THE ORIENTATION OF FISHES TO LOW FREQUENCY SOUND SOURCES AND THE ROLE OF THE LATERAL LINE SYSTEM.

by

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of

ZOOOLOGY

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

AUGUST, 1966
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Department of **Zoology**

The University of British Columbia
Vancouver 8, Canada

Date **22nd August 1966**
A theoretical analysis of the acoustic field around a sound source suggested that fish would be able to locate a sound source by detecting the associated near field displacements with their lateral line system.

Blinded goldfish and Mexican blind cave characins were able to locate both stationary objects and sound sources. The lateral line system was implicated as the directionally sensitive organs involved.

Blind cave fish were able to locate both stationary objects and a sound source against a background noise. The existence of a noise suppressing mechanism to the lateral line organs was suggested.

An efferent nervous supply was shown to innervate anterior lateral line organs of goldfish, and the inhibitory nature of the efferent nerves was demonstrated. The efferent nerves were found to be insensitive to the stimulation of acoustically sensitive organs on the fish, but responded to changing states of muscular activity in the fish.

Swimming goldfish changed hydrodynamically during respiration from bluff bodies, when their mouths were shut, to streamlined bodies, when their mouths were open. This change in configuration lead to the proposal that the anterior lateral line organs function both as velocity detectors and near field displacement detectors.
A central location was suggested for a neurophysiological noise attenuating system to the lateral line system, and the efferent nerves innervating the lateral line organs were suggested to form part of a mechanism reflexively controlling swimming velocity.
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GENERAL INTRODUCTION

Fish are supplied with two receptor systems which are thought to respond to acoustic phenomena. The first of these systems are the ears and these have been shown to be pressure receptors (Bekesy 1960). The second of these systems is the lateral line system of receptors which are displacement sensitive (Harris G.G. and Bergeijk 1962).

If a fish can localise a sound source under water, then either of these systems might be operative.

The present research programme is orientated towards determining if fish can localise a sound source under water, whether theoretically the lateral line organs can be implicated, and the role that efferent nerve fibers, that possibly innervate the lateral line organs, play in coding information received at the level of the receptor.

With these general questions in mind the following specific questions have been asked experimentally.

a) Which, if any, of the two systems could be used by a fish to localise a sound source under water?

b) Are fish able to localise a sound source under water in both quiet and noisy conditions?

c) Is there an efferent nervous supply to the lateral line and what role, if any, do these nerves play at the receptor?
d) Are the respiratory movements possibly used by a fish as an aid to localise a sound source?

The nature of a sound source and its associated acoustic phenomena should be considered first. The simplest sound source is a pulsating sphere undergoing changes in volume. As the sphere changes volume it concentrically and uniformly displaces water around itself. This displacement decreases in amplitude as the distance from the centre of the sphere increases because the water has to be displaced over a greater surface area (Figure 1a).

The displacement of the water (equation i) at a distance \( r \) from the centre of the sphere of radius \( A \) is proportional to the amplitude of the pulse \( D \) and the surface area of the concentric sphere of radius \( r \).

If water was incompressible this non-propagating displacement known as the near field displacement would be the only acoustic phenomenon produced by a pulsating sphere. Water is not incompressible and an elastic component, the pressure wave, is generated. This is a propagated wave and satisfies the conditions of the normal wave equation which describes how the amplitude of the wave is decreased at a rate inversely proportional to the distance from the centre of the sphere.

It follows that at a critical distance \( R \) away from the centre of a pulsating sphere, the displacement
Figure 1

A The relationship between the amplitude of pulsation $D$ of a sphere radius $A$, and the displacement $d$ of a point situated in the near field and a distance $r$ away from the centre of the sphere.

B The relationship between the displacement $\Delta$ of sphere radius $A$ undergoing dipolar oscillations, and the displacement of a point situated a distance $r$ from the centre of the sphere, and at an angle $\theta$ to the path of its oscillation.

$dr$ is the radial component of displacement.

$d\theta$ is the angular component of displacement.

C The illustration of a left, right confusion between receptors $AB$ and $AB_1$ where the same time and amplitude differences exist in receiving pressure waves from a point sound source $P$.

A time and amplitude difference can exist if receptors $ABB_1$ are moved to position $RSS_1$ where the time difference between $AB$ is greater than between $RS$, but the amplitude difference between $AB$ is less than between $RS$.

D This illustrates a servo system capable of locating point $P$. $A$ and $B$ receive the same time and pressure differences. $AB$ is then swung through angle $\theta$ to $AB_1$ and the value of the error signal thus generated is proportional to $\cos \theta$ and the sign and magnitude of the signal locates $P$. 
\[
d = \frac{A^2 D}{r^3} \quad \text{(i)}
\]
\[
dr = \frac{\Delta A^2}{r^3} \cos \theta \quad \text{(ii)}
\]
\[
d\theta = \frac{\Delta A^2}{r^3} \sin \theta \quad \text{(iii)}
\]
amplitude will be equal to the pressure amplitude.

Distances less than $R$ from the centre of the sphere fall into a zone known as the near field. This consists of both near field displacement waves and propagated pressure waves. At distances greater than $R$, a zone known as the far field exists. This essentially consists for the most part of pressure waves, because the displacement effect rapidly attenuates.

The near field displacement is a vector quantity having both magnitude and direction, as opposed to the far field pressure wave which has only magnitude and no direction. In the special case of the acoustic field associated with a pulsating sphere, a single displacement detector situated in the near field would be able to localise the source, whereas three pressure receptors would be required in the far field.

A sphere oscillating along one axis is the next simple source of sound to be considered. This motion is similar to the propulsive movements of fishes and invertebrates and is, therefore, one of the most common sources of vibrations found in the aquatic environment.

Figure 1b shows a sphere undergoing linear oscillations, pushing water ahead of it, and drawing water from behind. Figure 1b and equations ii and iii show that the displacement of water caused by this motion can be analysed
into two components, a radial component and an angular displacement. Parallel to the axis of motion only radial displacement exists, and at 90° to this axis only angular displacement exists. For this model a single displacement detector placed in the near field could give no information about the position of the sound source because the motion has two components, and the angle θ would have to be known, and knowing this would already presume knowledge of the position of the sphere.

The acoustic properties of the last described sound source require that at least two receptors, whether pressure receptors or displacement receptors, are needed to localise the sound source, providing the sound source and receptors are all in the same plane. If two displacement or pressure receptors A and B are placed in the field of sound source P as shown (Figure 1c) then B will receive the pressure wave earlier and with a greater amplitude than A. However, this configuration can lead to a left-right confusion if B is moved to B' where the same amplitude and time differences exist between A and B' as did between A and B. A time and amplitude confusion can also exist with this configuration if ABB' is moved closer to the sound source P to a new position RSS'. In this new position the time difference between A and B is greater than between R and S, but the amplitude difference is far less between A and B than between R and S.
Bergeijk (1963) who first described the two types of confusion suggested employing one of two methods to overcome this confusion. One solution was to include an extra pressure receptor giving a minimum of three receptors, and the other was to include the two receptors in a servo system. The servo system would orientate A and B in such a manner that they would both receive the same amplitude pressure wave at the same time (Figure 1d).

However, this would introduce another confusion, a front-back confusion, which could be overcome by deliberately swinging B through a known angle $\theta$ to a new position $B'$. This deflection would generate an error signal in the servo system, the magnitude and sign of which would then determine the position of P. In this case the magnitude of the error signal would be proportional to $\cos \theta$ which changes very slowly for small angles. A more sensitive configuration is represented by RSS' in figure 1d. In this case the error signal is proportional to $\sin \theta$ which changes rapidly for small angles.

It is concluded, from a theoretical point of view, that a maximum of three receptors, or two receptors and a servo system, is required to detect a sound source under water except for the special case where the sound source is a pulsating sphere. Under this condition only one displacement receptor need be required.
Although the ears of vertebrates are generally considered to be pressure receptors, the inner ear has been shown to be displacement sensitive (Bekesy 1960), and the middle ear serves the purpose of transducing pressure waves into displacement waves.

