STRUCTURE AND FUNCTION OF THE AUDITORY SYSTEM IN THE CLOWN KNIFEFISH, *NOTOPTERUS CHITALA*

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SUMMARY

Hearing sensitivity and the anatomy of the auditory system in the clown knifefish, *Notopterus chitala*, were studied using behavioural techniques and scanning electron microscopy. While many structural features of the inner ear were similar to those found in other teleost species, the saccular endorgan in *Notopterus* was unusual in having three discrete epithelial regions. These regions could be distinguished from one another by regional differences in portions of the otolith overlaying them, by hair-cell orientation patterns, and by their position relative to the swimbladder. *Notopterus* was found to be able to detect sounds from 100 to 1000 Hz and best sensitivity occurred at 500 Hz, where the mean threshold was − 30 dB (re: 1 microbar). For frequencies from 300 to 700 Hz there was substantial variation in threshold values and indications of a bimodal distribution of thresholds. One hypothesis that may tie the ultrastructural and behavioural results together is that the ear in *Notopterus* is anatomically and functionally differentiated to mediate independent detection of two sound properties: changes in pressure and particle motion.

INTRODUCTION

Recent comparative studies have revealed considerable variability in the auditory system of fishes, including structural features (Platt & Popper, 1981; Popper, 1977, 1978, 1981), auditory capabilities (Coombs & Popper, 1979; Fay & Popper, 1980; Hawkins, 1981; Popper & Coombs, 1980*b*), and sound detecting mechanisms (Chapman & Sand, 1974; Enger & Andersen, 1967; Fay & Popper, 1974, 1975). Functional studies, however, have been primarily limited to the goldfish and cod –

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only two of the more than 25 000 extant fish species. To enhance our understandin of fish audition, we are continuing a comparative survey of fish auditory systems with a combined anatomical and behavioural study on the clown knifefish, *Notopterus chitala*.

Notopterus belongs to the superorder Osteoglossomorpha, a group of fishes in which there is wide variation in structural features of the auditory system (Greenwood, 1973; Popper, 1979; Coombs & Popper, 1980; Popper & Coombs, 1982). *Notopterus* is of particular interest because of a close physical relationship between the inner ear and the swimbladder (Dehadrai, 1957). In general, species in which the swimbladder and inner ear are close to one another seem to perform 'better' than other species without such relationships in a number of auditory tasks, including simple sound detection (Popper & Fay, 1973; Tytler & Blaxter, 1979; Coombs & Popper, 1979), and more complicated tasks related to frequency analysis (Dijkgraaf & Verheijen, 1950; Jacobs & Tavolga, 1968; Fay, 1970; Coombs, 1981; Enger, 1981; Hawkins, 1981). While some of these capabilities are related to the pressure-transducing properties of the air-filled swimbladder (Coombs & Popper, 1979), others may not be (Coombs & Popper, 1981). Furthermore, we do not know why taxonomically unrelated species have evolved what, on the basis of current knowledge, appear to be similar sound detection and analysis systems (Popper & Coombs, 1980 *a, b*, 1982). The results presented here involve the use of anatomical and behavioural techniques to provide us with basic information on the auditory system in *Notopterus* and with an initial insight into sound processing in a species with a specialized auditory system.

MATERIALS AND METHODS

Behavioural and ultrastructural studies were conducted on specimens of *Notopterus* about 8 cm in length from snout to base of the tail except for a single 20 cm specimen used in ultrastructural studies.

Ultrastructural procedures

Tissue for studies of the sensory epithelia of the sacculus and lagena were dissected from four specimens and prepared as described earlier (Popper, 1977, 1981). Briefly, the fish were anaesthetized with ethyl-m-amino benzoate (Sigma), the crania opened, the brains aspirated away, the ears exposed, and the cranial cavity filled with cold fixative (4% glutaraldehyde in 0·2 M s-collidine buffer). After about 10 min the animals were decapitated and the bulk of the tissue surrounding the ear region of the cranium dissected away. The remaining tissue was placed in cold fixative for at least 48 h, washed in cold buffer (0·2 M s-collidine buffer with 13% sucrose), post-fixed in 1% osmium tetroxide for about 45 min, run through alcohols and finally stored in 70% ethanol until used.

Final dissections and preparation for scanning electron microscopy entailed opening the otolithic organs and lifting the otoliths from the sensory epithelia. A stream of ethanol or an ultrasonic cleaner (Corwin, 1977; Popper, 1977) was used to 'blow away' the otolithic membrane, which separates the epithelium from the otolith, off the epithelia. Tissue was then critical-point-dried, mounted on alumi
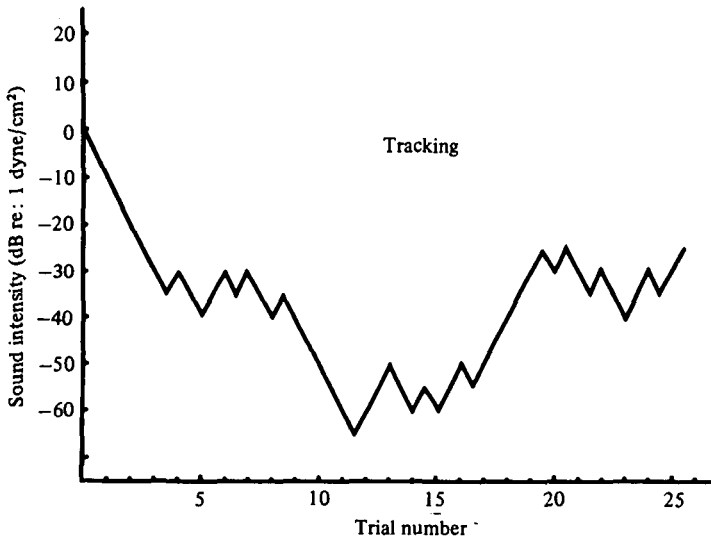
Fig. 1. Graph of a single day's threshold determination for an animal tested at 500 Hz, illustrating the tracking procedure used in determining thresholds. Each shift in direction in sound level is used to indicate a change from detection to non-detection. The figure also illustrates a special case in which two different threshold plateaus are present in a single test session. The threshold first stabilized at about −35 dB (re: 1 microbar) for several trials, dropped to about −55 dB and then returned to −32 dB for the final trials. Since thresholds for a single day are based upon the last eight reversals, the threshold for this day would have been calculated as −32 dB.

