

AUDITORY CAPACITIES OF THE MEXICAN BLIND CAVE FISH (*ASTYANAX JORDANI*) AND ITS EYED ANCESTOR (*ASTYANAX MEXICANUS*)

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Weber (1820) suggested that the ostariophysine fish are more sensitive to auditory stimuli than are many other fish since only the ostariophysines have a series of small bones (now known as the Weberian ossicles) connecting the swim bladder to the inner ear. These ossicles provide the means for direct energy transduction from the pressure receptor, the swim bladder, to the inner ear. Non-ostariophysines have no similar means for connecting the swim bladder to the inner ear and, consequently, energy transduction in most species is probably less efficient (Dijkgraaf 1960). More recently, the difference between hearing by ostariophysines and non-ostariophysines has been shown in behavioural experiments designed to measure the auditory capacity of fish. The ostariophysines have been found to hear a wider range of acoustical signals and they are more sensitive to lower intensity signals than are most non-ostariophysines (Stetter 1929; von Bouteville 1935; von Frisch 1936; Tavolga & Wodinsky 1963; Enger 1966; Jacobs & Tavolga 1967).

While data are available on the auditory sensitivity of over thirty non-ostariophysines (Lowenstein 1957; Moulton 1963), there are data on only nine species of ostariophysines (Moulton 1963). Different workers have arrived at different ranges of acoustic sensitivity for several of these ostariophysines (Lowenstein 1957), and in several species the wide acoustic range of the fish probably exceeded the range of the experimental apparatus. Consequently, the precise auditory sensitivity of only several of these nine species is known for certain.

The ostariophysines show a considerable range in adaptation to their environments (Greenwood et al. 1966). It is possible that this would be reflected in variation in the auditory capacities of different species, especially for those species inhabiting murky water where acoustic information plays a more significant role in the animals' behaviour than other sensory stimuli. There is

also some morphological basis for expecting variation and adaptation in auditory capacities of fish. Alexander (1962) has found considerable variation in the morphology of the Weberian ossicles in different ostariophysines and this may indicate variation in auditory capacities. However, not enough data are available on the precise range of acoustic sensitivity in enough ostariophysines in order to correlate this with morphological or environmental differences between species.

This paper reports upon an investigation of the auditory capacities of two species of ostariophysines of the genus *Astyanax*. These fish are members of the family Characidae, a group of ostariophysines that has received little attention in auditory studies. The two species selected are of especial interest since they are closely related (Hubbs & Innes 1936; Breder 1953) but inhabit widely different environments that are in close proximity to one another (Breder 1953). Thus, effects of strikingly different environments should be made apparent from a comparative study such as the one described here.

Astyanax mexicanus is found in rivers in Mexico whereas *A. jordani* (= *Anoptichthys jordani*), the Mexican blind cave fish, is found in La Cueva Chica, a cave in the state of San Luis Potosi. Viable and fertile hybrids between the two forms of *Astyanax* have been found in La Cueva Chica (Breder 1942) and it has been suggested that the blind cave fish has only recently started to evolve from the ancestral river fish (Kosswig 1965).

The major morphological differences between the two fish are that the blind cave fish has little or no body pigmentation while the river fish has a large dark line along the lateral line and the blind cave fish has only remnants of eyes and a thick layer of skin covers the optic region (Breder & Gresser 1941; Kuhn & Kähling 1954; Cahn 1958). Little or no hypertrophy or degeneration has been found in other sensory systems of the blind cave fish (Breder & Rasquin 1943;

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Grobbe & Hähn 1958; Schemmel 1967), but the auditory system has not yet been investigated.

Methods

Pure tone auditory thresholds were determined for eleven river fish (six male and five female) and six blind cave fish (three male and three female) from 40 to 50 mm in standard length. This size corresponds to the young adult stage for both species (Breder & Rasquin 1943). The blind fish (*A. jordani*) were aquarium bred animals obtained from the Aquarium Stock Company in New York City and the Key Aquarium in Staten Island, New York. Several river fish (*A. mexicanus*), four or five generations removed from rivers in Texas, were supplied by Dr Kenneth R. John from stocks that he maintains. Other river fish were collected in the vicinity of San Antonio, Texas by Dr Clark Hubbs.