The middle ear of fishes is considered to be the swim bladder, a gas bubble which, by obeying Boyle's Law, changes volume in response to surrounding pressure fluctuations. The volume changes are translated into displacements of the walls of the swim bladder which are conducted directly to the inner ear, as for example in the Clupeids, or indirectly by the Weberian Ossicles, as in the Osterophysi. Thus, fish possess two inner ears but only one pressure sensitive middle ear. Therefore, on theoretical grounds, the ears of fishes are precluded from being considered directionally sensitive to sound sources by virtue that they possess in effect only one pressure sensitive transducer between them.

Lateral line organs more than satisfy the two minimum conditions for a directionally sensitive, acoustically sensitive system. Firstly, they are displacement receptors, so that in the special condition of a sound source consisting of a pulsating sphere, a fish with a single lateral line organ could localise it if it was in the near field of the sphere. Secondly, fish possess many of these organs distributed over their bodies thereby satisfying the condition that fish require three receptors occupying different positions
in space to localise a sound source.

Touch receptors also fulfil the criteria required of a system capable of directionally localising a sound source. However, it is believed that their sensitivity to water displacement is less than that of the lateral line organs (Adrian 1929) and therefore the field of sensitivity of the touch receptors would be overlapped by the field of the lateral line organs.

In the experiments which follow, an investigation was made to see if fish have the ability to localise a sound source, and which organs, if any, can be implicated as those responding directionally.
BEHAVIOUR : PART I

Are fish able to locate stationary objects and low frequency sound sources by responding to stimuli other than visual stimulation.

INTRODUCTION

There is an apparent paucity in the field of marine bio-acoustics of information regarding the ability of fish to locate low frequency sound sources under water. This paucity may, partly be a result of a general acceptance, by workers in the field of marine bio-acoustics, of the conclusions drawn by the Germans Von Frisch and Dijkgraaf (1935) and Rheinhardt (1935), from their experiments that fish do not orientate to under water sound sources. Recently Bergeijk (1964) criticized the evidence presented by these workers on the grounds that no analysis of the stimulus field to which the fish were subjected, or the sensory apparatus with which the fish were responding, was given. Bergeijk deduced from the evidence presented in Von Frisch and Dijkgraaf's experiments that the fish were located for the most part in the far field of the sound sources used in their experiments, and this may be a reason why no responses were generally elicited from the fish. However, Von Frisch and Dijkgraaf did make the observation that fish used in the experiments orientated themselves with respect to the sound source when a high intensity sound was used, and the fish were in close proximity (10 - 20 cm) of the sound source. This evidence indicates that the fish
may have been orientated to the near field of the sound source.

Kleerekoper and Chagnon (1954), the only other investigators, prior to 1964, to study the directional behaviour of fish in the vicinity of a sound source, concluded that fish are able to orientate to sound sources. Unfortunately they gave no information on the nature of the acoustic field to which the fish were subjected and thus their evidence is difficult to interpret in the light of recent knowledge.

A major difficulty in this field of behaviour study is formulating the criteria by which a fish can be said to be locating or failing to locate a sound source. All of the workers engaged in this field of study prior to 1964 used the principal of the conditioned reflex in attempting to ascertain whether or not the fish were orientating towards the sound source. Observations were made on the head movements of the experimental fish to discover if they moved away from or towards the sound source depending upon the pre-conditioning stimulus given to the fish. This criterium was considered unsatisfactory because judgements were made on ill defined reactions of the fish. The fish was not observed to continue its initial head movements into an effort to escape from the field of the sound source, or to approach the sound source. This criticism is in particular levelled at the work of Kleerekoper and Chagnon.
The behaviour experiments in this investigation have purposefully been performed with unconditioned fish, and the experiments have been designed to elicit a clearly defined response from the fish when they were presented with the experimental situation.

The need to define the nature of the sound source used in these experiments, and to analyse the acoustic field surrounding the sound source was appreciated, and for this reason calculations of the displacement effect of the near field have been given for various points in the near field of the stimulus.
Figure 2

A  A perspective view of the small behaviour tank, and a plan view of the perforated plastic plate in which the 3 mm. glass rods were inserted.

B  The vibrating sound source consisting of a glass bead mounted on a thin rod attached to the micro stirrer driver unit.
DIAMETER 0.3 CM.

5 CM.

2.5 CM. 5 CM.

20 CM.

MICROMANIPULATOR ARM

SOLENOID

CLAMP

GLASS ROD

GLASS SPHERE

2.5 CM. 7 CM.

20 CM.

24 CM.

24 CM.
EXPERIMENTAL

In the first part of the experiment eight goldfish (*Carassius auratus*) approximately 6 cm. long were used. They all appeared healthy, and the scales covering their bodies were complete and undamaged. Twenty hours before the start of the experiments four of the goldfish were selected from the holding tank, which contained water at 12°C, and were blinded by cauterization. These fish were then returned to the holding tank.

In the second part of the experiment three blind Mexican Cave characins (*Anoptichthys sp.*) were maintained, for several days prior to the experiment, in a small aquarium containing water at 22°C.

Two behaviour tanks were used in these experiments, and both were constructed of a steel frame with glass sides and base. One tank measured 30 x 30 x 60 cm. and the other measured 24 x 24 x 24 cm.

A plastic plate with vertically mounted glass rods (Figure 2a) was placed in the smaller of the two tanks. Ten glass rods 7 mm. in diameter and 40 cm. long were suspended vertically, in two rows placed 10 cm. apart with 10 cm. between each rod, in the large tank so that one end of the rods rested on the bottom of the tank.

A sound source was used at one stage of the experiment. The source was a glass bead 1.5 mm. in diameter.
formed at one end of a fine glass rod, the other end of which was attached to the driver unit of a micro stirrer. A Sine wave generator connected to the micro stirrer caused the glass rod and hence the glass bead to oscillate back and forth. Unfortunately only two frequencies caused pure sinusoidal oscillations of the glass bead. These were 30 cps and 100 cps. The driver unit was mounted on the arm of a micro manipulator so that the rod hung vertically downwards, and the bead could describe movements along 1 axis in the horizontal plane (Figure 2b).

For the first part of the experiment all of the fish, the eight goldfish and the three blind cave fish, were subjected to the following test:-

A fish was removed from its holding tank and placed in a behaviour tank (the large tank for goldfish, and the small tank in the case of blind cave fish) which contained water at the same temperature as the water in the holding tank.

The release of the fish into the behaviour tank signalled the start of the test and the behaviour of the fish was observed as it approached the rods. An approach to a rod was considered to be made by the fish when it swam in such a direction that the rod lay directly in its path. An avoidance reaction was considered to have been made when the fish avoided hitting the rod by turning away from the rod when in close proximity to it. A distance of 2-5 mm in the case of blind cave fish.
This test was applied to each fish until the fish had approached a rod ten times, and the results of observations were recorded in tabular form.

In the second part of the experiment, using the above criteria, the ability of the fish to avoid a vibrating glass rod was tested.

The glass rods which were suspended in the behaviour tanks were removed, and a fish, taken from its holding tank, was placed in the appropriate behaviour tank. The sound source driver unit was mounted on the arm of the micro-manipulator above the surface of the water in the tank so that the fine glass rod attached to the driver unit was suspended vertically in the water with the glass bead a few centimeters above the bottom of the tank. The rod and bead were then moved about slowly until they lay in the path of the swimming fish, and the driver unit was switched on causing the bead to vibrate. The reactions of the fish, when confronted with the vibrating bead in this manner, were closely examined, and the observations were tabulated. This test was applied ten times to each fish.

At the end of these experiments the dimensions and amplitude of vibration of the glass bead in the tank were recorded.
RESULTS

The Reactions Exhibited by Goldfish when Approaching a Stationary Vertical Rod.

Blind goldfish made avoidance reactions only six times out of 40 trials, when making approaches to vertical rods which were obstructing their paths of locomotion. It was thought possible that goldfish behaviourally did not recognize the significance of a rod obstructing their paths, and thus might not make avoidance reactions even with their eyesight intact. This hypothesis proved to be incorrect because none of the unoperated goldfish failed to avoid rods obstructing their paths after a total of 40 trials (Table I).