stubs, coated with gold–iridium, and viewed with an ETEC Autoscan scanning electron microscope.

Behavioural procedures

Behavioural auditory sensitivity was determined using operant conditioning techniques similar to those used for other species in our laboratory (see Coombs & Popper, 1979; Popper & Clarke, 1979). Animals were trained to cross a hurdle in the centre of a small test aquarium when a sound was presented. Failure to cross the barrier elicited a mild pulsed shock (7 V RMS, 60 Hz at the source, one 40 ms pulse per s). A trained animal was one that would successfully cross the hurdle prior to shock onset, and thereby avoid getting any shock for 80 % of the trials on three successive days. Such avoidance was used as a measure of sound detection, whereas failure to avoid shock onset was interpreted as non-detection.

Hearing sensitivity was measured using the 'up–down staircase' method of tracking the threshold (Fig. 1). In this procedure (see Tavolga & Wodinsky, 1963; Jacobs & Tavolga, 1967) the sound pressure level (SPL) was decreased by 5 dB following each avoidance response and increased in 5 dB steps following each trial in which the animal did not detect the sound. Each detection/non-detection alternation between successive trials was used to determine a single threshold value which was measured as the sound level half-way between those of the two trials. The last eight individual thresholds of a single day's test session were averaged to determine one threshold value for that day. The last eight values, rather than all

Table 1. *Standard deviations and range (in parentheses) of the sound pressure
levels measured in water at ten different locations in each test tank*

| | Standard deviation and range (dB) | | | |
| | Single-speaker system | | Multiple-speaker system | |
Frequency (Hz)	Tank 1	Tank 2	Tank 1	Tank 2
100	0·8 (2·3)	0·6 (1·6)	1·1 (3·2)	0·7 (2·0)
200	3·0 (10·7)	2·7 (7·7)	4·3 (15·1)	3·0 (8·3)
300	6·5 (20·0)	3·5 (10·0)	6·3 (23·4)	5·0 (16·0)
400	6·8 (20·1)	7·6 (20·0)	4·3 (10·0)	5·7 (18·4)
500	3·4 (11·7)	3·7 (9·8)	6·3 (16·0)	4·5 (16·0)
600	5·3 (17·8)	6·3 (16·7)	5·3 (15·2)	6·0 (21·3)
700	3·4 (10·9)	3·5 (13·0)	5·6 (17·0)	4·8 (16·7)
800	3·4 (10·9)	5·3 (14·1)	5·7 (14·4)	3·4 (9·5)
1000	4·4 (13·7)	4·0 (13·0)	1·9 (6·1)	5·7 (19·7)

of the 13–20 that may be determined for a single day, were used in order to allow ample time for the animal to reach a stable threshold. Unstable thresholds (those with a standard deviation greater than 7 dB) were rejected.

The experiments were controlled using a minicomputer which monitored behavioural responses of the fish through a photocell system, presented and attenuated sounds, presented shocks and timed all aspects of the experiments.

Experiments were conducted in test tanks placed in the centre of either of two sound-attenuated rooms. The rooms had walls 20 cm thick filled with sand (see Popper & Clarke, 1979), which were sufficient to attenuate ambient noises in the frequency range above about 50 Hz by at least 20 dB (and more as frequencies increased). The sound system was similar to that described earlier (Coombs & Popper, 1979), except that sound was presented through a single 20·3 cm diameter speaker above the test tank rather than from an array of small speakers. Sound-pressure levels were measured with a hydrophone placed at ten different positions in each tank. The range and standard deviation from the mean of these measures are shown in Table 1 for each tank and the ranges are plotted in Fig. 2A as a function of frequency. The median output of the hydrophone recordings in each tank was used as the final calibration value for each test frequency. Velocity levels of particle motion were also measured for the single speaker system with a velocity hydrophone sensitive on the vertical axis. Due to the size of the velocity hydrophone, it was not possible to measure levels in the same ten positions that were used for pressure measurements. Instead, velocity levels accompanying a 0 dB re: 1 μbar pressure signal were measured at four different locations in the tanks. The mean of these values averaged 16 dB re: 1 μVar (1 μVar = 6·7 × 10^{-6} cm/s) and ranged from 6 dB re: 1 μVar at 800 Hz to 26 dB re: 1 μVar at 400 Hz. For any one frequency, the average range in velocity levels at different tank positions was 12 dB, with the largest range at 800 Hz and the smallest at 400 Hz.

