergeijk, 1967; Hawkins, 1973). Pressure transduction therefore may increase hearing sensitivity.

In tests of underwater hearing in terrestrial mammals, such as man (Smith, 1969), particle motion energy has been suggested to be the mode of acoustic mechanoreception. The ear of many frogs, such as species of *Rana*, although possessing unique features, has a middle ear structure apparently adapted for aerial hearing and similar to that of terrestrial mammals. However, the frog ear also shares common features with the ears of fishes, such as the mechanism of producing relative shearing of the hair cell cilia and overlying tectorial structure (Wever, 1974). Studies on fish hearing suggest both the inertial and pressure modes may be utilized and that different systems may be involved in pressure and particle motion sensitivity (Fay & Popper, 1975, 1980; Buwalda, 1981). The swim bladder of many fishes can act as a pressure transducer, and such pressure sensitivity is generally considered most important at higher frequencies (above about 200 Hz) while particle motion sensitivity may be more significant at lower frequencies (Fay & Popper, 1980).

Analysis of differential sensitivity to sound pressure and particle motion requires a methodology in which the ratio of pressure to particle motion (acceleration, velocity and displacement) for a given frequency can be experimentally manipulated. Techniques using far-field travelling waves provide no basis for such analysis since the ratio of pressure to particle motion remains constant with both decreasing equally with distance from the source. In the acoustic near-field, the ratio changes with distance since particle motion decreases more rapidly than pressure, but the near-field is small, especially for higher frequencies (about 0·05 m at 1000 Hz). Furthermore, practical problems discourage free-field studies and detailed analysis of near-field effects within enclosed containers is difficult because complex sound fields are produced by reflections off walls and particle motions are typically higher than expected for given pressures (Parvalescu, 1964; Popper, 1972; Hawkins, 1973).

Techniques using standing waves provide the simplest and most useful methodology for testing sensitivity to both pressure and particle motion since in a standing wave these two components are 90° out of phase and their respective maxima and minima are spatially separated. Various standing wave apparati have been used in studies of fish (see Hawkins & MacLennan, 1976, for review).

A relatively simple standing wave tube apparatus is used in this study consisting of an upright tube made of a rigid material filled with water. A speaker placed at the base of the tube sets up standing waves between the speaker surface and the

air–water interface. Placement of specimens is simple and the water need not be drained during experiments. Since similar tubes can be used for calibrating velocity hydrophones (Bobber, 1970), it was expected that the apparatus would be suitable for the experimental purpose.

MATERIALS AND METHODS

Standing wave tube apparatus

The standing wave tube was constructed of an upright steel pipe (Schedule 40 steam pipe) 3 m long with a 15 cm internal chamber and 0·63 cm thick walls. Sine wave signals were produced by a function generator (Wavetek Model 186) and amplified to drive a speaker (University Sound Model UW-30) (Fig. 1). Sound produced by the speaker created standing waves with particle motion and pressure 90° out of phase along the length of the tube. Calculation using a formula for deformations in pressure vessels (Roark, 1954):

$$D = \frac{R}{E}\left(\frac{P^R}{t} - \frac{vP^R}{2t}\right), \tag{1}$$

where D = radial displacement, $R = \frac{1}{2}$ outside diameter, P = pressure, t = wall thickness, E = elastic modulus (steel: 2×10^{11} N m^{-2}), and v = Poisson's ratio (steel: 0·26) indicates that at pressures of 1 Pa (well above threshold levels established for *Rana catesbeiana*, Lombard *et al.* 1981), radial displacements at a pressure antinode due to expansion and contraction of the tube walls is very small ($< 10^{-11}$ m). Vertical displacements associated with standing waves at the same pressure level were calculated to be at least 10^2–10^3 times greater than the radial displacement at the range of frequencies used.

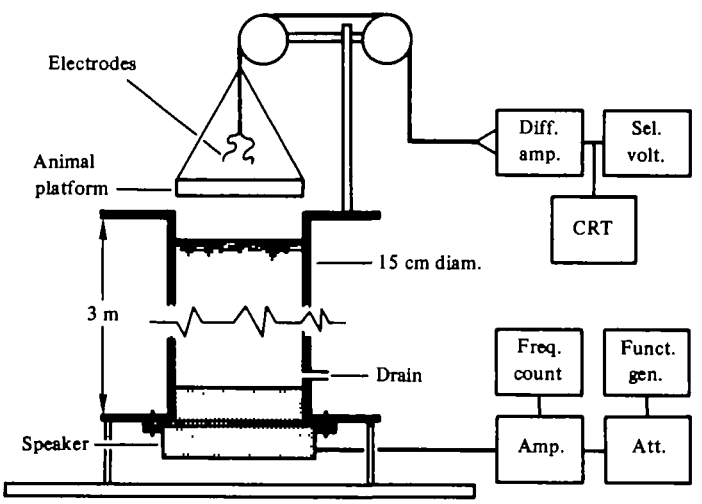

Fig. 1. Schematic diagram of standing wave tube and electronic system for sound stimulus production (bottom) and microphonic response measurement (top). Animal platform cable moves through a pulley system at the top of tube. A wooden platform was constructed around the tube for easy access to the top and to hold instrumentation.

Standing wave calibration

Calibration measurements of pressure and particle motion were made with a Wilcoxon Research Self-Amplified Hydrophone and a GeoSpace GSC-20D Subminiature Digiphone respectively. The hydrophone was tied into a configuration with the head lying in the horizontal plane and the cable marked with a depth scale in centimetres. The digiphone was rigged into a replica of the specimen holder used in experimentation. The specimen holder was made of an open grid plastic platform of rectangular shape, approximately 14·5 cm by 6 cm and 0·3 cm deep. The platform was tied with monofilament line to the inside of a lead strip collar approximately 2·5 cm deep and 0·15 cm thick. This collar lay flush against the inside of the tube when the specimen holder was placed inside. The lead collar provided sufficient weight to counteract the buoyancy of the experimental animals. This specimen holder produced negligible distortion of the sound field inside the tube when tested by suspending it with a hydrophone or digiphone.