The Reactions Exhibited by Mexican Blind Cave Fish to the Presence of a Stationary Vertical Rod blocking their Paths of Locomotion.

The results of this series of tests (Table II) clearly indicate the ability of the blind cave fish to make avoiding reactions when their paths are obstructed by glass rods. A series of measurements presented with these data indicate the mean distances away from the rods when the fish first started to make an avoidance reaction.
The reactions exhibited to blind goldfish to the presence of a vertical rod blocking their paths of locomotion.

<table>
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<th>No. of Avoidances</th>
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<td></td>
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<td>2</td>
</tr>
<tr>
<td>Blind B</td>
<td></td>
<td>10</td>
<td>0</td>
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<tr>
<td>Blind C</td>
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<td>10</td>
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<tr>
<td>Blind D</td>
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<td>10</td>
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<tr>
<td>Normal E</td>
<td></td>
<td>10</td>
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<tr>
<td>Normal F</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Normal G</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Normal H</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Diameter of Rod 0.7 cms.

Temperature of water 12°C ± 1°C.
TABLE II

The reactions exhibited by blind Mexican cave fish to the presence of a vertical rod blocking their paths of locomotion.

<table>
<thead>
<tr>
<th>Identification of Cave Fish</th>
<th>No. of Approaches</th>
<th>No. of Avoidances</th>
<th>Mean Distance from S.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0.3 cms.±0.1 cms.</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
<td>0.3 cms.±0.1 cms.</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0.3 cms.±0.1 cms.</td>
</tr>
</tbody>
</table>

Diameter of Rod 0.3 cms.

Temperature of water 22°C.
TABLE III.

The reactions made by blinded goldfish when approaching a sound source.

<table>
<thead>
<tr>
<th>Identification of Goldfish</th>
<th>Frequency of Sound Source</th>
<th>No. of Approaches</th>
<th>No. of Avoidances</th>
<th>Distance from Sound</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100 cps.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>100 cps.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>100 cps.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>100 cps.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>30 cps.</td>
<td>10</td>
<td>9</td>
<td>0.6 cm.</td>
</tr>
<tr>
<td>B</td>
<td>30 cps.</td>
<td>10</td>
<td>8</td>
<td>0.8 cm.</td>
</tr>
<tr>
<td>C</td>
<td>30 cps.</td>
<td>10</td>
<td>10</td>
<td>0.8 cm.</td>
</tr>
<tr>
<td>D</td>
<td>30 cps.</td>
<td>10</td>
<td>9</td>
<td>0.8 cm.</td>
</tr>
</tbody>
</table>

\(+\) 0.2 cms.
TABLE IV

The reactions of Mexican blind cave fish when approaching a sound source.

<table>
<thead>
<tr>
<th>Identification of Cave Fish</th>
<th>Frequency of Sound Source</th>
<th>No. of Approaches</th>
<th>No. of Avoidances</th>
<th>Distance from Sound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>2</td>
<td>100 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>3</td>
<td>100 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>1</td>
<td>30 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>2</td>
<td>30 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>3</td>
<td>30 cps.</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
</tbody>
</table>

Diameter of glass bead = 0.15 cms.

Amplitude of vibration at 100 cps. = 0.4 cms.

Amplitude of vibration at 30 cps. after adjustment = 0.4 cms.
The Reactions made by Blinded Goldfish when Approaching a Sound Source.

The oscillations of the glass bead constitutes an approximate dipolar source of vibration. This means the acoustic field around the vibrating sphere is not uniform but the water displacement is maximum along the axis of vibration, and minimum at right angles to this axis. For this reason the sphere was always set in the paths of swimming fish so that the axis of vibration was approximately parallel to the swimming path of the fish.

The data presented in Table II indicates that none of the fish made an avoidance reaction to the vibrating bead when it vibrated at 100 cps., but avoided it when it oscillated at 30 cps. Included with the data are measurements indicating the mean distance away from the source when each fish took avoiding action.

The Reactions made by Mexican Blind Cave Fish when Approaching a Sound Source.

The cave fish were able to locate the position of the vibrating bead at both 100 cps. and 30 cps. They started to make their avoidance reactions at a distance of approximately 4.5 cms. from the glass bead at both frequencies, which suggested that their sensitivity to the acoustic field of the vibrating rod, between 30 and 100 cycles per second (Table IV), was independent of frequency,
At the termination of these tests the amplitude of vibration of the rod was measured visually, and the dimensions of the glass bead taken (Table IV).
The Displacement of Water Required to Elicit a Response in Goldfish and Blind Cave Fish to a 30 cps. and 100 cps. Dipole Sound Source.

According to the results recorded in Table III, goldfish first responded to the sound source at distances which varied between 0.5 cms. and 0.8 cms. from the glass bead.

The displacements at these points may be calculated using the relationship given by Harris and Bergeijk (1962) for a dipole source.

\[ dr = \Delta \left( \frac{A^3}{r^3} \right) \cos \theta \]

\( dr \) = the radial displacement.
\( \Delta \) = the displacement amplitude of the sound source.
\( A \) = the radius of the sound source.
\( r \) = the distance from the sound source.
\( \theta \) = the angle between the device sensing the vibrations from the sound source, and the axis of oscillation of the sound source.

In these experiments \( \Delta A^3 \cos \theta \) is a constant = 0.25 cm.; \( \cos \theta = 1 \) (because \( \theta \) is approx. 0); \( A = 0.15 \text{ cm}^2 \).

Therefore \( \Delta A^3 \cos \theta = 8.725 \times 10^{-4} \).
The displacement to which fishes A, B and D responded to is given by
\[
\frac{d_r}{0.8^3} = 8.725 \times 10^{-4} = 1.7 \times 10^{-3} \text{ cms.}
\]
Fish C orientated to a displacement given by
\[
\frac{d_r}{0.6^3} = 8.725 \times 10^{-4} = 4.0 \times 10^{-3} \text{ cms.}
\]
Therefore the blind goldfish responded directionally to displacement which varied between 1.7 x 10^{-3} cm. and 4.0 x 10^{-3} cms. in amplitude at 30 cps.

The goldfish did not respond to the 100 cps. sound source.

Blind cave fish responded to both the 100 cps. and the 30 cps. sound source.

In all but one case the distance at which the fish first responded to the source was 4.5 cms.
\[
\frac{d_r}{4.5^3} = 8.725 \times 10^{-4} = 9.7 \times 10^{-6} \text{ cms.}
\]
Therefore blind cave fish respond directionally to displacements of 9.7 x 10^{-6} cms. in amplitude at frequencies of 30 cps. and 100 cps.
DISCUSSION

The results of the first part of the experiment indicate that blind cave fish are better able to locate stationary rods than blind goldfish even though the rods used in the tests to ascertain the cave fishes directional ability were thinner. If the goldfish had failed to locate the rods obstructing their paths of locomotion every time in the forty tests, then it could be argued that either the fish had failed to sense their presence or had sensed them but failed to localise them. However, the goldfish did localise the rods six times out of forty which indicates that fish must have not only sensed the rods but also located them. It is therefore possible to consider that in the other thirty-four tests the goldfish were unable to localise the rods rapidly enough to take avoiding action.

Why then were the goldfish unable to make an avoiding action in time? The ability of the fish to avoid an object depends upon the swimming speed of the fish as it approaches the rod, the distance away from the rod when the fish is first able to detect it and the response time of the fish to convert the information it receives about the position of the rod into an avoiding action. If the product of the approach speed and the distance away from the rod at which the fish first detects it, is greater than the response time it will collide with the rod.
The ability of fish to detect objects at a distance by mechanical means has been noticed by several people including Dijkgraaf (1963) who noticed that blinded fish were able to detect the approach of objects moved slowly through the water towards them.