The velocity levels reported here are higher than would be expected in a true far-field situation, where a sound pressure of 1 μbar is accompanied by a particle
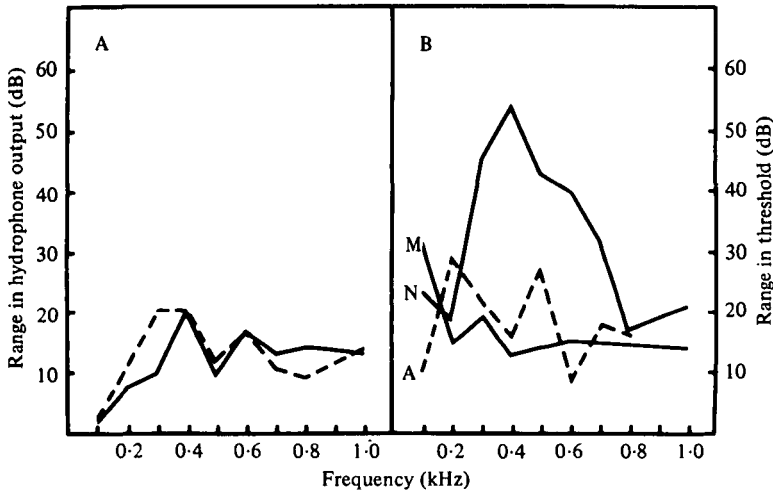
Fig. 2. Graphs comparing the range in sound-pressure levels calibrated for different locations in the two test tanks (dotted and solid lines in A) to the range in threshold values measured for three different species of fish tested in the same tank (B). M, *Myripristis kuntee*; N, *Notopterus chitala*; A, *Adioryx xantherythrus*. Sound-pressure ranges are shown for the single-speaker system used for *Notopterus*, but are nearly identical to those resulting from use of the multiple-speaker arrangement used for *Myripristis* and *Adioryx* (see Table 1 and Coombs & Popper, 1979). Note that velocity ranges are within the sound-pressure ranges shown here.

velocity of 1 μVar (Siler, 1969). The velocity levels are also higher than what was previously predicted for acoustic systems similar to those used here (Parvulescu, 1967; Jacobs & Tavolga, 1967; Hawkins & McLennan, 1976; Hawkins & Johnstone, 1978).

<div align="center">RESULTS</div>

Anatomical studies

Gross dissections and radiographs confirmed a close association between the ear and anterior projections of the swimbladder (Fig. 3), as first described by Dehadrai (1957). The projections attach to the lateral surface of the bony bulla encompassing each of the inner ears, in the vicinity of the saccular endorgan. Both the bulla and swimbladder walls are appreciably thinner than elsewhere in the area of attachment.

The membranous chamber of the sacculus in *Notopterus* is attached intimately to the surrounding bone and, as a result, it was impossible to dissect out the ear intact. However, the gross structure of the ear (Fig. 4a) could be ascertained from a number of partial dissections. Considerable care had to be exercised to remove the saccular sensory epithelium (macula) intact, and a complete macula could only be obtained from the largest of the specimens. However, the shape and size of the macula was demonstrable in each specimen since it was removable in large sections.

The saccular otolith is ellipsoid, with a long anterior projection (Fig. 5) that fits snugly into the surrounding region of the saccular membranous chamber and which is lodged in a groove of bone in the cranium (see Dehadrai, 1957). The saccular macula consists of three discrete regions (Fig. 4), each of which is associated with

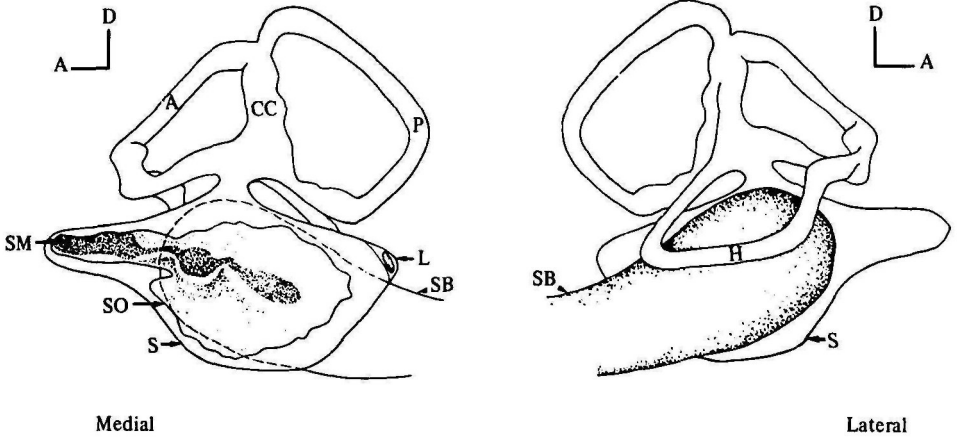Medial   Lateral

Fig. 4. Drawings of the medial and lateral views of the relationship between the anterior projection of the swimbladder (SB) and the ear in *Notopterus*. The swimbladder is actually separated from the saccular chamber (S) and otolith (SO) by a thin bony membrane not shown here. Note that the swimbladder projection does not extend over the anterior one-third of the saccular macula (SM) nor over the entire lagenar chamber (L). Further, note that the saccular macula is divided into three 'regions' connected by thin bridges.
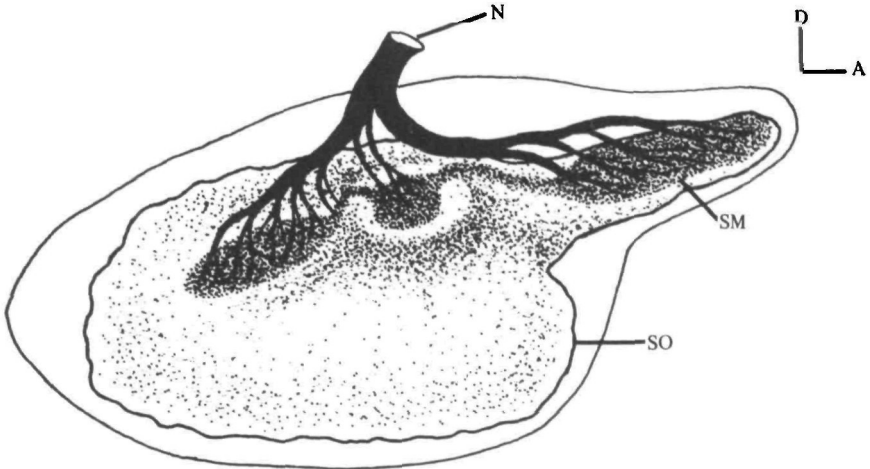