The digiphone was suspended above the platform of a specimen holder by tying it to the lead collar with monofilament lines. Fine insulated copper wire was soldered to the digiphone terminals and attached to a shielded cable, marked with a centimetre depth scale, for lowering the entire assemblage. The cable and copper wires did not support the digiphone which was restrained only by the monofilament line supports.

Both outputs of the hydrophone and digiphone setup were fed directly into a Hewlett-Packard 3581-C Selective Voltmeter for measurement at the frequency of

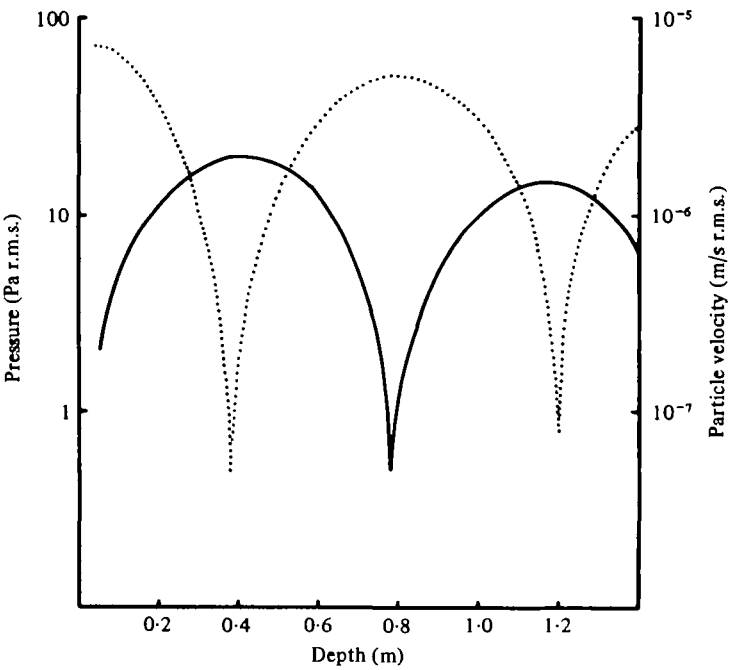

Fig. 2. Semi-log plot of control pressure (solid line) and particle velocity (dotted line) readings in standing wave tube at 894 Hz.

the sound stimulus. Calibration curves of sound pressure and particle motion within the tube containing a specimen holder showed successful spatial separation of these sound components over the entire frequency range of interest (approximately 200–3000 Hz) (Fig. 2). The standing wave tube had a resonant frequency (1 λ) at about 440–450 Hz depending on water temperature. Pressure and particle motion were maximum at multiples of one-half the resonant frequency (beginning at about 220 Hz) and about 10–20 dB above levels at intermediate frequencies at equal speaker outputs. Frequencies below about 220 Hz produced standing waves of less than one-half wavelength. In such cases, the amplitude profile along the length of the tube was truncated at the bottom (speaker surface), with the water surface maintaining a particle motion maximum and pressure minimum. Since pressure always increases rapidly below the surface while particle motion decreases or stays constant, sound fields with spatially distinct pressure and particle motion tracks could be produced at very low frequencies (down to about 20 Hz). However, pressure and particle motion maxima were progressively lower at frequencies below about 0·2 kHz, and testing at such frequencies was hindered by limited intensities.

Absolute measures of pressure were calculated using calibration data for the hydrophone (0·8123 mV/Nm^{-2} rms). Calibration of the digiphone output was achieved by calculating the expected particle motion (velocity) maximum over a range of frequencies at a given depth and pressure in the standing wave tube using the formula:

$$\mu = \frac{P}{\rho c \tan kd}, \tag{2}$$

where μ = particle velocity, P = pressure, ρ = density of water, c = sound velocity, k = wave number, and d = distance from surface of water column (Bobber, 1970). Calculated velocity values were consistently higher (about double) than the values obtained by using the manufacturer's calibration data for the digiphone and its measured output. It is assumed, therefore, that the digiphone suspension system and/or impedance mismatch between the digiphone and water decreases the sensitivity of the digiphone. The calibration value calculated for the digiphone was 13 v/m/sec.

During experimentation measurements of pressure and particle motion were made with whole animals and partial head preparations (described below) within the tube. During such measurements, the animal or head preparations were tied next to the head of the hydrophone or onto the platform suspended below the digiphone. Such measurements provided an accurate characterization of the sound field under such experimental conditions.

Spectral analysis of hydrophone pressure measurements using the sweep mode of the selective voltmeter and an X–Y Recorder (Hewlett-Packard 7035B) at a range of frequencies showed the fundamental frequency of sound stimuli always to be at least 40 dB above harmonic frequencies. A 45 Hz signal, perhaps representing a resonant frequency of the tube support system, was also found, although it was at least 30 dB below the fundamental of the stimulus.

Experimental animals

A total of 56 specimens of four species of frogs were examined. Three species of the genus *Rana*, including *R. catesbeiana*, *R. clamitans*, and *R. pipiens* were used. All three species have similar ear structure, and basically amphibious lifestyles, spending a good deal of time both on land and in water. Both males and females were used, and body size ranged from about 7–13 cm in length and 35–130 gm in weight.

Six specimens of *Xenopus laevis* were also used. *Xenopus* are almost exclusively aquatic frogs, and have a middle ear morphology somewhat different from that found in *Rana*. The tympanum is present but not visible externally, being covered by skin and connective tissue, and a small middle ear cavity exists which does not open into the mouth cavity (no Eustachian tube). Three females of relatively large body size (7·0, 7·4 and 8·2 cm in length) and three smaller males (5·5, 5·9 and 6·6 cm in length) were used.