The organs credited with being sensitive to this "distance touch" are the displacement sensitive lateral line organs and most of the evidence relating to the displacement sensitivity of the lateral line organs has been reviewed by Dijkgraaf (1963) who draws evidence to support this concept from behavioural, morphological and physiological studies.

The lateral line organs are considered not only to be displacement sensitive, but are also considered to be directionally sensitive. Flock and Wersall (1962) demonstrated from electron microscopical evidence, that the lateral line organs of fish are morphologically polarised. This evidence is supported by the neurophysiological recordings of Sand (1937) in elasmobranchs, and other workers such as Murray (1955) and Dijkgraaf (1961) in amphibia, that at least two forms of afferent nerve fibers innervate the lateral line organs, those which fire when the cupular of the lateral line organs is deflected in one direction, and those which fire when it is deflected in the opposite direction. Thus, the lateral line organs are both morphologically and physiologically polarised.
Dijkgraaf assumed that fish responded to the damming effect of the water built up ahead of bluff bodies as they came closer to the fish. It is also true that swimming fish build up a damming effect ahead of them as they swim. This has been demonstrated recently by Walters (1966) who used a polarometric method to observe the damming phenomena built up ahead of blind cave fish as they swam. Because the front of the head of the fish is bluff, water is first pushed ahead of the fish before it is able to escape to the side. Thus, a swimming fish is likely to experience a constant displacement of its lateral line organs, and is able, as Dijkgraaf puts it, "to detect the resistivity of the water ahead of the fish."

If a rod is placed in the field of the damming effect (Figure 3b) the configuration of the bow wave will be distorted, and the velocity of the water flow between the object and the fish will increase as water from the bow wave is forced to travel through the restricted aperture. In this way the fish would be able to detect the object either by measuring the changing configuration of its bow wave, or by the changing velocity of water past its head, or by both.

Although the bluffness of the goldfish's head is not as great as that of the blind cave fish, the greater size of the goldfish used in these experiments means they would send out bow waves ahead of them of an extent at least as great as those sent out by the blind cave fish. This
Figure 3

A The bow wave set up ahead of a swimming Mèxican blind cave fish.

B The interruption of the bow wave by the presence of an object in the path of a blind cave fish.
assumption was investigated later and found to be correct.

The goldfish in these experiments travelled at least three times the speed of the cave fish, which meant they covered the width of their bow wave three times as quickly as the blind cave fish, and would, therefore, have only one third of the time to respond in, and take avoiding action, if an object were detected in its bow wave.

Thus, it is probable that the goldfish hit more rods than the cave fish because they had less time in which to take avoiding action after detecting the rod.

In the second part of the experiment goldfish were able to locate a sound source of 30 cps. but were unable to locate a source of 100 cps. This indicates a limiting frequency response in the organs detecting the vibrations from the sound source.

As previously discussed, it is theoretically possible to discount the ears as being able to locate sound sources under water. This is indirectly supported by one of the results of these experiments and the work of Enger (1966). Enger demonstrated from electrophysiological experiments that the acoustic frequency range of the ears of the goldfish extend over a range of about 50 cps. to several thousand cycles per second. Therefore, if the goldfish was capable of using its ears for localising sound sources it should have been able to localise the sound source at 100 cps., but it failed to do so, and only localised it when it vibrated at 30 cps., which
is believed to be within the frequency range of response of the lateral line organs.

The amplitude of the near field displacements at the positions near the sound source where the goldfish and the blind cave fish first began to take avoiding action were calculated with the intention of finding the order of magnitude of displacements to which the fish were sensitive. It is realised that these values may not reflect the true magnitude of sensitivity of these two species, but goldfish in particular appear to be several orders of magnitude less sensitive to water displacement than blind cave fish. It is suggested that the method by which these measurements were made may give an indication of the goldfishes response time of reaction to the sound source rather than its displacement sensitivity. For instance, the goldfish used in this experiment were many times heavier and swam approximately three times faster (at 3 cm. sec.\(^{-1}\)) than the blind cave fish. This implies that the goldfish would have more difficulty in stopping and taking avoiding action than blind cave fish because of their greater momentum. Also the goldfish had recently been deprived of their eyesight and it is probable that their nervous system had not yet compensated for this loss of input. However, it may be noted that similar experiments - performed with goldfish blinded eight weeks previously and held in tanks - yielded similar results.
It is evident that, under the experimental conditions imposed upon them, goldfish and blind cave fish are capable of both detecting stationary rods and low frequency sound sources. In both cases the lateral line organs, with the possible assistance of touch receptors, are indirectly implicated as the organs capable of directional response.
Are Fish able to localise a Sound Source against Constant Background Noise?

INTRODUCTION

The previous experiments demonstrated the ability of both blind cave fish and blind goldfish to locate the positions of stationary and vibrating objects. Under these circumstances the objects were located against a background noise of the fish's own body movements. The present experiments were designed to investigate the ability of blind cave fish to locate sound sources against an experimentally imposed background noise.

A series of tests were designed firstly to determine if the fish is able to distinguish between concurrent unidirectional, vibratory displacements in the water, and secondly if a fish is able to discriminate between two vibratory displacement waves orientated in planes at right angles to each other, including tests to show if fish are able to distinguish between two vibrational displacements orientated at right angles to each other and of the same frequency, and similar amplitude.

The experiment is intended to test the directional properties of the sensory system which the fish uses for directional localization. If the fish is able to differentiate between two displacement waves vibrating at right angles
to each other and is able to use the characteristics of one of the waves to localise a sound source or an object, then the ears can be excluded from being considered the sensory system in question. This is because the ears respond to pressure waves and would receive the sum of the two pressure waves (Harris and Bergeijk 1962) which accompany the displacement waves and would be unable to determine the direction of travel of the wave. In order to localise an object by information carried by the pressure wave which travels primarily in the horizontal plane, the fish would have to be capable of breaking down the complex pressure wave it detects into two components, and to do this would presume a knowledge of the direction of travel of one of the pressure waves.
Figure 4

A A diagram of the arrangement of the apparatus used in the experiment to measure the acoustic field of the sound generated in the small tank by the eight inch loud speaker.

B The vibrational movement of the base of the tank caused by the vibration of the diaphragm of the loud speaker.

C A quadripolar sound source. Forces $F_1F_1$ applied together cause the sphere to form an ellipsoid with its major axis in the vertical plane. Force $F_2F_2$ causes an ellipsoid to be formed with its major axis in the horizontal plane.

$N_1N_2$ and $N_3N_4$ represent two horizontal nodal rings.

$N_1N_4$ and $N_2N_4$ represent two vertical nodal rings.

The line through $N_1N_2$ is for the comparison of the motion of the sphere above this line with the motion of the base of the tank.
PRE-AMPLIFIER - OSCILLOSCOPE

HYDROPHONE

TANK

AUDIO GENERATOR

AMPLIFIER

8" SPEAKER

BASE OF TANK

F2

N1

F1

N2

N3

N4

QUADRIPOLAR SOUND SOURCE
EXPERIMENTAL

The experiments were carried out on blind cave fish held in the small aquarium tank described in this previous section.

The apparatus and experimental procedures used in the previous experiment were retained for this experiment except that the experiments were repeated against a constant background noise. The noise was achieved by placing the small tank, containing water at the same temperature as the water in the holding tank for the blind cave fish, directly over the diaphragm of an eight inch loud speaker (Figure 4a). Single tone signals lasting the duration of each test were delivered through this speaker from an audio generator via an 11 watt amplifier.

The frequency and amplitude of the pressure wave caused in the water of the small tank was monitored first on an oscilloscope screen by recording the voltage output of a small hydrophone placed in the water on the tank. The hydrophone had previously been calibrated by the "null displacement" method, first described by Trott and Lide (1955), and was found to have a flat frequency response with an output of 1 micro volt per micro bar over a frequency range of 0-650 cps.

The hydrophone was mounted on a rod, clamped on the arm of a micro manipulator and moved around the tank,
measuring the hydrodynamic pressure at each point developed throughout the tank. The diaphragm of the speaker oscillated up and down at frequencies between 25 and 500 cps. during the transmission of a tone signal. These vibrations were conducted to the base of the small aquarium which also oscillated up and down at the same frequency as the speaker diaphragm. The bottom of the tank acted like a plunger pushing the water up and down uniformly so that at any instant the hydrodynamic pressure, and water displacement was uniform throughout the tank.