The fish were individually housed in 2.5-litre aquaria with continuous aeration and filtration. For training and testing, the fish were transferred by net to the test tank (shuttlebox) which had neither aeration nor filtration. Water temperature in the home tank and in the shuttlebox was maintained between 23.8° and 26.8°C and the fish were fed daily after testing, with a dried meal preparation.

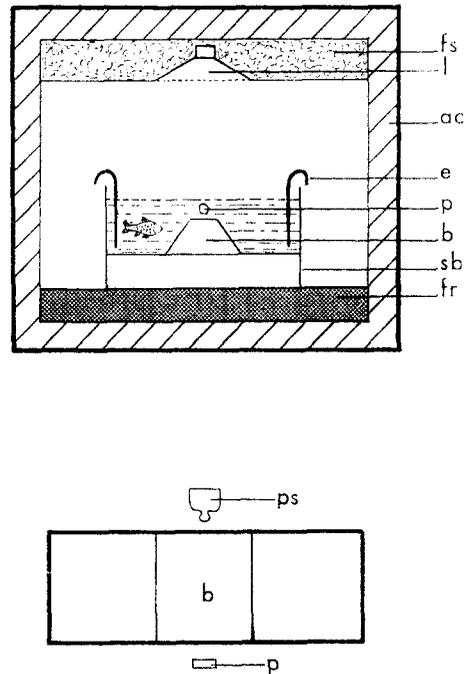
Test Tank (Shuttlebox)

The acrylic plastic experimental tank was 27.5 cm long, 7.5 cm wide and 11.0 cm deep (Fig. 1). A flat-topped barrier, 4.5 cm high and 2.5 cm long, divided the tank into two equal compartments. A photocell mounted on the top of the barrier monitored the responses by the fish.

Stainless-steel screen electrodes were placed at either end of the tank and the electrical field (unconditioned stimulus) was presented through the entire tank instead of only being on the side of the tank with the fish. The shock caused the fish to become agitated but did no damage to the fish. Shock was continued throughout the testing sessions and was presented whenever the fish did not respond to the sound stimulus by crossing the barrier.

Training Procedure

Thresholds were determined using the avoidance conditioning technique developed by Behrend & Bitterman (1962) and modified by Tavolga & Wodinsky (1963). The animals were trained to cross the barrier in the shuttlebox in response to a 500 Hz pure tone sine wave (conditioned stimulus). If the fish did not respond to the sound within 10 s, it received a



TOP VIEW OF SHUTTLE BOX

Fig. 1. Shuttlebox and acoustic chamber used in determinations of the auditory thresholds in the two species of *Astyanax*. The loudspeaker, light source photocell and electrodes are attached to the control apparatus through jacks on the side of the chamber. The chamber was darkened and rested on double deflection rails (not shown) to help eliminate low frequency sounds. ac, acoustic chamber (10-cm walls); b, barrier; e, electrodes; fs, fibreglass; fr, expanded polystyrene foam; l, loudspeaker; ps, photocell light source; p, photocell; sb, shuttlebox.

single 8-ms 60 Hz pulsed shock (10 V a.c. measured at the source) each second until it crossed the barrier (escape response). By crossing the barrier within the 10 s when only sound was present, the fish did not receive a shock (avoidance response). When the fish crossed the barrier, it broke the beam of light to the photocell and both conditioned and unconditioned stimuli were stopped. Training continued until the animal crossed the barrier in response to the sound in 90 per cent of twenty-five training trials given each day and maintained this level for three consecutive days.

No stimuli were presented during a 1 min intertrial interval (ITI) between each sound presentation and barrier crossings during the ITI were monitored continuously. If the fish crossed the barrier more than three times during one ITI, the water level was lowered in 1-mm

steps. This cut the ambient crossing rate to levels that did not permit false responses during the CS period and also did not hinder responses to the CS.