Fig. 5. Drawing of the saccular chamber in *Notopterus*. Note the anterior projection and the 'bump' in the centre of the saccular otolith (SO). These regions on the otolith correspond to the anterior and medial regions of the saccular macula (SM), which is shown here with its innervating nerve (N).

a specific feature of the otolith (Figs. 4, 5). The most rostral epithelial region lies along the anterior otolith projection and is connected by a thin epithelial 'bridge' to the middle epithelial region which lies along a central 'bump' on the otolith. The middle region is connected by another 'bridge' to the caudal epithelial region just behind the otolith bump. The middle and posterior epithelial regions are associated with portions of the otolith closest to the swimbladder diverticulum.

The lagena is quite similar to that found in many other fishes (e.g. Popper, 1977) and contains a small rounded otolith. It is located on the dorsal–posterior quadrant
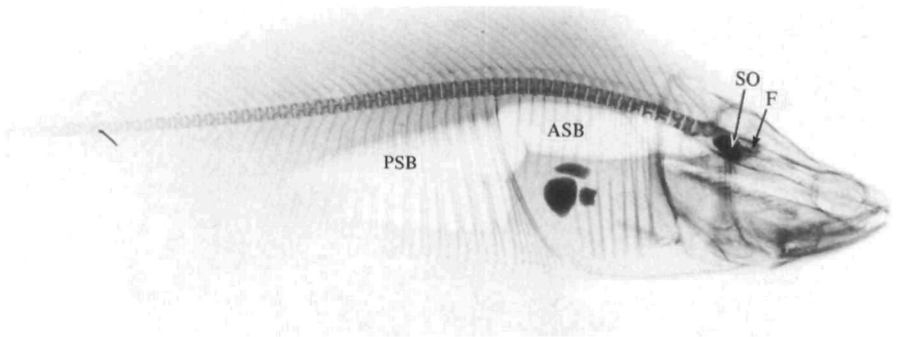
Fig. 3. Radiograph showing a lateral view of the swimbladder/inner-ear relationship in *Notopterus*. Fish were anaesthetized before being X-rayed. Darkened areas represent radio-opaque structures, such as bones or otoliths, whereas lighter areas outline radio-translucent structures such as air-filled cavities. Note the anterior finger-like projection (F) of the saccular otolith (SO) and the proximity of the anterior region of the swimbladder (ASB) to the posterior portion of the saccular otolith. A detailed drawing of this region is shown in Fig. 4. The darkened patches below the swimbladder are probably stones that have been swallowed and become lodged in the gut. PSB, Posterior swimbladder region.