Microphonic recordings

Animals were anaesthetized with 20% ethyl carbamate using intraperitoneal injections of about 0·02 ml/gm body weight. A small area (approximately 2–3 mm in diameter) of skin and muscle overlying the otic capsule was removed from the dorsal surface of the head and a small hole about 0·2 mm in diameter drilled into the dorsolateral part of the otic capsule above the perilymphatic cistern region of the inner ear. The animal was then tied to the platform of the specimen holder and an insulated tungsten electrode with an exposed tip approximately 50 μm in diameter placed within the perilymphatic cistern. Histological examination was done on four typical experimental preparations to verify correct placement of the electrode. Microphonic potentials were therefore made from outside the endolymphatic sac containing the auditory papillae, as done previously by Strother (1959). A tapered layer of insulation near the electrode tip allowed the recording electrode to be firmly wedged in place in the otic capsule. A reference electrode was placed into the dorsal musculature medio-posterior to the otic region. Both electrodes were attached to leads contained within a shielded cable attached to the specimen holder platform. The animal and specimen holder were then lowered into the tube using the pulley system (Fig. 1). Frogs were tested in both horizontal (animal lying flat on platform) and vertical (animal tilted 90° and lying on side) orientations since particle motion responses may be directionally sensitive to plane of motion. Scanning electron microscopic studies of the auditory papillae of the inner ear of frogs show the amphibian papilla to lie predominantly in a horizontal plane, with most hair cell orientations directed medially or laterally (Lewis, 1978). Animals lying on their side might therefore provide maximum responses to the vertical particle displacements in the tube since the latter would be parallel to the hair cell orientations. The basilar papillae lies in a more intermediate position with respect to the vertical and horizontal axes of the animal (Lewis, 1978). Most hair cells have medio-dorsal orientations (at least in the frog species used in this study), and would probably provide responses to particle motion within the tube with animals both lying flat

and on the side. All experiments were run at water temperatures of approximately 21–23 °C.

Experimental procedure involved recording microphonic responses while lowering or raising the animal through the sound field and plotting responses against simultaneous pressure or particle motion measurements. Microphonic responses were first amplified and then sent to an oscilloscope for visual examination and to a selective voltmeter for measurement at the given frequency (Fig. 1). At frequencies below about 1000 Hz, microphonic responses of all species tested showed a large second harmonic component as observed previously in *Rana catesbeiana* (Capranica, 1966; Paton, 1971). Measurement of microphonics below 1000 Hz was therefore done at double the frequency of the stimulus. Microphonic responses above about 1000 Hz showed a large fundamental component in all species, although a second harmonic component could also be tracked at amplitudes about 5–15 dB below the amplitude of the fundamental. Since artefactual signals unrelated to auditory responses could sometimes be observed at the fundamental frequency, microphonic measurements above 1000 Hz were also made at the second harmonic frequency. Differences between microphonic potentials below and above 1000 Hz appear related to directional orientations of the hair cells contained in the amphibian and basilar papillae respectively (Capranica, 1966). Most tests were run at multiples of one-half the resonant frequency of the standing wave tube (beginning at about 220 Hz) because pressure and particle motion levels were maximum for a given speaker output at such frequencies. The frequencies used in separate tests often differed due to variation of the resonant frequency primarily caused by slight fluctuations in water temperature (21–23 °C) or water level.

Partial head preparations

Entire frogs tended to produce major distortion of the sound field within the tube (see Results), and it was necessary to use preparations including only the part of the head surrounding the middle and inner ear region. Such preparations (total of 30) produced little sound field distortion and provided other experimental benefits (see Results). Frogs were first prepared for microphonic recordings, and several used in whole body experiments, before being decapitated. The lower jaw, nasal chambers, and contralateral side of the head were removed. The middle ear cavity remained intact and full of air. Such preparations produced good microphonic responses for at least 20 minutes at 21–23 °C before noticeable deterioration was observed. Head preparations were tied directly to the specimen platform with a fine wire, and orientated in both horizontal (head flat) and vertical (head tilted 90°) planes.

It also became necessary during experimentation to produce preparations as free of extraneous air bubbles as possible. One method used was to briefly place the preparations, which were constantly kept submerged in water, in a vacuum to remove air bubbles adhering to cut edges of skin and tissues. This procedure did not seem to diminish microphonic responses in any way.

Middle ear experiments

Certain experimental manipulations were made with both whole animals and head preparations to analyse mechanical pathways of sound reception. Only specimens of *Rana catesbeiana* and *Rana clamitans* were used in such experiments. To investigate the role of the air-filled middle ear cavity, the middle ears of certain head preparations were filled with water by cutting a small hole (approximately 1 mm in diameter) in the tympanum and drawing out the air underwater with a fine pipette. Air could be restored for control purposes by draining the cavity and covering the small hole with a thin layer of silicone grease.

The role of the stapes-tympanum complex in underwater hearing was analysed by experimentally impairing this system. The tympana of both whole frogs and head preparations were weighted with a flat, coiled piece of metal wire weighing approximately 1 gm attached with a thin layer of silicone grease. This weight was probably much greater than the combined weight of the stapes and tympanum, and, rather than merely shifting the frequency response of the complex, probably severely dampened its response to the entire frequency range involved in hearing. As an additional and perhaps more direct technique, the stapes was severed in certain experiments. The stapes was cut near its midpoint and both the plectral ligament and stapes itself were completely severed. In the case of whole frogs, the mouth was opened and the stapes cut by placing a fine pair of scissors through the broad Eustachian tube. In head preparations the operation was again done simply through the Eustachian tube. Visual examination immediately after the operation and again after experimentation confirmed a wide gap (usually about 1 mm wide) between the two parts of the stapes. Animals or head preparations were first tested at a range of frequencies (about 250–2500 Hz) before the operation or attachment of weights, and again after the operations or addition of tympanic weights. Tympanic weighting had the advantage of subsequent removal of the weights for control purposes.