Threshold Determination

The test shuttlebox was placed in an acoustic chamber (Industrial Acoustics Company model AC-3, Fig. 1) which cut ambient noise levels by 60 dB when measured with a band pass filter set for wide band noise. The chamber was on double-deflection rails and was lined with a 5-cm thick pad of foam rubber. The test signal was presented through a 20.3-cm air loudspeaker suspended 20 cm above the surface of the water. The speaker was surrounded by fibreglass and expanded polystyrene foam to dampen back-waves and produce an infinite baffle. An incandescent lamp was left on in the chamber when the river fish were tested since they stopped responding when placed in complete darkness.

Thresholds were determined using the up-down staircase method (Dixon & Massey 1951; von Békésy 1960). The threshold level was the point at which the fish responded in 50 per cent of the trials at that sound level. If the fish responded to the sound and crossed the barrier during the 10-s period when sound alone was present, the sound level for the next trial was lowered by 5 dB. If the animal did not respond to the sound, it received a shock and in the next trial the sound pressure level was increased by 5 dB. The mean level of twenty of these changes constituted the basis for the threshold for an animal each day. Each fish was tested at least three times at each frequency and the data for all animals of each species were pooled.

After determining the threshold at the training frequency, the animal was transferred to other tones in 100 to 500 Hz steps. The fish were given one day's training at each new frequency without sound attenuation.

Equipment

The experiments were controlled using either of two solid-state apparatuses that have been described in detail by Tavolga (1966). Training of both species, and testing of the river fish, was done using the Audio-Ichthyotron Mark IV and testing of the blind fish was with the Audio-Ichthyotron Mark V. Both apparatuses monitored intertrial response rate and latency of responses, presented and pulsed the shock, and turned the sound stimulus on through a photoconductive switch. The experimenter initiated each trial on the Mark IV, a semi-automatic

device, and attenuator settings were adjusted on an attenuator set (Hewlett-Packard 350D). The Mark V was fully automatic and controlled the intertrial interval, attenuator settings, and printed the number of intertrial responses, latency of response, and attenuator setting on a seven channel printer. An attenuator built into the Mark V was automatically changed in response to the behaviour of the fish.

The unconditioned stimulus (shock) was produced by an internal source in the Mark IV and by a variable transformer (Fisher Powerstat model 136) attached to the Mark V. The voltage was measured at the source, and no attempts were made to determine the shock intensity received by the fish.

Sound Calibration

Sound was calibrated with a hydrophone (Chesapeake Instrument Company model SB 154 C) placed in the wells of the test tank and this signal was amplified through a transistorized pre-amplifier with a flat gain of 51 dB. Calibration was checked several times during the course of the study. Signal intensity varied by 1 or 2 dB in different parts of the shuttlebox but the random movement of the fish compensated for this variation (Tavolga & Wodinsky 1963).

Results

Threshold Determinations —River Fish

Auditory thresholds for the river fish were determined at seventeen frequencies from 50 to 7500 Hz. Frequencies below 50 Hz were not used in either study since the speakers did not reproduce low frequency signals without considerable distortion and loss of signal stability.

Thresholds for the river fish are shown in Table I and are represented graphically in Fig. 2. At 50 Hz, the mean threshold for four fish was -14.9 dB (re 1 μ b) with a range of -8.8 to -28.0 dB μ b. The threshold at 7500 Hz was $+2.8$ dB μ b and the lowest threshold for the river fish was at 1000 Hz where the threshold was -40.5 dB μ b.

Statistical comparisons of the river fish thresholds are shown in Table II. The thresholds from 100 Hz (-32.7 dB μ b) to 500 Hz (-30.6 dB μ b) varied significantly ($P=0.005$), while the thresholds from 800 to 1500 Hz varied insignificantly ($P=0.1$). The thresholds from 2000 to 3000 Hz were alike ($P=0.1$), but there was significant difference between the thresholds from 4000 to 5200 Hz ($P=0.05$).