The floor of the tank was considered to behave in a similar manner to that of a quadripolar sound source.

A quadripolar motion is produced by a sphere when it is constricted by two forces which are applied to the sphere periodically and alternately at right angles to each other (Figure 4b and c). When $F_1$ is applied the sphere forms an ellipsoid with its major axis in the vertical plane, and when $F_2$ is applied it forms an ellipsoid with its major axis in the horizontal plane. If $F_1 = F_2$ then the sphere will perform this oscillation about 4 nodal rings indicated by $N_1N_2N_3N_4$ (Figure 4) which are fixed and unaffected by the oscillations. The base of the tank shown in b has a motion similar to the motion of the segment of the sphere undergoing quadripolar oscillations demarcated by the line, and so it is justified in applying equations defining
quadripolar motion to the motion of the base of the tank.

The hydrodynamic pressures were recorded from the tank and converted into water displacement values by applying the equation (Table V) describing the relationship between pressure and water displacement in the near field of a quadripolar sound source Harris (1963).

When the acoustic field of the source of noise had been monitored at various frequencies, a series of tests were performed to see if blind cave fish were still able to detect the presence of a rod placed in their path despite the presence of a background noise.

The same procedure was used in these experiments as was used in the experiments where no background noise was present. The fish were allowed to approach the rods, or sound source ten times for each frequency of the background noise used, and the results of the behaviour of the fish under these conditions were recorded in tabular form.
RESULTS

The Measurement of the Acoustic Field of the Noise Source.

The hydrodynamic pressure changes in the small tank caused by the noise source were measured by the hydrophone for frequencies of 25, 50, 100, 200 and 500 cycles per second. The amplifier intensity level was kept at a fixed value so that power input to the loudspeaker was always constant.

It was found that the hydrodynamic pressure in the tank was uniform for any particular frequency except for a small volume of water near the side of the tank. For calculation purposes, the pressure was measured always at a point 5 cm. above the centre of the floor of the tank because this was the height at which the blind cave fish usually swam.

The frequency of the noise source and the pressure and displacement amplitudes generated in the small tank were measured or calculated and are tabulated in Table V.

To Determine if Blind Cave Fish are still able to detect a Rod placed in their paths despite the Presence of a Background Noise of Constant Amplitude and Frequency.

Only in 9 tests out of a total of 150 did the blind cave fish fail to locate vertical rods placed in their paths despite the presence of background noises which ranged from 43.5 to 57.5 decibels above 1 m Bar or,
in terms of displacement, from 0.3 microns to 35 microns.

The oscillations of the water caused by the background noise were in the vertical plane, whereas the displacements caused by the fish as it approached the rods were in the horizontal plane. These tests indicate that the fish were able to discriminate between periodically changing water displacements in the vertical plane, and unidirectional displacements in the horizontal plane.

The distance from the rods where the fish first made an avoidance reaction was approximately 0.3 cm. (Table VI) which is of same distance as in the tests when there was no background noise (Table II).

**To Determine if Blind Cave Fish are still able to Detect the Presence of a Sound Source placed in their Paths despite the Presence of a Background Noise.**

Blind cave fish are clearly able to locate a vibrating bead placed in their paths despite the presence of a background noise, as indicated by the results tabulated in Table VII.

These results indicate that the fish differentiate between vertical oscillations, caused by the noise source, and horizontal oscillations of the water caused by the vibrating bead which is the sound source set in their paths of locomotion.
The calibration of the pressure changes generated in the small tank by background noise for different frequencies.

<table>
<thead>
<tr>
<th>Frequency cps.</th>
<th>Pressure U Bars</th>
<th>Displacement Angstroms</th>
<th>Decibel Scale ref. to 1 u Bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>150</td>
<td>35,000</td>
<td>43.5</td>
</tr>
<tr>
<td>50</td>
<td>250</td>
<td>10,000</td>
<td>47.9</td>
</tr>
<tr>
<td>100</td>
<td>350</td>
<td>3,500</td>
<td>50.8</td>
</tr>
<tr>
<td>200</td>
<td>600</td>
<td>1,500</td>
<td>55.6</td>
</tr>
<tr>
<td>500</td>
<td>750</td>
<td>300</td>
<td>57.5</td>
</tr>
</tbody>
</table>

Decibel scale is calculated from the relationship

\[ n = 20 \log_{10} \frac{P_1}{P_2} \]

where \( n \) = decibels

\( P_1 \) = measured pressure

\( P_2 \) = reference pressure

The sound source used for the background noise was considered to be an approximate quadripolar source, therefore the relation below was used to obtain the displacement caused by the sound source and set up in the water of the tank.

\[ d = \frac{P}{4\pi^2 fpc} \]

where \( \lambda = c/f \)

\[ d = \text{displacement in cms.} \]

\( P = \text{pressure in u Bars.} \)

\( r = \text{distance of point of measurement from sound source in cms.} \)

\( f = \text{frequency in cps.} \)

\( p = \text{density of water.} \)

For this experiment

\( r = 5 \text{ cms.} \)

\( p = 1 \text{ gm. cm.}^{-3} \)
The effect of noise of constant amplitude and frequency on the ability of blind cave fish to detect a rod placed in their path.

<table>
<thead>
<tr>
<th>Noise Frequency (cps.)</th>
<th>Fish Identification</th>
<th>No. of Approaches</th>
<th>No. of Avoidances</th>
<th>Nearest Distance of Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>8</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>10</td>
<td>9</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>8</td>
<td>0.3 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0.3 cm.</td>
</tr>
</tbody>
</table>

\[ \pm 0.1 \text{ cm.} \]

Diameter of rods = 0.3 cms.
TABLE VII.
The effect of noise of constant amplitude and frequency on the ability of blind cave fish to detect a vibrating bead placed in their path.

<table>
<thead>
<tr>
<th>Frequency (cps.)</th>
<th>Fish Identification</th>
<th>No. of Approaches</th>
<th>No. of Avoidances</th>
<th>Nearest Distance of Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>4.4 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>4.6 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4.9 cm.</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4.4 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4.6 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4.5 cm.</td>
</tr>
</tbody>
</table>

Diameter of glass bead = 0.15 cms.
Amplitude of vibration = 0.4 cms.
Frequency of vibration = 100 cps.

Amplitude of water displacement 4.5 cms. from glass bead.

\[ = 9.7 \times 10^{-6} \]

\[ = 970.0 \text{ Å} \]
They are able to locate the sound source independent of the frequency of the background noise, even in the case where the frequency of the background noise is the same as the sound source, and the amplitude of the water displacements caused by the background noise are four times those caused by the sound source. Also, the sensitivity of the cave fish to the sound source was not impaired by the presence of the noise as can be seen by comparing the nearest distances of approach to the sound source given in Tables IV and VII.
DISCUSSION

Blind cave fish possess a facility for locating objects and a sound source against a background noise, according to results drawn from these experiments. This ability could be discussed in terms of the fish's ability to detect pressures against background pressures or in terms of displacement because both acoustic phenomena are present in the experimental conditions, and the fish respond to both of them.

The pressure at any point in the experimental tank would consist of components from a large number of sources. Under the experimental conditions a pressure wave would be generated from the floor of the tank which is the source of background noise, and this in turn would produce reflected waves from the walls and floor of the tank. Considering the dimensions of the experimental tank the reflected pressure waves would be hardly attenuated at the frequencies of sound used. The fish would produce pressure waves generated by its own body movements, and these in turn would interact with objects placed in the tank as well as the walls of the tank and produce component waves. These waves would then interact to produce a pressure with magnitude only and no direction. Physically the fish with its single swim bladder would be unable to locate the sources of these pressure waves.