RESULTS

Whole animal experiments

Whole frogs of the sizes used produce major distortions of the standing wave sound field. Fig. 3 includes pressure and particle motion tracks at 650 Hz with a frog attached to the hydrophone and digiphone platform. These results are generally representative for all frequencies examined. Pressure and particle motion are largely in phase when an animal is inside the tube, except in the regions near a pressure minimum. At such points, pressure characteristically decreases and increases rapidly on either side of the node, while particle motion decreases to that point and levels off, increasing only very gradually. This observed effect of whole animals is almost certainly caused by the air inside the animals. Tests with small balloons (approximately 2–10 ml in volume) tied to the hydrophone and digiphone accurately mimic the effect of whole frogs. It appears that such balloons and whole animals act as partial air–water interfaces to such a degree as to set up standing waves between themselves and the speaker surface. Much of the particle motion measured probably

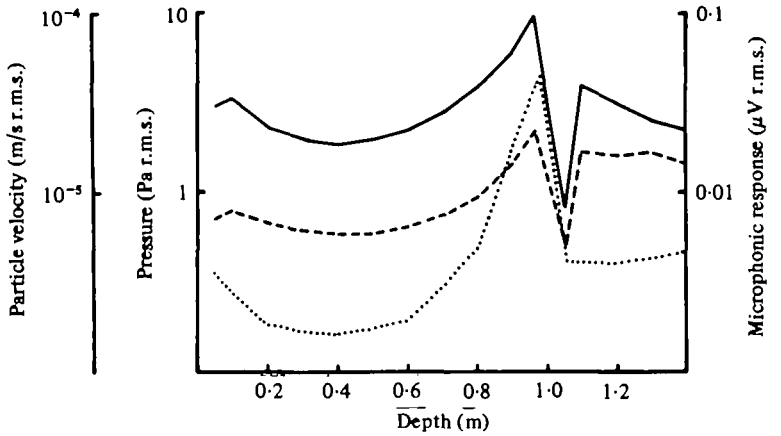

Fig. 3. Semi-log plot of pressure (solid line) and particle velocity (dotted line) readings and microphonic responses (dashed line) for whole *R. catesbeiana* at 650 Hz. Note: compare pressure and particle velocity amplitude profiles with control profiles in Fig. 2.

represents near-field displacements caused by the pulsations of air cavities produced by pressure fluctuations. Therefore, to some degree, pressure and digiphone output are in phase. The independence of pressure and particle motion at pressure minima is difficult to explain. Other complications occur when bubbles are placed inside such a tube, such as the phenomenon of bubble imaging (Meyer, 1957).

Figure 3 also shows microphonic responses for a whole *R. catesbeiana* at 650 Hz. Responses increase sharply in amplitude with pressure after a pressure minimum, rather than levelling off as in the case of particle motion and therefore provide suggestive evidence of pressure sensitivity. Such results were found at all frequencies producing standing waves of about one wavelength or more (thereby enabling testing at at least one pressure minimum) from about 400–3000 Hz. Specimens of *Rana* and *Xenopus* produced similar sound field distortion and similar microphonic tracks. On the whole, experiments aimed at distinguishing between pressure and particle motion sensitivity with whole animals were not considered satisfactory because of the difficulty in understanding the sound field stimulus inside the tube.

Tests with whole frogs did provide a basis for determining the range of frequencies found to produce clear microphonic responses. The range of frequency sensitivity of whole frogs was found to apply to partial head preparations as well. Responses to frequencies below about 200 Hz were consistently very small (less than about 0·1 μV) even at relatively high sound pressure levels (above about 100 Pa) for all species. Microphonic responses above about 200 Hz in all species appeared to generally track underwater midbrain threshold curves for *Rana catesbeiana* (Lombard *et al.* 1981), gradually becoming more difficult to distinguish above about 1500 Hz but often clear to 3000 Hz. Microphonic responses in this frequency range could usually be obtained at pressure levels 20–40 dB above midbrain threshold levels.

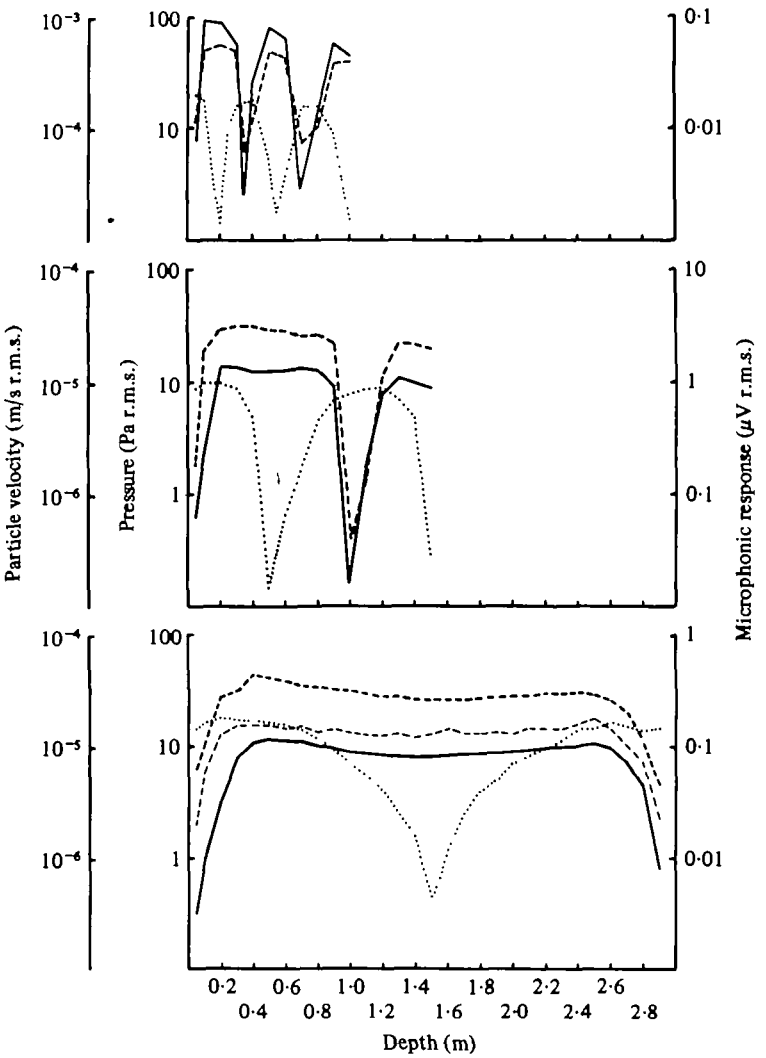

Fig. 4. Semi-log plots of pressure (solid lines) and particle velocity (dotted lines) readings and microphonic responses (dashed lines) for *R. catesbeiana* head preparation at 1986 Hz (top), 661 Hz (middle), and 224 Hz (bottom). Included at bottom are microphonic responses of *Xenopus* head preparations at 222 Hz (fine dashed line). Pressure and particle motion readings for bottom graph pertain to *R. catesbeiana* preparation at 224 Hz and are essentially the same as readings for *Xenopus* preparation.