Fig. 6

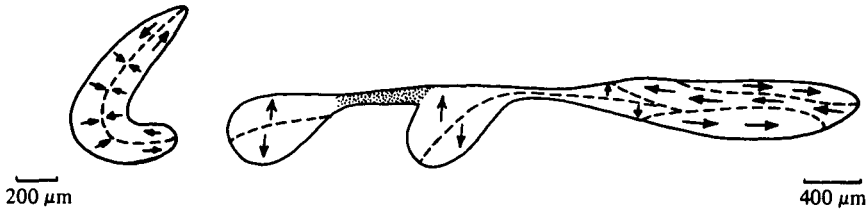200 μm   400 μm
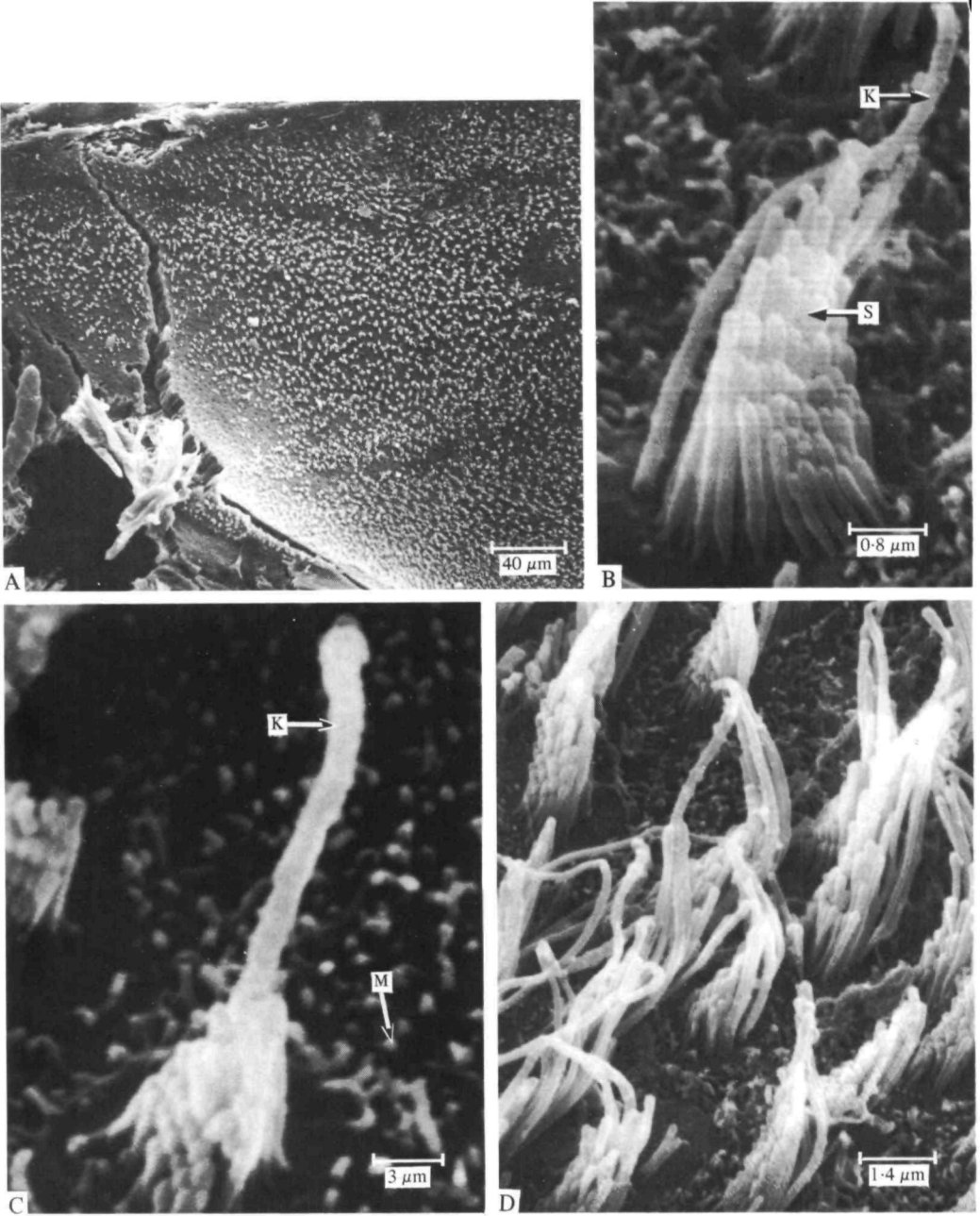
Fig. 7. Schematic drawings of the hair cell orientation patterns on the saccular (right) and lagenar (left) maculae in *Notopterus*. The tips of the arrows indicate the side of the sensory cells in each region at which the kinocilium is located. Dashed lines indicate approximate dividing lines between orientation groups. The stippled area indicates a region without sensory cells.

of the saccular chamber and appears to be outside the area of direct swimbladder impingement (Fig. 4).

As typical of all vertebrate inner ear sensory epithelia, both the sacculus and lagena in *Notopterus* contain numerous sensory hair cells (Fig. 6A–D) surrounded by supporting cells (Fig. 6B,C). The apical surface of each sensory cell has a ciliary bundle consisting of a single kinocilium located at one side of a large number of stereocilia (Fig. 6B–D). The hair cells on the saccular and lagenar epithelia are organized into groups, with all of the ciliary bundles in each group having their kinocilia on the same side of the bundle (Fig. 6). Orientation maps indicating the position of the kinocilia with respect to the stereocilia for the saccular and lagenar sensory epithelia are shown in Fig. 7. The rostral epithelial region of the saccular macula has three groups of cells, aligned rostrally and caudally along an axis about 15° above the animals' horizontal plane, and directly along the anterior projection of the saccular otolith. Two other groups of cells at the posterior tip of the rostral epithelial region are orientated a few degrees off the animals' vertical plane, one group dorsally and the other ventrally. The same vertical pattern is found on the middle and caudal epithelial regions.

SEM investigations of the inter-regional bridges show that the more rostral of the bridges contains a few sensory cells oriented dorsally and ventrally, while the second bridge contains only supporting cells.

Fig. 6. Scanning electron micrographs showing various types of sensory cells on the saccular and lagenar sensory epithelia in *Notopterus*. (A) Low-power photograph of the caudal portion of the most anterior of the three saccular macula regions. Note the V-shaped line on the region. This represents a very thin line of cells with ciliary bundles removed, presumably due to treatment in the ultrasonic cleaner (see Methods). Significantly, this bundleless line divides the vertically from horizontally oriented cell groups (see Fig. 7). (B) Type FI ciliary bundle from the sacculus. This type of ciliary bundle has a kinocilium just longer than the longest of the stereocilia. The FI bundle is the most widely found ciliary bundle type in *Notopterus*, as it is among many of the teleost species that have been studied to date (Popper, 1977, 1978, 1979, 1981; Platt & Popper, 1981). Note that the kinocilium is at one side of the ciliary bundle. The microvilli (M) adjacent to this bundle are on the apical side of the surrounding support cells. (C) Type II ciliary bundle. This bundle is often found at the very edges of the sensory epithelia surrounding the bulk of the macula. The kinocilium is substantially longer than the longest stereocilia. The stereocilia themselves are often very short. (D) Ciliary bundles typical of those found in the centre of the three saccular sensory epithelial regions. These bundles are slightly longer than the FI bundles. K-kinocilium; S-stereocilia; M-microvilli on supporting cells.