The displacement of water at any one point in the tank would be the sum of displacement waves originating from
the same sources as the pressure waves. But the contributions of reflected displacement waves, from the sides and surface of the tank, would contribute to the resultant displacement to a far lesser extent than did the reflected pressure waves to the resultant pressure. This is because of the rapid attenuation of displacement waves with distance from a sound source. At any point in the tank a resultant displacement wave possessing both magnitude and direction may be detected.

As previously mentioned, the lateral line organs of fish and amphibians are directionally sensitive. Sand (1937) demonstrated that lateral line organs in Rays are only sensitive to displacements of water along the long axis of the lateral line canals, and Dijkgraaf (1956) showed that the lateral line organs of Xenopus are sensitive only to displacements directed along the stitch of neuromasts which compose the lateral line organ.

Blind cave fish have lateral line organs arrayed in lines parallel to the long axis of the body, for example the main trunk lateral line, and other lines of organs, such as the hyomandibular, are arrayed at right angles to this. A single lateral line organ, depending upon its orientation on the fish will respond only to one particular component of any compound displacement in the water about the fish. The fish (as a whole) has, therefore, the facility to analyse complicated compound displacement waves impinging upon it, into the separate component waves which are orientated into horizontal and vertical components. This
system would be more than adequate for the fish used in these experiments, and under the conditions to which they were exposed. They would be able to discern between the vertically oscillating background displacements, and the horizontal displacements emanating from the sound source or the horizontal displacement caused as a result of the interaction between the fishes bow wave and a stationary object in its path. Having made this separation the fish would then be in a position to localise sources contributing to displacements in both the vertical and horizontal planes.

If touch receptors only were considered to be involved in localising the sound source, then several difficulties would be incurred. Firstly, electrophysiological evidence on touch receptors in fishes is scarce and it has to be assumed that they possess properties similar to those found in higher vertebrates. Namely, they respond in an "all or none" manner, so that they give only one ungraded response to an above threshold stimulus. They are none-directional, and they are quickly adapting.

Therefore, the direction of the source giving out displacement waves can only be ascertained centrally by the fish. The brain must analyze the number of touch receptors firing all over its body, and find the location where the greatest density of firing is to be found.

Amplitude could be ascertained only by detecting the total number of touch receptors firing in a particular
area. Frequency detection by the touch receptors would
depend upon the adaptation rate of receptors, the information
carrying capacity of the nerves to the touch receptors, and
the presence of an association centre in the brain capable
of recognizing frequency. It is possible that under the
conditions of this experiment the fish may have rapidly adapted
to the constant background noise and have detected changes
in the amplitude of vibrations from the sound source. This
is unlikely in the special case where the background noise
was exactly the same frequency as the sound source. Under
these circumstances if the fish adapted to frequency, it should
not have located the sound source and should have been
insensible to it.

It is concluded that the evidence drawn from these
experiments excludes the ears from being directional receptors
and implicates the lateral line organs as the probable
candidates. Touch receptors cannot be ignored but appear to
be unlikely candidates as directional receptors capable of
locating a sound source.
Anatomical evidence was presented first to show the existence of an efferent nervous supply to the hair cells of the mammalian cochlear by Rasmussen (1946). He traced nerve fibers which originated in the superior olivary nucleus of one side of the brain, and crossed to the contralateral vestibular nerve which they accompanied to the intraganglionic spiral bundle of the cochlear. He demonstrated the efferent nature of these fibers by making appropriate placed lesions in them and observed their nervous degeneration towards the cochlear. The efferent fiber tract was named the crossed olivo-cochlear bundle. Later, in 1960 he was able to demonstrate the existence of afferent fibers to the cochlear which originated in the ipsilateral superior olivary nucleus.

Galambos (1956) was the first to investigate the neurophysiology of the crossed olivo-cochlear bundle. He substantiated Rasmussen's anatomical evidence by finding them to be efferent fibers, and also he found them to be strongly inhibitory. Sound evoked potentials generated in the afferent vestibular nerve were strongly inhibited by electrical stimulation of the crossed olivo-cochlear fibers. This investigation was pursued further by Fex (1962) and
Desmedt (1960)(1962) who separately showed that the nervous activity of the olivo-cochlear fibers could be increased by stimulating the ipsilateral ear by sound, and that this activity could be further increased, they believe facilitated, by electrically stimulating the vestibular nerves of the contralateral ear. Fex (1962) considered the efferents to be part of a mechanism for feedback control of the sensitivity of the cochlear to sound. Both workers realised that the efferents coded the information carried by the afferent vestibular nerve, and Desmedt thought it was important to see how the efferents were related to psychophysiological problems of conscious auditory perception. In particular how an animal could detect a series of complex sounds from a background noise.

The presence of an efferent nervous supply to the hair cells of the mammalian cochlear invited investigations of other hair cell systems to see if they too possessed efferent innervation. In 1962 Flock and Wersall described two types of innervation to the hair cells of the lateral line organs of the cottid, Lota vulgaris. One type of nerve synapsed onto the hair cell with a vesiculated synapse and the other type possessed an unvesiculated synapse, and vesicles and granules were present in the cytoplasm of the hair cell in the region of this synapse. Flock (1966) inferred from his evidence that the unvesiculated synapses
might be efferent, but could offer no assurance that they were not recurrent collaterals from afferent fibers.

From the previous behaviour experiments it does appear that fish possess a facility for localising a particular sound from a background noise. Desmedt and Fex realised that terrestrial mammals possess this facility, and from their experimental results implicated the olivo cochlear efferent bundle as part of a mechanism for neurophysiologically suppressing the effect of background noise. Flock and Wersall's discovery of a possible efferent nervous supply to the lateral line organs of fish has prompted this present neurophysiological investigation to see if efferents innervation to the hair cells of the lateral line organs does exist and to see what role, if any, it plays in overcoming psychophysiological problems associated with auditory perception in fishes.
EXPERIMENTAL

The experiments were carried out on fifty goldfish (Carrassius auratus) of approximately six inches in length. The fish were kept prior to the experiment in large holding tanks and were fed on a reduced diet in order to limit the deposition of fat on the bodies of goldfish, particularly about the orbits.

A fish was selected from the holding tank and placed in a clamp which held the fish rigidly for the duration of the experiment.

This clamp (Figure 5a) consists of a shaped plate of \( \frac{3}{4} \) " perspex to the middle of one side of which is screwed a saddle shaped piece of perspex. The fish's body was placed across the saddle and the tail clamped to the rectangular end of the plate. The head was clamped to the other end of the plate by means of a plastic tube which was inserted into the mouth. This tube was also clamped to the plate. The head end of the plate was cut away so that the orbit on the side of the fish adjacent to the plate was accessible for operation.

The fish clamp was then attached to a platform mounted in the operating tank by means of a bolt through a hole in a piece of perspex which was attached to the clamp and served as a base.

The perspex operating tank (Figure 5b) had a perspex platform \( \frac{3}{4} \)" thick at one end, 2" below the top edge of the tank. In the middle of the platform was a bolt.
Figure 5

A  The clamp used for holding the fish rigidly throughout the experiment.

B  Schematic arrangement of the apparatus used during the neurophysiological experiments.
for securing the clamp. At the end of the tank, opposite the platform, there were three tubes which pierced the walls of the tank. One tube, the water inlet, was situated in the end wall, while the other two tubes were situated on the lower edges of the side walls three inches from the end walls, and were the drainage tubes.

The clamp was bolted to the platform and a flexible plastic tube was connected between the tube in the fish's mouth and the water inlet tube. Dechlorinated water was circulated through the tank by a small rotary pump driven by an electric motor with a variable speed control. The passage of water in the system was through the inlet tube, over the fish's gills, out through the drainage pipes, into the pump, through a cold water cooling coil, and back again to the fish. The water level in the tank was controlled by adding more water to the tank or by draining water off through a tap. Before each experiment fresh water was added to the system. Care was taken not to raise the water level above the lower edge of the orbit so preventing water from coming in contact with tissue exposed by dissection.

A drawing of the completely dissected preparation has been given in Figure 6 and is the result of 26 dissections. All dissection was performed under a Zeiss operating microscope with floor stand.