Partial head preparations

Fig. 4 displays pressure and particle motion tracks at 224 Hz (lower graph) for a *R. catesbeiana* head preparation. The relationship between pressure and particle motion is approximately that expected from control measurements (Fig. 2). The microphonic response clearly tracks pressure. Also plotted are pressure and particle motion tracks and microphonic responses at 641 and 1986 Hz and, again, the responses track pressure. All of the above microphonic responses were obtained at sound

pressure levels approximately 40–80 dB above midbrain thresholds, and cover frequencies considered 'low' (224 Hz), 'middle' (661 Hz), and 'high' (1986 Hz) for most frog species (see Capranica, 1976, for review). All specimens of *R. catesbeiana*, *R. clamitans* and *R. pipiens* showed pressure tracking within this frequency range.

Figure 4 also shows representative data from a head preparation of *Xenopus laevis*. Pressure tracking is evident at this frequency as well as other frequencies examined. One specimen of *Xenopus* produced a pressure tracking at the lowest frequency for which any microphonic response track could be discerned (170 Hz). Head orientation ('horizontal' or 'vertical') was found to have no effect on the microphonic responses of the head preparations of several *Rana* and *Xenopus* examined.

Microphonic responses from whole animals and isolated head preparations from the same animal were similar in amplitude for the same sound levels. Although the shapes of the response curves differed (Figs. 3 and 4), both ranged over the same general range of amplitude and both had similar maximum responses.

Middle ear experiments

The air-filled middle ear cavity of partial head preparations appeared responsible for the pressure sensitive tracking of such preparations. Figure 5 shows microphonic response curves of a representative *R. catesbeiana* preparation at 648 Hz. Filling the middle ear cavity with water markedly reduces microphonic responses, by about 40% (4–5 dB) at points of maximum response. Re-filling the cavity with air partially restored the response. This effect was observed in several other preparations of *R. catesbeiana* and *R. clamitans* tested at about 220 Hz (two preparations), 880 Hz (one preparation), 1300 Hz (two preparations), and 1800 Hz (two preparations).

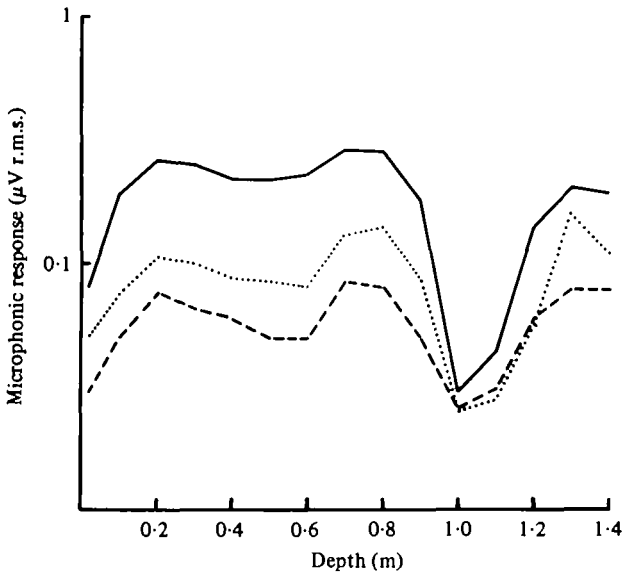

Fig. 5. Semi log plot of microphonic responses from *R. catesbeiana* head preparation at 648 Hz with normal air-filled middle ear cavity (solid line), water-filled middle ear cavity (dashed line), and subsequently drained middle ear cavity (dotted line).

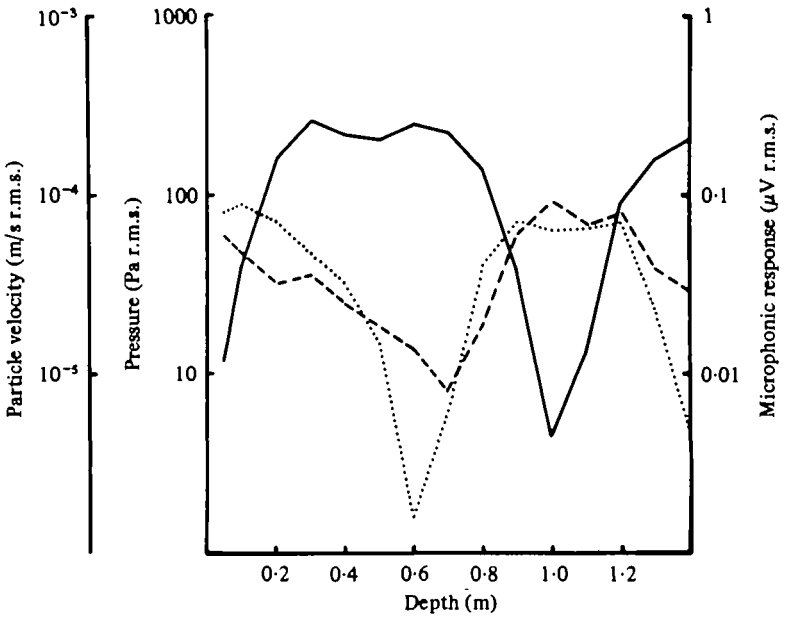

Fig. 6. Semi-log plot of pressure (solid line) and particle velocity (dotted line) readings and microphonic responses (dashed line) for *R. catesbeiana* head preparation with water-filled middle ear cavity at 661 Hz indicating particle velocity tracking by microphonic responses.

It should be noted that even with the middle ear cavity filled with water, a general pattern of pressure tracking is still discernible. Small air bubbles adhering to the preparation appear responsible for such pressure sensitivity. Briefly exposing the preparation to a vacuum depresses such pressure tracking and produces relatively flat, low amplitude responses. Such preparations occasionally showed particle motion sensitivity under relatively high sound levels. Fig. 6 shows particle motion tracking in a *R. catesbeiana* preparation at 661 Hz. Peak pressure levels in this figure are approximately 100 dB above midbrain threshold levels and about 40 dB above levels producing clear pressure tracking in the same preparation with a normal air-filled ear. Particle motion tracking was observed only at frequencies below about 800 Hz, and then in only certain cases, in *R. catesbeiana* and *R. clamitans* preparations. No comparable tests with *Xenopus* specimens were done. Sound intensities required to produce discernible particle motion sensitivity were near levels causing distortion artefacts with the speaker used, so additional testing at greater particle velocities was not feasible. Microphonic responses associated with particle motion sensitivity showed a dominant second harmonic component as seen in pressure sensitive responses. Orientation of the head preparations did not appear to alter sensitivity to particle motion, with insignificant differences seen between vertical or horizontal head positions.