The intra-specific threshold determinations at 1000 Hz for the river fish were compared. An

Table I. Auditory Thresholds in the River Fish, *Astyanax mexicanus*

Frequency (Hz)	Mean threshold (dB re 1 μ b)	Standard deviation	No. of determinations	No. of animals
50	-14.9 (-8.8 to -28.0)*	6.41	14	4
100	-32.7 (-27.3 to -37.8)	8.88	12	4
200	-39.1 (-35.5 to -46.5)	6.10	9	3
300	-31.2 (-21.0 to -40.0)	6.61	13	4
500	-30.6 (-23.2 to -43.8)	5.85	16	5
800	-37.0 (-30.5 to -44.5)	3.75	12	4
1000	-40.5 (-32.1 to -46.6)	5.13	12	4
1500	-37.1 (-27.7 to -53.3)	7.49	13	4
2000	-19.8 (-7.9 to -34.0)	8.52	12	4
2500	-20.3 (-14.9 to -26.1)	5.05	15	5
3000	-23.8 (-15.8 to -39.0)	6.10	12	4
3500	-32.4 (-18.5 to -42.5)	8.58	18	7
4000	-22.9 (-17.0 to -28.0)	3.29	8	3
4500	-29.4 (-15.8 to -42.8)	6.98	11	5
5200	-23.9 (-19.1 to -30.8)	3.75	10	4
6400	-10.3 (-8.3 to -14.5)	2.32	9	3
7500	-2.8 (-6.8 to +9.7)	3.67	9	3

*Range.

analysis of variance of the thresholds of the four fish tested at 1000 Hz showed that the mean thresholds were all similar to one another ($P=0.1$) and similar results were obtained at all other frequencies tested.

Auditory Thresholds—Blind Cave Fish

Auditory thresholds for the blind cave fish

were determined at sixteen frequencies from 50 to 6400 Hz. Table III shows the thresholds, standard deviations, range, and number of determinations at each frequency. At 50 Hz, the mean threshold was -19.7 dB (re 1 μ b) for ten determinations on three animals. The threshold at 6400 Hz was $+1.5$ dB μ b for one

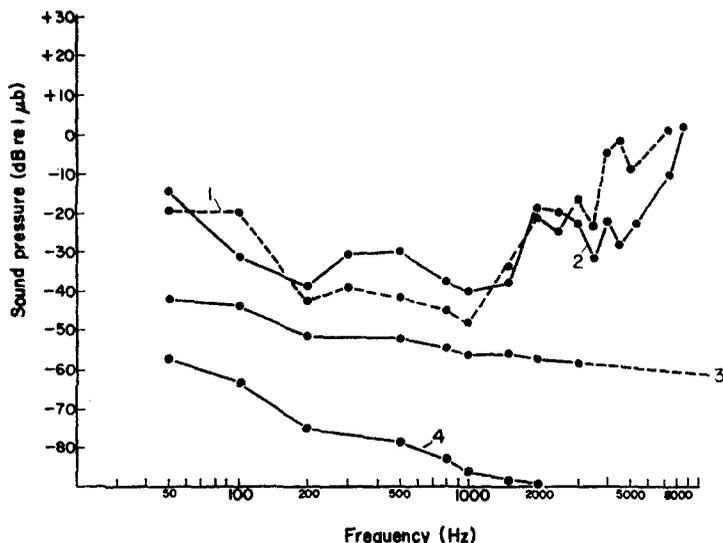


Fig. 2. A comparison between the auditory thresholds for the two species of *Astyanax* and the ambient noise levels in the acoustic chamber. Bandwidth noise levels were determined using a band-pass filter up to 2500 Hz. Above 2500 Hz the ambient noise levels could not be determined due to electrical noise in the system. Spectrum levels of ambient noise (noise per cycle) were calculated from the bandwidth noise level. 1, mean threshold blind fish; 2, mean threshold river fish; 3, bandwidth noise level; 4, spectrum level of ambient noise.

determination. The greatest auditory sensitivity in the cave fish was at 1000 Hz at which the threshold was -48.2 dB μ b with a range of -35.1 to -56.1 dB μ b. The thresholds at 1000 Hz and at 800 Hz did not differ significantly ($P=0.1$).