The fish's eye on one side was first removed by cutting through the optic muscles and the optic nerve, Connective tissue and fatty tissue was removed from the walls of the orbit by dissection and aspiration. Care was
Figure 6

Diagram of the dissected ramus buccalis facialis on the right side of the head of a goldfish.

$N_1$-$N_{10}$ represent lateral line organs in the sub-orbital lateral line canal.

$N_{11}$-$N_{15}$ represent lateral line organs in the nasal canal.
taken not to disturb blood vessels or nerves, although inevitably some blood vessels, such as the retinular artery were cut. When the orbit had been dealt with in this manner, blood and loose pieces of tissue were washed away with oxygenated Cautland's saline which was at the same temperature as the water flowing in the operating tank.

The first sub orbital bone was severed from its underlying connective tissue and removed from the skull. An incision was next made in the lachrymal bone along a line A-A (Figure 6) dividing the bone into two halves. The lower half of the lachrymal and the second sub-orbital bone were reflected back in one piece, and any underlying connective or muscular tissue impeding this reflection were cut. The reflected position of these two bones was maintained by a fine hook retractor.

This initial dissection revealed three branches of the buccal branch of the facial nerve, these have been termed branches X, Y and Z in Figure 6 Herrick (1899) was consulted in order to help identify the various nervous components of the orbit.

The usual procedure was to select one of the branches X, Y or Z, and free it from the underlying connective tissue, and follow the nerve branch until it disappeared as thin fibers through the boney wall of the lateral line canal it innervated. Only the buccal branches of the facial
Figure 7

Schematic arrangement of electronic recording apparatus and stimulating apparatus used in the neurophysiological experiments.
nerve innervated the neuromasts of the sub-orbital canal and parts of the nasal canal, and it was possible to trace the nerves until they split up at the base of each organ.

Throughout the clearing process, care was taken to ensure that the nerves were always kept moist with saline. When one nerve branch to an organ had been cleared, very carefully to prevent damage to it by scraping or stretching, recording electrodes were slipped carefully under the nerve. Saline was removed from around the electrodes and nerves by aspiration and paraffin oil pipetted into the orbit and over the electrodes to act both as an insulator and to reduce dessication of the nerves. When activity had been found (in a nerve branch) the water level in the tank was raised to cover the lateral line organ innervated by the nerve branch from which recordings were being made.

A schematic diagram of the recording apparatus used in these experiments has been given in Figure 7. The electrodes used in these experiments were differentially recording electrodes. One type consisted of a pair of parallel fine silver wire hooks which were shielded close to their tips, and mounted on a thin perspex rod. The shielding was continued as the ground electrode, which was clipped onto the fish. The perspex rod was clamped into the arm of a Palmer micro manipulator. Another type of
electrode was used for extremely fine nerve fibers of short length. This consisted of a very fine platinum hook which was placed under the nerve, a fine silver wire indifferent electrode placed in the tissue close to the nerve, and a ground electrode which was clipped to the fish.

The electrodes were connected by double cored, screened cable to the input of a differentially balanced pre-amplifier. The frequency response of the amplifier, a Tektronix 122, was set to reject all signals greater than 10,000 cps. or less than 80 cps., and the gain was set at 1000. The output of the pre-amplifier was fed into one channel of a Tektronix 502A dual beam oscilloscope, and also into one channel of a Tanberg stereo A.C. tape recorder.

One experiment required the use of an electrical pulse generator for stimulating the nerves. A Grass S4 stimulator was used, and this could deliver both D.C. and square wave pulses with variable amplitude, frequency duration and delay. This machine had three output leads; two to the oscilloscope, one of which monitored the impulse signal, and the other which triggered the time base of the oscilloscope. The third lead was the stimulation lead and this fed into a stimulus isolation unit, a Grass S.U.4. The purpose of this transformer device was to reduce stimulus artifact by raising the stimulating current above ground. The stimulating current was delivered through two fine silver
hooks set close together, about 0.5 mm. apart, and mounted on a perspex rod which was clamped in the arm of a Palmer micro manipulator. Selected results were photographed with a Polaroid Tektronix CL2 oscilloscope camera mounted on the oscilloscope. Information stored on tape was played back through the oscilloscope, analysed and photographed at the end of the experiment.
RESULTS

Evidence of an Efferent Nervous Supply to the Lateral Line Organs in the Lateral Line Canals in the Head of Goldfish.

A total of eleven fish was used in these experiments. Initially a branch of the facial nerve supplying, or appearing to supply, a lateral line organ was selected, and silver or platinum bipolar recording electrodes were slipped under it. In each case an irregular spontaneous discharge was recorded from the nerve. The nerve was cut and recordings made of any activity in the proximal part of the nerve. Vigorous activity usually was noticed immediately after cutting the nerves, and this was thought to be injury potentials which had come about as a result of the nerve section. However, these rapidly died down and persistent activity continued sometimes for six hours after cutting the nerve.

This activity had a frequency range of less than one impulse per second to a maximum of 30. The frequency varied with the fish and the lateral line organ the nerve was innervating.

It was thought that this activity might originate from either a visceral efferent nervous supply to blood vessels, somatic efferent supply to muscles, sensory efferent supply to lateral line organs, or collateral afferent nerve fibers from afferent sensory nerves from the lateral line organs. The close proximity of nerve fibers from the
TABLE VIII.

Impulses recorded from proximal end of cut nerve originally innervating lateral line organ n9 (Figure 6).

The canals were stimulated by water jet from a syringe.

<table>
<thead>
<tr>
<th>Time from Beginning of Experiment Seconds</th>
<th>Start, Duration and end of Stimulus</th>
<th>No. of Impulses Per Second</th>
<th>Lateral Line Canal Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>11</td>
<td>Nasal Canal</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>15</td>
<td>Sub-Orbital Canal</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>Trunk Canal</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td>Hyomandibular Canal</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
Diagramatic representation of the results presented in Table VIII.

A, B, C and D represent the result of stimulating different lateral line canals by water jet, and recording from the efferent nerve supplying lateral line organ n9.

A  nasal canal
B  sub-orbital canal
C  trunk canal
D  hyomandibular canal
A

![Graph A](image1)

B

![Graph B](image2)

C

![Graph C](image3)

D

![Graph D](image4)
Figure 8

A  Bipolar recordings of action potentials from a fine branch of the ramus mandibularis V innervating the muscle adductor mandibular 3.
B  Bipolar recordings of action potentials from nerve Y of the ramus buccalis facialis.
facial and trigeminal roots in the orbit was the predication factor in making these assumptions.

In two subsequent experiments a fish was prepared as previously described and bipolar silver stimulating electrodes were slipped under nerve Y in one case, and nerve Z in the other. These nerves were stimulated by D.C. and square wave pulses over a wide range of frequency from 1 per second to 1,000 per second and a duration of 0.1 micro-second to 1 millisecond, with a voltage range, at the beginning of the experiments of 0.001 volt to 10 volts at the end. At the same time as the stimulation, the blood vessels in the proximity of the orbit were observed to see if they changed in diameter, and muscles in and surrounding the orbit were observed to see if they twitched in response to stimulation. No change in diameter of the blood vessels, or twitching of the muscles was observed.

In one further experiment a fine branch of the mandibullar branch of the vagus nerve of a goldfish was dissected free. Weak D.C. electrical stimulation of this resulted in the contraction of the adductor mandibular 3 muscle. Bipolar silver recording electrodes were slipped under this nerve and it was then cut distally. The record from this nerve (Figure 8a) was very regular and clearly palsatile, unlike the characteristically irregular discharge from the fine nerve branches which apparently innervate the lateral line organs (Figure 8b).
**TABLE IX.**

Impulses recorded from ipsilateral proximal end of cut nerve supplying lateral line organ n7 (Figure 6) stimulate contralateral side of head with vibrating air bubbles.