The lagenar macula contains sensory hair cells that are divided into two groups (Fig. 7). Basically, cells on the lagenar macula are orientated along the animal's vertical plane, with ciliary bundles on the rostral side orientated dorsally and the ciliary bundles on the caudal side of the macula orientated ventrally. There is some deviation from these basic orientations, as is typical of most other lagenae that have been investigated (e.g. Popper, 1977, 1981; Popper & Coombs, 1982). In certain areas of the macula, ciliary bundles in each group are orientated more towards the mid-line than in either dorsal or ventral directions. However, this intra-group variation is marked by gradual transitions between orientations with many intermediary directions between any two extremes. Inter-group differences, in contrast, are marked by sharp transitions between one orientation group and another.

The ciliary bundles, particularly with respect to the relative sizes of the kinocilia and stereocilia, are typical of those seen in other teleost species. In general, several 'rows' of cells on the periphery of both the saccular and lagenar maculae have long kinocilia and short stereocilia (Type F2 of Popper, 1977) (Fig. 6C), while the bulk of the cells on the saccular macula have kinocilia that are just a bit longer (less than 1 μm) than the longest of the graded stereocilia (Type F1 of Popper, 1977) (Fig. 6B). The ciliary bundles in the middle of the three saccular regions are slightly longer (approximately 7 μm v. 6 μm total length) than those on the other two saccular regions (Fig. 6D). The ciliary bundles on the lagenar hair cells are of two types. The bundles on the dorsal region of the macula resemble type F2 bundles, although the stereocilia are not as short as the F2 ciliary bundles found at the periphery of the maculae. The more ventral portion of the lagenar macula has F1 ciliary bundles.

Support cells between the sensory cells generally have a high density of microvilli on their apical surfaces, but the support cells on the second saccular bridge, between the middle and posterior macula regions, have a higher microvillar density than seen in other regions.

Behavioural results

Data obtained from the two test chambers were very similar. While it was impossible in this study to measure sensitivity to pressure and particle motion independently, thresholds are expressed in terms of both pressure (Table 2, Fig. 8) and displacement (Fig. 9) for comparison. The frequency range of hearing for *Notopterus* extends from at least as low as 100 Hz (the lowest undistorted frequency reliably achieved with speakers used in the experiments) to about 1000 Hz with best sensitivity occurring at 500 Hz.

Fig. 2 shows that thresholds for *Notopterus* vary by as much as 55 dB at 400 Hz and substantially more than for two other species for frequencies between 300 and 700 Hz. Furthermore, variability in thresholds could even be seen within a single test session for a few isolated cases. The testing session represented by Fig. 1 shows how the threshold appeared to stabilize at a high level for several sound trials before the level abruptly dropped to a much lower level where it again stabilized and then finally returned to the higher sensitivity level. Examples of more than one threshold plateau during any single test session were not restricted to any one fish and were found only for frequencies from 300 to 600 Hz.
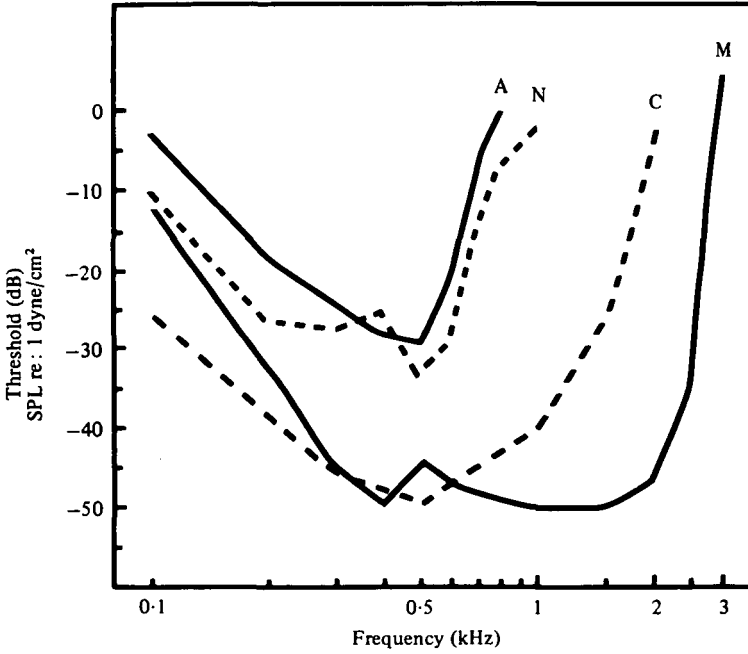
Fig. 8. Pressure-sensitivity curves based on the mean of pooled data for several individuals for each of the three species tested in the same test apparatus. A, *Adioryx*; N, *Notopterus*; M, *Myripristis*. Data for *Adioryx* and *Myripristis* are from Coombs & Popper (1979). Data from the goldfish (C) is included for comparison (from Popper, 1972).
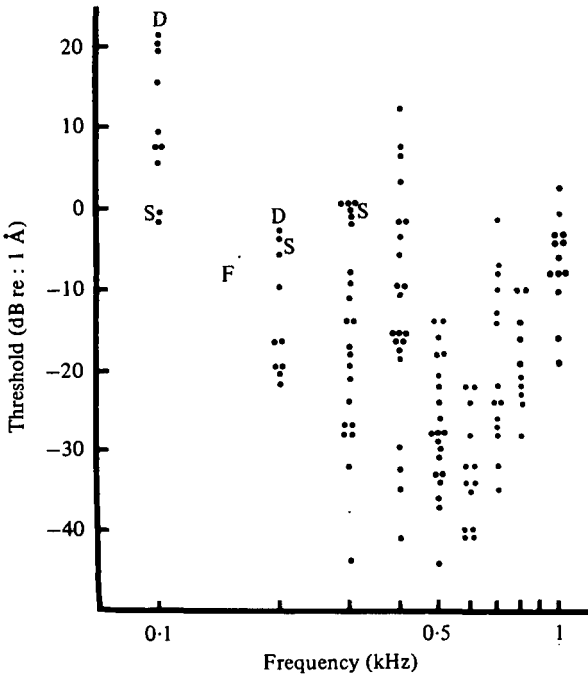