The auditory thresholds of the blind cave fish are represented graphically in Fig. 2. Statistical analyses of the thresholds were made to determine if the thresholds at frequencies close to one another were statistically similar. Table II summarizes the comparisons of the t -tests and analyses of variance. The thresholds at 50 Hz (-19.7 dB μ b) and 100 Hz (-20.1 dB μ b) were similar ($P=0.5$) as were the thresholds from 200 to 500 Hz ($P=0.3$) and 4000 to 5200 Hz ($P=0.1$). There was significant variation in the thresholds from 2000 to 3500 Hz ($P=0.01$).

The auditory thresholds did not vary significantly between individual blind fish. Mean thresholds were calculated for individual fish at 500 Hz and the thresholds were found to be insignificantly different from one another ($P=0.1$). Similar analysis of the data at 1000 Hz also showed the similarity between the mean thresholds of different animals ($P=0.1$).

Audiogram Comparisons

A comparison of the audiogram of the blind

fish and river fish was made using the Mann-Whitney U test. The differences between the two complete audiograms (Fig. 2) were not significant ($P=0.1$). Comparisons between individual points on the two audiograms were made using the Student's t -test, and significant variation was found at individual frequencies from 100 to 1000 Hz and from 3000 to 6400 Hz. The Mann-Whitney U test was used to compare the portions of the two audiograms from 50 to 1500 Hz and from 2000 to 6400 Hz; the differences indicated by the t -test were shown to be not significant ($P=0.097$ and $P=0.052$, respectively) when the segments of the audiogram were considered together.

Astyanax mexicanus was tested up to 7500 Hz and *A. jordani* up to 6400 Hz but it is likely that both species can probably detect pure tones up to 8000 or 9000 Hz. Thresholds could not be determined at these frequencies since test signals before attenuation must be at least 30 dB above the presumed threshold level. At high frequencies this would have necessitated use of signals of over $+30$ dB μ b, but the loudspeaker tended to distort high frequency signals at higher intensities.

Behavioural Controls

In order to determine if thresholds changed

Table II. Results of Statistical Tests Between Auditory Thresholds at Different Frequencies

(A) River fish		(B) Blind fish	
Frequency (Hz)	P=*	Frequency (Hz)	P=*
50 } 100 }	0.05	50 } 100 }	0.005
200 } 300 } 500 } 800 } 1000 }	0.1 0.1 0.05 0.1	100 } 200 } 300 } 500 } 800 } 1000 }	0.005 0.005 0.005 0.1
2000 } 2500 } 3000 } 3500 }	0.05	1500 }	
4000 } 4500 }	0.01	2000 } 2500 } 3000 } 4000 } 4500 } 5200 }	0.01 0.005 0.005 0.005 0.05

*Student's *t*-test used for testing significance between two frequencies and a one-way analysis of variance used for testing significance between three or more thresholds.

over time, they were determined for one blind fish at 500 Hz 4 months after the initial testing. During the intervening 4 months, the fish was tested at other frequencies. Thresholds before and after the 4-month period were virtually identical.

Since a 1-min intertrial interval was used throughout the experiments, steps were taken to ensure that the fish were responding to the sound and not to the time interval. At times, fish were tested with intertrial intervals that were randomly varied between 1, 2, 3, 4 and 5 min in length. These thresholds were not different from thresholds determined with the 1-min ITI. Fish were also tested with a silent CS period in order to determine whether the fish were responding to unintentional stimuli that might have been present along with the CS. No appreciable rate of barrier crossings was found during the 10-s silent conditioned stimulus periods.

No intra-specific threshold differences were found that could be attributed to size or sex of the specimens or the 3°C temperature range permitted during testing and training.

Ambient Noise Levels

Bandwidth noise levels and sound spectrum level, determined using methods previously described by Jacobs & Tavalga (1967), are shown in Fig. 2. Bandwidth noise levels above 3000 Hz had to be extrapolated from the values determined below 3000 Hz since electrical noise masked the ambient noise levels at those frequencies. However, the noise levels above 3000 Hz would be expected to continue to decrease since the major portion of the noise in the laboratory consisted of low frequency sounds produced by movements in the laboratory, street noise, and sounds from the subway seven floors below.