Severing the stapes or weighting the tympanum produced very similar effects in significantly lowering sensitivity over a broad range of frequencies (about 200–2500 Hz). Fig. 7 shows control microphonic responses recorded from a *R. clamitans* head

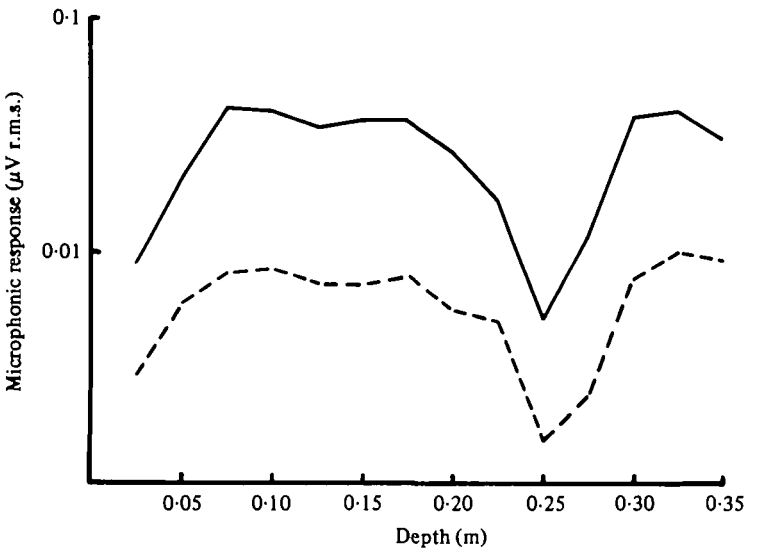

Fig. 7. Semi-log microphonic responses for *R. clamitans* head preparation at 1326 Hz with intact stapes (solid line) and severed stapes (dashed line). Stimulus pressure levels ranged from about 2 Pa (minimum) to 60 Pa (maximum).

preparation at 1332 Hz and the responses after severing the stapes. The general tracking is still pressure sensitive, but sensitivity is lowered throughout and in the region of maximum response is reduced approximately 80% (14 dB). Weighting the tympanum produced generally identical results, and upon removal of the weight the response returned to normal. Degree of reduction in sensitivity due to severing the stapes and weighting the tympanum was also very similar for both whole frogs and partial head preparations.

Reduction in sensitivity was not identical, however, over the entire frequency range investigated. Depending on body size, reduction was often very slight (less than 10% or 1 dB) at frequencies between about 400–1000 Hz. Fig. 8 shows a graph of percentage microphonic response after severing the stapes compared to initial control responses of head preparations from four male *Rana catesbeiana*: two relatively large specimens (125 and 129 mm in body length; tympanum diameters of about 15 mm) and two smaller specimens (76 and 80 mm in body length; tympanum diameters of about 10 mm). Measurements were made at microphonic maxima (pressure maxima) for both control and experimental conditions. Frequencies involving minimum reduction of microphonic responses are seen to vary with body size, generally being lower for the large frogs (about 400–700 Hz) and higher for the small frogs (800–1100 Hz). The general zone of minimum effect is shifted towards lower frequencies in the large frogs compared to the smaller specimens. Estimates of middle ear volumes were obtained for all four specimens after subsequent fixation. The middle ear cavities were filled with water, and the water then pipetted out and measured. Volumes for the smaller frogs were approximately 0·2 ml, and about 0·4 ml for the larger animals.

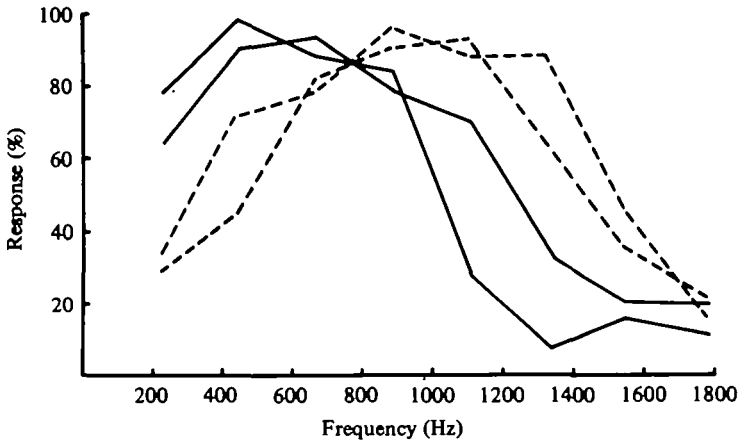

Fig. 8. Percent microphonic responses remaining after severing the stapes in head preparations of 2 'large' (solid line) and 2 'small' *R. clamitans* plotted against stimulus frequency. See text for details of size categorization. Microphonic responses were recorded at response maxima (pressure maxima) both before and after operation.

Tests done with frogs of intermediate size tended to show minimum microphonic reduction at intermediate frequencies. Figure 8 portrays the extremes of the size range used to clearly distinguish the size-related trend. The same trend in body size effects was observed in whole frogs and with the alternate technique of tympanic weighting.