Fig. 9. Thresholds expressed as displacement thresholds for *Notopterus* (dots). Behavioural estimates of displacement sensitivity in other fish species are indicated by letters: D, dam-selfish (Myrberg & Spires, 1980), S, salmon (Hawkins & Johnstone, 1978), F, two species of flatfish (Chapman & Sand, 1974).

Table 2. *Threshold determinations, in dB re:* 1 μbar (1 dyne/cm²) *for three specimens of* Notopterus: *the mean, standard deviation and total number* (N) *of pooled data are shown below*

				Frequency				
Fish no. 100	200	300	400	500	600	700	800	1000
N-6								
0	−32	−26	−16	−23	−34	−28	−12	1
−11	−29	−31	−24	−22	−25	−9	−2	4
−13	−32	−29	−9	−35	−56	−3	−9	−6
		−39	−11	−34	−15	3		−4
		−30	−32	−51	−25	−4		
			−30	−36	−16	−9		
			−25	−41	−28	−20		
			−47	−38		−21		
				−42				
N-9								
−5	−16	−14	−16	−29	−33	−23	−5	1
−13	−18	−11	−2	−61	−27	−21	1	2
1	−15	−12	−8	−38	−34	−31	−11	−14
		−13	−7	−33		−22	1	7
		−11	−11	−31				−12
		−40	−30	−33				0
		−36	−31	−31				
		−21	−31	−31				
		−33	−24	−27				
		−39						
		−40						
		−44						
N-10								
−1	−22	−11	−30	−19	−27	−18	−10	−3
−22	−33	−23	−33	−18	−18	−6	−16	−4
−21	−34	−20	−49	−19	−33	−24	−7	−1
−15	−29	−56	−56	−23	−21			
		−26	−44	−32				
				−26				
				−43				
				−33				
Mean −10	−26	−27	−25	−33	−29	−16	−7	−2
S.D. 8·4	7·7	12·0	10·3	10·3	10·1	10·2	5·7	5·9
N 10	10	22	21	25	13	15	10	13

DISCUSSION

While a number of features of the auditory system in *Notopterus* are typical of those found in other fishes (Popper, 1977; Platt & Popper, 1981), the gross morphology and ultrastructure of the sacculus are unusual among teleosts (Popper, 1977, 1978, 1981), and quite divergent from the patterns found among other members of the Osteoglossomorpha (Popper, 1979, 1981). As far as is known, no other vertebrate saccular macula is divided into distinct regions along the otolith as it is in *Notopterus*. These regions can be distinguished from one another by (1) the narrow epithelial strands which separate them, (2) the regional differences in the portions of the otolith overlying them, and (3) their hair-cell orientation patterns. It is reasonable to suggest that this anatomical differentiation may underlie a functional differentiation within the saccular macula and one such possibility is discussed later.

While hearing sensitivity in *Notopterus* is within the range of hearing capabilities

established for a number of other fish species (e.g. Popper & Fay, 1973; Popper & Coombs, 1980*b*; Hawkins, 1981; Popper, 1982), it is different in a number of ways from sensitivity measures of other species, even those tested in the same experimental system (Fig. 8). These differences include the relatively high thresholds in *Notopterus* despite a close coupling between the swimbladder and ear, and the large variability in sensitivity for frequencies between 300 and 700 Hz as compared to *Adioryx* and *Myripristis*. Fishes with specialized connexions between the swimbladder and inner ear, such as *Myripristis* (Nelson, 1955), have generally been thought to have much better sensitivity than species without such anatomical linkage (Popper & Fay, 1973; Coombs & Popper, 1979; Platt & Popper, 1981; Popper, 1982). Thus, the closeness in sensitivity measured for *Notopterus*, a fish with an intimate swimbladder/inner-ear connexion, to the auditory sensitivity found in *Adioryx*, a fish without any such connexion (see Fig. 8), seems at first paradoxical. However, it must be emphasized that these comparisons are based on mean data. As such, the data for *Notopterus* do not reflect the large variability in threshold measures for frequencies between 300 and 700 Hz (Fig. 2B). In fact, a number of individual threshold determinations approach sensitivity measurements for *Myripristis* (see Table 1 and Fig. 8) and so the mean data for *Notopterus* may not accurately reflect the sensitivity of this species.

It is possible that the wide range in *Notopterus* thresholds may represent two populations of sensitivity measures. In some cases it appears as if thresholds may be clumped into two groups (see Table 1), with modes near the low and high ends of the threshold range. However, due to sample size, it is difficult to determine whether the threshold variation for frequencies between 300 and 700 Hz represents a bimodal, rather than uni-modal, distribution. A bimodal distribution is difficult to demonstrate statistically because treatment groups are not involved; therefore *a priori* comparisons cannot be made. Furthermore, to place thresholds into groups for *a posteriori* comparisons would involve arbitrary assignments. However, results from single test sessions in which two distinct threshold plateaus occur (see Fig. 1) lend some support to the notion that more than one set of threshold values are being represented, with perhaps one set more closely aligned with sensitivity values obtained from *Myripristis*.