The bandwidth noise level and the sound

Table III. Auditory Thresholds in the Mexican Blind Cave Fish, *Astyanax jordani*

Frequency (Hz)	Mean threshold (dB re 1 μ b)	Standard deviation	No. of determinations	No. of animals
50	-19.7 (-15.9 to -26.3)*	3.31	10	3
100	-20.1 (-12.8 to -28.7)	4.45	12	3
200	-42.3 (-27.1 to -58.2)	9.33	16	3
300	-39.2 (-28.7 to -46.6)	4.93	12	3
500	-42.1 (-35.9 to -51.2)	3.54	27	5
800	-45.5 (-35.8 to -60.2)	5.76	21	4
1000	-48.2 (-35.1 to -56.1)	6.55	19	5
1500	-34.8 (-30.2 to -43.7)	3.82	16	4
2000	-21.8 (-14.3 to -30.5)	6.18	14	4
2500	-25.9 (-19.5 to -36.1)	5.10	9	3
3000	-17.3 (-10.5 to -35.8)	7.48	9	3
3500	-24.0 (-15.5 to -30.9)	4.90	8	3
4000	-5.76 (-2.2 to -8.8)	2.75	6	3
4500	-2.2 (-9.2 to +6.5)	—	4	2
5200	-9.0 (-8.1 to -9.1)	—	2	1
6400	+1.5	—	1	1

*Range.

spectrum level (noise per cycle) were respectively at least 15 and 30 dB below the thresholds for both species (Fig. 2). The bandwidth noise level did not vary significantly during the course of these studies.

Behaviour of the Fish During Training and Testing

The two species of *Astyanax* did not respond in the same way to training and testing procedures. The blind fish had difficulty learning to cross the barrier in response to the shock since they could not visually orient to it. The

blind fish learned the escape problem in about 7 days during which time they were prodded in the correct direction in about 25 per cent of the trials with a plastic paddle. After 1 week of learning to orient to the barrier and to escape, the blind fish generally needed an additional 3 weeks to learn the avoidance problem to the criterion level. Avoidance-trained blind fish remained fairly still in the water during the inter-trial interval and faced the barrier. The time between onset of the sound and crossing of the

barrier was very similar to the latency for the trained river fish.

River fish generally learned to escape on the first day of training but they would not reach a satisfactory level of avoidances when trained in the open training boxes used for the blind fish. In order to eliminate ambient visual cues, which seemed to 'distract' the fish, they were placed into the visually isolated acoustic chamber and they reached criterion level of 90 per cent in 2 weeks after the start of training.

More river fish than blind cave fish were used for threshold determinations since the river fish had difficulty in the transfer of avoidance response from the training tone, 500 Hz, to other frequencies. Consequently, no single river fish was used at all of the frequencies for which data are reported. The blind fish, however, had no difficulty making response transfers and several fish were used at most test frequencies.

When river fish were initially transferred from 500 to 300 Hz (in 100-Hz steps), they did not respond to the 300 Hz signal. The fish resumed avoiding when they were returned to 500 Hz. They could then be transferred to higher frequencies, but above 2500 Hz the fish stopped responding at criterion level, even after being transferred to these frequencies in small steps and after a week or more of retraining at these frequencies. In order to obtain data above 2500 Hz, several new river fish were given initial training at 3000 Hz and these were successfully tested up to 7500 Hz. Training time at 3000 Hz was the same as at 500 Hz and thresholds for both groups of river fish, at overlapping frequencies, were identical.

Thresholds at 300 Hz and lower frequencies for *A. mexicanus* were obtained by shifting fish to these frequencies after threshold determinations at higher frequencies had been made for a month or more. The fish responded reliably down to 50 Hz, where the experiment was terminated.