DISCUSSION

The frog species tested utilize the pressure component of sound in underwater hearing. Pressure-related sensitivity was observed down to 20–40 dB above midbrain threshold levels determined for *Rana catesbeiana* by Lombard *et al.* 1981. The frequency range over which pressure has been found to be the effective stimulus (about 200–3000 Hz) corresponds with the general frequency ranges of the amphibian papilla (about 100–1000 Hz) and basilar papilla (about 1000 Hz and above) of the ranid inner ear in aerial hearing (Capranica, 1976). Particle motion sensitivity was difficult to demonstrate within available intensity ranges, although particle motion tracking was established at certain frequencies at sound levels approximately 100 dB above threshold levels. Such responses were found only in preparations in which pressure sensitivity had been abolished, and would normally be obscured by pressure-related responses of much higher amplitude. Testing at frequencies lower than about 200 Hz was difficult since maximum intensities at such frequencies were limited in our standing wave tube apparatus. Particle motion may be a significant stimulus at such low frequencies, especially for the sacculus which appears highly sensitive to terrestrial vibration below about 100 Hz in various frog species (Lewis, pers. comm.). Over the general range of the anuran auditory papillae, however, pressure appears the relvant stimulus.

The middle ear cavity appears primarily responsible for underwater pressure

sensitivity. Both whole animals and partial head preparations show microphonic responses of similar amplitude, suggesting that the mouth cavity and lungs may not be involved significantly in pressure transduction. The mode of stimulation of the auditory papillae underwater appears similar to that in air, with the tympanum and stapes acting as the route of acoustic energy. Severing the stapes or weighting the tympanum causes significant reduction of microphonic responses over the general frequency range of 200–2500 Hz, although reduction was usually slight at frequencies between about 400–1000 Hz. This latter effect was body-size dependent, however with larger frogs having lower frequencies of minimum reduction. It is suggested that this phenomenon is related to the resonance frequency of the middle ear cavity. The middle ear volumes of the smaller frogs used in these tests was approximately 0·2 ml, and that of the larger frogs approximately 0·4 ml. If the middle ear is initially assumed to be a spherical air cavity, then the approximate radii of these cavities would be about 0·36 and 0·46 cm respectively.

Using a generalized formula for the natural resonance frequency of a spherical air bubble (monopole source) in a sound field (Meyer, 1957):

$$f_n = \frac{0{\cdot}328}{R}, \tag{3}$$

where R = the radius and f_n = the natural (resonant) frequency of the bubble, spherical air cavities with radii of about 0·36 cm and 0·46 cm would have resonance frequencies of approximately 900 Hz and 720 Hz respectively. Tests involving severing the stapes found minimum reduction of microphonic responses at about 800–1100 Hz in the smaller frogs and about 400–700 Hz in large frogs. The calculated and experimental values would not be expected to correspond completely, since the middle ear cavity is not spherical and has tissue boundaries that would probably alter resonance characteristics. Nonetheless, there is an approximate concordance in that both calculated and measured values for small frogs are higher than those for large frogs.

It is hypothesized that at such resonant frequencies the middle ear cavity pulsates to such an extent as to produce auditory stimulation without requiring the tympanum–stapes pathway. Stimulation would occur rather through a shaking of the entire otic capsule produced by the pulsations of the adjacent middle ear cavity. At frequencies substantially different from the resonance frequency, sound pressure would produce pulsations of the ear cavity of much lower amplitude that might be damped by the tissue surrounding the cavity. Under such conditions, however, the small pulsations of the cavity would still produce significant movements of the tympanum and corresponding displacements of the footplate of the stapes, thereby setting up compression waves within the inner ear. The more compliant tympanum may undergo greater displacements than the other tissue borders (at least in *Rana*) in response to pressure fluctuations at such frequencies, thereby allowing for increased sensitivity. At resonant frequencies, the tympanum–stapes pathway, while perhaps contributing to overall sensitivity (slight microphonic reduction does occur upon severing the stapes at resonant frequencies), is not as functionally significant as at lower or higher frequencies. It is hypothesized, therefore, that the pattern of stimulation via the tympanum–stapes

pathway at non-resonant frequencies is similar to that operating in aerial hearing. At resonant frequencies, stimulation may also be achieved by pressure effects causing pulsations of the middle ear cavity which are transmitted directly to the inner ear through the surrounding tissues.

It is perhaps unexpected that air cavities other than the middle ear cavity appear to have little role in underwater hearing. Certainly any air in the mouth cavity or lungs is, as seen in the effects of whole frogs on the standing wave sound field, easily effected by sound pressure. One might expect that these air cavities would pulsate with pressure changes, producing near field displacements that might stimulate the ear. Over the frequency range examined, any such effect of the mouth and lungs is probably swamped by stimulation via the middle ear. It is possible that the mouth cavity and lungs might be more significant at frequencies below 0·2 kHz which could not be satisfactorily tested in the standing wave tube. The lungs, which may have volumes greater than 2–3 cc in large frogs, might especially have low resonant frequencies at which they might produce high amplitude near-field displacements to drive the ear. Interaction of the mouth cavity and lungs with the pressure-transducing characteristics of the middle ear cavity via direct connections with the latter might also be expected. The middle ear cavity has a broad opening with the mouth cavity in the species of *Rana* tested; the middle ear cavity of *Xenopus laevis* is separate from the mouth cavity and self-enclosed. In the former, this connection might be expected to alter the pressure sensitive features of the ear cavity, and the lungs, which might at times form a continuous air cavity with the mouth and middle ear, could also be involved. None the less, partial head preparations suggest little difference in sensitivity with or without the mouth cavity or lungs over the frequency range examined. In *Rana*, the ear cavity might be effectively separated from the mouth cavity by close adpression of the lower jaw to the roof of the mouth, and the lungs are probably effectively sealed by the epiglottis.

The demonstration of pressure sensitivity in underwater hearing in *Rana* and *Xenopus* is significant in that the ears of most tetrapod vertebrates are assumed to respond to particle motion underwater via 'bone conduction'. This however, is the first case in which differential sensitivity to pressure and particle motion components of underwater sound has been tested in a tetrapod. Much previous work on underwater hearing in mammals has stressed bone conduction as the basis of hearing in such forms as pinnipeds (Repenning, 1972; Terhune & Ronald, 1972), cetaceans (see Popper, 1980, for review) and man (Smith, 1969). However, virtually all predominantly aquatic tetrapods have air-filled middle ear cavities, and such air cavities could certainly function as pressure transducers at least at certain frequencies. Of interest would be possible correlation of frequencies of maximum sensitivity with estimated or measured resonance frequencies of such cavities. The middle ear cavities of aquatic tetrapods also frequently display unique features. For example, the ear of dolphins is surrounded by an extensive system of air-filled space (Purves, 1966), and fossil evidence from reptile lineages which have evolved aquatic forms, such as various turtles and lizards (mosasaurs), show consistent trends in the elaboration and enlargement of the middle ear cavity (Bramble, pers. comm.). While such modifications could have several interpretations, such as providing increased

isolation of the ear from the skull to maximize directional information via bone conduction, such morphology also might be related to improved stimulation of the ear by pulsations of surrounding air cavities. Underwater pressure transduction by middle ear cavities as demonstrated here in anuran amphibians may be more widespread among aquatic tetrapod vertebrates than currently recognized.