If two threshold populations are represented in our data, the next question concerns underlying mechanisms. It is possible that the animal is in different locations in the test tank when the thresholds are being measured. Since intensity varies as a function of location (see Table 1 and Fig. 2A for measures of this variability), the measured thresholds will also vary. However, while the range in threshold values for other species falls within the range of sound-pressure levels calibrated for different locations in the test tanks, this is not the case for *Notopterus* (see Fig. 2). This indicates that the variability is not a function of sound-pressure variation within the test tanks. Moreover, the range in velocity levels also falls within the same range as pressure levels, making it unlikely that velocity variation within the tanks was responsible. It is also unlikely that the large range of thresholds results from 'practice' effects, or extended training beyond initial response acquisition, since there was no consistent tend for thresholds to become lower as testing progressed.

Another possible explanation for the wide range in the data involves different

sound detecting mechanisms. Unlike terrestrial vertebrates which detect sound(primarily by detecting changes in ambient pressure, fishes may also detect particle motion (also referred to as particle displacement, velocity or acceleration) associated with the sound pressure wave (Popper & Coombs, 1980a, b). Thus, a pressure-sensitive system and a motion-sensitive system would yield two different threshold measures for the same stimulus. Similar results from earlier studies have been interpreted as evidence for two separate sensory systems – a motion-sensitive system involving the lateral line, and a pressure-sensitive system involving the inner ear (Tavolga & Wodinsky, 1963; Cahn, Siler & Wodinsky, 1969). However, this particular interpretation may not be entirely correct for Notopterus. In all (of the few) cases studied, lateral line sensitivity has been restricted to extremely loud sounds for frequencies below 300 Hz (Kuiper, 1956, 1967; Kroese, Van der Zalm & Van den Bercken, 1978; Strelioff & Honrubia, 1978), whereas threshold variability for Notopterus appears at frequencies generally above this. Moreover, recent studies on the roach by Sand (1981) indicate that the trunk lateral line is virtually insensitive to sound-induced motion even at high intensities. Consequently, it appears highly unlikely that the motion sensitivity of the trunk lateral line in Notopterus was being measured in this study. However, this does not preclude the possibility that the head canal system in this animal was involved. What little information we have on these organs in Notopterus shows that there are massive neuromasts located in large head canals which may be coupled to the inner ear via a flexible lateral window in the skull (Greenwood, 1973; Omarkhan, 1949; M. Braford, personal communication). This situation may turn out to be somewhat analogous to the arrangement found in Clupeids, for which the lateral line, because of its intimate connexion with the inner ear and swimbladder bubble, has been found to be pressure-sensitive (Blaxter, Denton & Gray, 1981). Thus, differences in pressure sensitivity between the head canal organs and the ear is a viable hypothesis which merits further exploration.

An alternative hypothesis for 'dual' thresholds does not involve lateral line organs. It is now clear that the unaided fish inner ear is motion-sensitive, but that with the aid of a pressure-to-displacement transducer, such as the swimbladder, it effectively becomes pressure-sensitive (Fay & Popper, 1974, 1975; Chapman & Sand, 1974; Buwalda & Van der Steen, 1979). Thus, it is possible that the inner ear can account for motion thresholds as well as pressure thresholds, depending on whether or not the swimbladder affects all areas of the ear equally. The potential for unequal stimulation of different ear regions by the swimbladder can be seen in Notopterus, where only the two most posterior saccular epithelial regions appear to be directly associated with the swimbladder. Thus portions of the ear unaffected by the swim-bladder may be motion-sensitive, whereas portions in a position to be stimulated maximally by the swimbladder may be pressure-sensitive.

While it is impossible to determine whether some of the thresholds for Notopterus reflect motion sensitivity and others pressure sensitivity, it is conceivable that velocity levels used in this study were sufficiently high to stimulate the ear directly. Fig. 9 shows that at least a portion of the data expressed as displacement thresholds falls within the realm of behavioural estimates of displacement sensitivity for other fish species. Likewise, there are a number of thresholds which are clearly below these estimates, making it likely that these values represent pressure sensitivity.

Whether or not the anatomical differentiation observed for the saccular macula in *Notopterus* translates into a functional differentiation of pressure and motional inputs remains to be seen. It is impossible to predict the biomechanical behaviour of the system based solely on the anatomy, and our description of how the system in *Notopterus* might behave is purely speculative at this time. Clearly, neurophysiological investigations into the response properties of different portions of the macula are needed to help resolve this issue, but obtaining such data is hindered by the difficulty in surgical access to the ear in this species.

Regardless of whether the saccular macula in *Notopterus* is adapted specifically for independent pressure and motion detection, the current anatomical data lend further support to the idea that a substrate for complex signal processing may occur prior to the central nervous system (Fay & Popper, 1980; Platt & Popper, 1981; Popper, 1982). The results from *Notopterus* also provide additional evidence that the primary advantage accrued by fishes with swimbladders close to, or contiguous with, the sacculus of the ear is not necessarily enhanced hearing sensitivity and extended frequency ranges of hearing (Fay & Popper, 1980, for further discussions). While the significance of such an intimacy may very well be important in hearing sensitivity for some species, such as *Myripristis* (Fig. 8 and Coombs & Popper, 1979), there may be other advantages as well, such as those associated with independent detection of pressure and particle motion (see Buwalda & Van der Steen, 1979; Schuijf & Buwalda, 1980). Future studies must then consider the functional significance of specialized connexions between gas-filled cavities and the inner ear.

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