Discussion

Auditory thresholds for *A. jordani* and *A. mexicanus* do not differ significantly, indicating that there has been no discernible alteration in the pressure detecting system of the blind cave fish as a result of entry into a new environment. The significance of the extensive auditory capacity of the cave animal for survival in its natural environment is not clear. Breder (1942) gave a detailed description of La Cueva Chica but he did not mention any sounds in the cave and there is no evidence to indicate that these

species are sound producers. The only other animals in the cave were small invertebrates that probably produce no sounds or very low frequency sounds. Bats inhabiting the cave are known to be insectivorous (Breder 1942) and their sounds would probably be at too high a frequency to be detectable by the blind fish. In addition, none of the organisms are known to serve as food or be predators of the blind cave fish, and it is therefore unlikely that the blind fish auditory mechanism would become adapted to detect sounds produced by these invertebrates.

The auditory thresholds of the blind cave fish, when compared to the eyed river fish, indicates that the cave species has neither an hypertrophied nor degenerated pressure detecting system. There are several possible reasons for the maintenance of the auditory capacity in the blind cave fish, beyond the explanation that hearing a broad range of sounds is advantageous for the species. It has been suggested that *A. mexicanus*, the ancestral form of the blind cave fish, entered the cave only recently (Kosswig 1965) and thus selective pressures, if present, have not had time to work on the complex ear mechanism of *A. jordani*. It is also possible that the interrelationships between the ear and other structures that start to develop early in the developmental process of the fish are so complex that a gene altering the ear of the fish would disastrously alter other organ systems of the species.

The multitude of roles played by the ear in the behaviour of the cave fish may also preclude changes in the auditory capacity. Poulson (1963) found that the inner ears of cave Amblyopsidae, the only other cave species whose ears have been studied, are larger than those of epigeal relatives and he suggested that an enlarged ear helps the fish maintain equilibrium in the absence of eyes. Hypertrophy of the ears of *A. jordani* may not occur if the ears of *A. mexicanus* already had sufficient sensitivity to provide the blind cave fish with the equilibrium information necessary to compensate for eye loss. Likewise, the ear of the blind cave fish may not have degenerated if it is supplying necessary equilibrium information to the fish.

Indeed, both Mexican blind cave fish and blinded river fish can orient themselves in a fish tank and avoid bumping into stationary objects (John 1957). Although this may partially depend upon the use of the lateral lines to detect objects (Walters & Liu 1967), maintenance of equilibrium would be an important factor for the

learned portion of this behaviour.

The pure tone auditory capacities of the two species of *Astyanax* is wider than for any other species tested in a quantitative manner. It is difficult to make comparisons of specific auditory thresholds with other species since techniques differ considerably and this may seriously affect the resultant thresholds (Dalton 1968). It is possible, however, to compare the thresholds obtained for the two species of *Astyanax* with those obtained for the goldfish, *Carassius auratus*, by Jacobs & Tavolga (1967) since basically the same testing situation was used in both experiments. The goldfish, a cyprinid, could detect pure tones to 3000 Hz. Comparisons using the Mann-Whitney U test between the absolute thresholds of *A. jordani* and the goldfish show that the auditory thresholds for the two are almost identical at all frequencies up to 1500 Hz ($P=0.5$). Above 1500 Hz, the thresholds for the goldfish increase rapidly while those for *Astyanax* stay low. It is unlikely that the similarity in thresholds is due to the technique, since thresholds on a variety of other non-ostariophysines have been tested and none show the similarity shown by *Carassius* and *Astyanax* (Tavolga, unpublished manuscript; Tavolga & Wodinsky 1963). Although the behavioural differences between the goldfish and *Astyanax* may be attributed to environmental differences, it is more difficult to correlate the behavioural differences with the morphological differences that provide the basis for different auditory capacities. Alexander (1962) reported that the inner ears of the goldfish and *A. abramoides* were similar to one another. However, there are differences in the structure of the Weberian ossicles between the goldfish and the two species of *Astyanax* reported here (Popper, unpublished manuscript). It is possible that the similar thresholds between *Carassius* and *Astyanax* up to 1500 Hz may result from similarities in the structure of the inner ear while dissimilar thresholds above 1500 Hz may result from differences in signal transduction through the structurally different Weberian ossicles.