This study was supported by a National Institutes of Health postdoctoral fellowship (1 F32 NSO6531-01) to TEH and a National Science Foundation grant (DEB 8002619) to REL. The authors wish to thank John Gilpin for his help in constructing the experimental apparatus, Joan Hives for her careful typing and editing of the manuscript, and Shirley Aumiller for her help in preparing the figures. Richard Fay and Cynthia Hillery read a preliminary manuscript and we are grateful for their comments and criticisms.

REFERENCES

BOBBER, R. J. (1970). Underwater Electroacoustic Measurements. Naval Research Laboratory. U.S. Government Printing Office, Washington, D.C.

BUWALDA, R. J. A. (1981). Segregation of directional and nondirectional information in the cod. In *Hearing and Sound Communication in Fishes* (ed. W. N. Tavolga, A. N. Popper and R. R. Fay), pp. 139–171. New York: Springer-Verlag.

CAPRANICA, R. R. (1966). Vocal responses of the bullfrog to natural and synthetic mating calls. *J. Acoust. Soc. Am.* **40**, 1131–1139.

CAPRANICA, R. R. (1976). The auditory system. In *Physiology of the Amphibia*, vol. III (ed. B. Lofts), pp. 443–466. New York: Academic Press.

FAY, R. R. & POPPER, A. N. (1975). Modes of stimulation of the teleost ear. *J. exp. Biol.* **62**, 379–387.

FAY, R. R. & POPPER, A. N. (1980). Structure and function in teleost auditory systems. In *Comparative Studies of Hearing in Vertebrates* (ed. A. N. Popper and R. R. Fay), pp. 3–42. New York: Springer-Verlag.

HAWKINS, A. D. (1973). The sensitivity of fish to sounds. *Oceanogr. Mar. Biol. Ann. Rev.* **11**, 291–340.

HAWKINS, A. D. & MACLENNAN, D. N. (1976). An acoustic tank for hearing studies on fish. In *Aquaculture and Fisheries Science*: Symposium on Sound Reception in Fish, 1975 (ed. A. Shuijf and A. D. Hawkins), pp. 149–169. Amsterdam: Elsevier.

LEWIS, E. R. (1978). Comparative studies of the anuran auditory papillae. In *Scanning Electron Microscopy*, 1978, vol. II, pp. 633–642. SEM, Inc., AMF O'Hare, Illinois.

LICHT, L. E. (1969). Comparative breeding behaviour of the red-legged frog (*Rana aurora aurora*) and the western spotted frog (*Rana pretiosa pretiosa*) in southwestern British Columbia. *Can. J. Zool.* **47**, 1287–1299.

LOMBARD, R. E., FAY, R. R. & WERNER, Y. L. (1981). Underwater hearing in the frog, *Rana catesbeiana*. *J. exp. Biol.* **91**, 57–71.

MEYER, E. (1957). Air bubbles in water. In *Technical Aspects of Sound*, Vol. 2. Ultrasonic range, underwater acoustics (ed. E. G. Richardson), pp. 222–239. Amsterdam: Elsevier.

PARVULESCU, A. (1964). Problems of propagation and processing. In *Marine Bio-acoustics* (ed. W. N. Tavolga), pp. 87–100. Oxford: Pergamon.

PASSMORE, N. I. & CARRUTHERS, V. C. (1979). *South African Frogs*. Johannesburg, South Africa: Witwatersrand University Press.

PATON, J. A. (1971). Microphonic potentials in the inner ear of the bullfrog. Master's thesis, Cornell University.

POPPER, A. N. (1972). Pure-tone auditory thresholds for the carp, *Cyprinus carpio*. *J. Acoust. Soc. Am.* **52**, 1714–1717.

POPPER, A. N. (1980). Sound emission and detection by delphinids. In *Cetacean Behavior: Mechanisms and Function* (ed. L. M. Herman), pp. 1–52. New York: John Wiley.

PURVES, P. E. (1966). Anatomy and physiology of the outer and middle ear in cetaceans. In *Whales, Dolphins and Porpoises* (ed. K. S. Norris), pp. 321–376. Los Angeles: University of California Press.

REPENNING, C. A. (1972). Underwater hearing in seals: functional morphology. In *Functional Anatomy of Marine Mammals*, vol. 1 (ed. R. J. Harrison), pp. 307–331. Oxford: Academic Press.

ROARK, R. J. (1954). *Formula for Stress and Strain*. New York: McGraw-Hill.

SMITH, P. F. (1969). Underwater hearing in man. 1. Sensitivity. Submarine Medical Research Laboratory, Naval Submarine Medical Center, report no. 569, pp. 1–23.

STROTHER, W. F. (1959). The electrical response of the auditory mechanism in the bullfrog (*Rana catesbeiana*). *J. comp. Physiol. Psychol.* **52**, 157–162.

TERHUNE, J. M. & RONALD, K. (1972). The harp seal, *Pagophilus groenlaundicus* (Erxleben, 1777), III The underwater audiogram. *Can. J. Zool.* **50**, 565–569.

VAN BERGEIJK, W. A. (1967). The evolution of vertebrate hearing. *Cont. Sens. Physiol.* **2**, 1–49.

WEVER, E. G. (1971). The mechanics of hair-cell stimulation. *Ann. Otol. Rhinol. Lar.* **80**, 786–804.

WEVER, E. G. (1974). The evolution of vertebrate hearing. In *Handbook of Sensory Physiology*. Vol. V/I: Auditory system (ed. H. H. Autrum, W. D. Keidel and W. D. Neff), pp. 423–454. Berlin: Springer-Verlag.