The Weberian ossicles play a significant role in the auditory differences between ostariophysines and non-ostariophysines, and it may be the specific morphology of the apparatus that determines the actual extent of acoustic transduction performed by the ossicles. It is not at all clear what characteristics of the ossicles play the most significant role in acoustic trans-

mission but investigations of more ostariophysines, both behaviourally and morphologically, should provide some answers to this question.

The thresholds represented for the two species of *Astyanax* are, in all likelihood, absolute thresholds. Background noise levels (Fig. 2) were considerably lower than the thresholds, and Buerkle (1968) and Tavolga (1967) have shown that noise over 20 dB below thresholds will not have an effect on changing auditory thresholds of fish.

The stimuli used here were limited to pure tone sine waves and the stimulus impinging upon the fish were dominantly pressure stimuli, although some displacement stimuli, which affects the lateral line (Harris & van Bergeijk 1962), may have been present. Parvulescu (1964, 1967) has determined that a small body of water in a closed area stimulated by a speaker in air will have a pure pressure field. In addition, pressure thresholds obtained in a similar apparatus for *Haemulon sciurus* (Tavolga & Wodinsky 1963) were very similar to pressure thresholds determined by Cahn, Wodinsky & Siler (1967) using a different apparatus which allowed for isolation and measurement of pressure and displacement stimuli. It would be of considerable interest to determine whether displacement thresholds for the two species of *Astyanax* are similar since there are some data that indicate that *A. antrobius*, another species of Mexican blind cave fish, has a more extensively developed system of free neuromasts than does *A. mexicanus* (Dr Kenneth R. John, personal communication).

The two species of *Astyanax* behaved differently to the change of test frequencies, and the behavioural differences may have been related to how the species perceived the acoustical stimuli.

The blind fish had no difficulty at 300 Hz, or between 2500 and 3000 Hz, as did the river fish, possibly indicating that the blind fish and the river fish had learned to respond to different aspects of the stimuli. The blind fish may have learned to respond to 'sound' stimuli in general, while the river fish, a visually oriented species (Breder 1953; John 1964), may have learned to respond to a 500 Hz signal or the specific qualitative composition of the 500 Hz tone. The breakdown of the river fish behaviour at 300 Hz probably occurred because the fish learned to respond to the pressure stimulus present at 500 Hz, and they became confused when presented with a combined pressure and displacement stimulus present at 300 Hz and

below. After considerably more training to pressure stimuli above 500 Hz, the river fish probably effectively 'ignored' all non-pressure aspects of the low frequency signal and gave only pressure thresholds. This suggests an improved method for training fish for threshold determinations. Rather than train fish initially to a single frequency, the training stimulus should alternate between different signals so that the fish learns to respond to 'sound' and not to a specific signal or specific signal content. Although this method may not make training faster, it would make stimulus transfer easier, and retraining during testing would no longer be necessary.

Summary

The auditory capacities of two species of characid fish, *Astyanax mexicanus* and *A. jordani*, were determined using avoidance conditioning techniques. Pressure thresholds for both species were as low as those reported for other ostariophysines and the minimum threshold for both species was at 1000 Hz. Both species of fish could hear a wider range of sounds than that reported for any other species of fish tested in a quantitative manner. There is no evidence that the Mexican blind cave fish can detect pressure stimuli any better or more poorly than its ancestral eyed relative. There is no obvious selective advantage for maintenance of a wide auditory range upon entry into the cave since no source of sounds above about 1000 Hz are present there. There may be selective pressures for maintenance of the ear for less obvious reasons, such as the need for the equilibrium information that the ear provides.

Although not enough ostariophysines have yet been investigated, these data substantiate earlier findings that they can hear a wider range of sounds and lower intensities than non-ostariophysines.

The behaviour of the fish in the avoidance apparatus differs strikingly during initial training and during testing, and this is probably due to the different orientation of these two species.

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