<table>
<thead>
<tr>
<th>Time from Beginning of Experiment Seconds</th>
<th>Duration of Stimulus</th>
<th>No. of Impulses Per Second</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>10</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>9</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>5</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>4</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>9</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>10</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>7</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>2</td>
</tr>
<tr>
<td>J</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>10 secs.</td>
<td>5</td>
</tr>
<tr>
<td>60</td>
<td>10 secs.</td>
<td>4</td>
</tr>
<tr>
<td>90</td>
<td>10 secs.</td>
<td>8</td>
</tr>
</tbody>
</table>
Diagramatic representation of the results presented in Table IX.

E, F, G, H, I and J represent a consecutive series of recordings ten minutes apart obtained from the efferent nerve supplying lateral line organ n7 during the stimulation of the contralateral side of the head with air bubbles.
E

IMPULSES PER SECOND

TIME, SECONDS

F

IMPULSES PER SECOND

TIME, SECONDS

G

IMPULSES PER SECOND

TIME, SECONDS

H

IMPULSES PER SECOND

TIME, SECONDS

I

IMPULSES PER SECOND

TIME, SECONDS

J

IMPULSES PER SECOND

TIME, SECONDS
It was concluded that the discharge originated either from efferent fibers to the lateral line organs or from collaterals from afferent fibers supplying these organs.

Fine fibers innervating single lateral line organs were exposed in 3 fish in 3 consecutive experiments and bipolar recording electrodes were placed under them. Recordings were made from fibers innervating lateral line organs $N_6, N_7, N_9,$ and $N_{14}$ (Figure 6). These fibers were cut distally and recorded from proximally. The effect of stimulating ipsilateral lateral line canals, by water jets from a fine syringe, or from touch by a fine brush, on the discharge from the proximal ends of these nerves was observed. Each lateral line canal was stimulated individually by concentrating the stimulus over the area of the canal. Results for stimulation (by water jet) of the nasal canal, the hyomandibular canal, the main trunk canal, and the canal in the first sub-orbital bone on the discharge in the nerve fibers supplying the lateral line organ $N_9$ of a single fish, have been presented in tabular and graphical form in Table VIII and graphs A, B, C and D respectively. No relationship could be drawn between the rate of discharge and the application of the stimulus.

This test was applied on fifteen fish in subsequent experiments with the same result, namely no increase, or decrease, in discharge rate of the proximal cut end of these fibers could be correlated with the application of a stimulus to neighbouring ipsilateral canals.
In a further series of experiments involving five fish, fine fibers to lateral line organs \( N_6, N_7, N_9, \) and \( N_{14} \) were exposed and the fibers cut distally so that discharges, originating in the proximal ends of these fibers, could be recorded.

Contralateral-lateral line canals in the fish's head were stimulated, once every 30 seconds for periods of 10 seconds each, by air bubbles which were released at a rate of 2 per second from a 3 mm. diameter tube, connected to a compressed air source. The tube was placed close to the side of the head opposite to that being recorded from, and in such a manner that the air bubble brushed the side of the fishes head as they ascended to the surface. Table IX lists a series of discharge rates obtained from the proximal cut end of the nerve supplying lateral line organ n 7 of a single fish during stimulation of contralateral-lateral line canals by air bubbles. These results have been represented graphically in graphs E,F,G,H,I and J, and only in graphs E and F is there any correlation between discharge rate and stimulus, where there is an increase in discharge rate during stimulus.

This increase in discharge rate was found to be correlated with movement of the fish during the stimulus, and no increase in rate was noticed when the fish remained stationary during stimulus.
The Nature of the Efferent Nervous Supply to the Lateral Line Organs in the Lateral Line Canals in the Head of the Goldfish.

Nerves Y and Z of the buccal branch of the facial nerve were exposed on one side of the head of a fish. Hooked silver bipolar electrodes were slipped under nerve Y and the spontaneous discharge from this nerve bundle was recorded for several minutes. The nerve bundle was severed proximally and the nervous discharge of the distal part of the nerve was recorded. It was noticed that the discharge rate of this part of the nerve was greater than that previously recorded from the nerve when it had been intact.

An increase in discharge rate was not expected, because the discharges recorded in both nerves Y and Z were thought to have been contributed to by at least two sources, namely afferent nerves from the lateral line organs, and efferent nerves supplying these organs. Effective removal of one of these sources, the efferent nerves, by severing, in this particular instance nerve Y, proximally, and recording distally, was expected to decrease the discharge rate.

The increase in discharge rate was not transitory but was at a sustained level until the experiment was terminated. This experiment was subsequently repeated, on nerve Z of the same fish, and nerves Y and Z of three other fishes, with similar results. Results of an experiment similar to the ones described above have been recorded in tabular and graphical form in Table X and Graph K respectively.
TABLE X.

The effect of an application of strychnine to the lateral line organs of the nasal canal upon the discharge of the fibers from the buccal branch of the facial nerve supplying the organs (Nerve Z, Figure 6).

Strychnine sulphate was applied locally to the organs at a dose rate of 0.5 mg. per kg. of fish.

<table>
<thead>
<tr>
<th>Time in Minutes from beginning of Experiment</th>
<th>Application of Strychnine</th>
<th>State of Nerve Z</th>
<th>Impulses per Second</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
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<td>2</td>
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</tr>
<tr>
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<td>Nerve Intact</td>
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</tr>
<tr>
<td>4</td>
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</table>
The diagramatic representation of results presented in Table X.

The effect of an application of strychnine to the lateral line organs of the nasal canal upon the discharge of the fibers from the buccal branch of the facial nerves supplying the organs. (Nerve Z Figure 6).

The activity levels of discharges recorded from nerve Z have been drawn in for normal conditions, and after cutting the nerve and recording distally.

A sample of nervous activity displayed under the appropriate activity levels.
TABLE XI.

The effect of cutting nerve Z (Figure 6) of the buccal branch of the facial nerve, upon the discharge recorded in the distal part of the fiber, and then applying strychnine locally to the organs innervated by this fiber in the nasal canal.

Strychnine sulphate applied locally to receptors at a dose rate of 0.5 mg. per kg. fish.

<table>
<thead>
<tr>
<th>Time in Minutes from beginning of Experiment</th>
<th>Application of Strychnine</th>
<th>State of Nerve Z</th>
<th>Impulses per Second</th>
</tr>
</thead>
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</table>
Diagramatic representation of the results presented in Table XI.

The effect of cutting nerve Z of the buccal branch of the facial nerve upon the discharges recorded in the distal part of the fiber, and then applying strychnine locally to the organs innervated by this fiber in the nasal canal.

The activity levels of discharges recorded from nerve Z has been drawn in for normal conditions, after cutting and recording distally and after the application of strychnine.

A sample of nervous activity is displayed beneath the appropriate activity levels.
One possible solution to the paradoxical results obtained in these experiments was that the efferent nervous supply to the lateral line organs had an inhibitory action on the generation of nerve impulses in the afferent nerve fiber. Bearing in mind the electron-microscopical work of Flock and Wersall (1962), in which they showed the existence (to the lateral line organs of *Lota vulgaris*) of two types of innervation, in close proximity to one another it was considered possible that the site of action of any inhibition, if it existed, was at the point where efferent and afferent nerve fibers synapse on the hair cell.

In experiments performed successively on four fish, nerves Y and Z were exposed. Spontaneous nervous discharge from nerve Z was recorded for several minutes, and then strychnine sulphate, dissolved in saline, was injected in the tissue underlying the neuromasts innervated by the nerve being examined. The strychnine was administered at a dose level of 0.5 mg. per Kg. of fish. A rapid, and sustained rise in discharge rate was noticed, and this could be observed for a period of 11 minutes (Graph L, Table XI) or for periods up to 1 hour in other experiments.

The nerve was severed distally and recording was continued proximally, with the result that an immediate drop in discharge rate was observed. This decreased rate was maintained without recovery, at a level slightly above that initially recorded in the intact nerve, until the termination of the experiment. Similar results were obtained for experiments performed on both Z and Y for each of the four fish.
A series of control experiments were performed on four more fish. Spontaneous activity was recorded in the intact nerves Y and Z for several minutes. The nerve was cut and the recording continued from the distal part of the nerve. An immediate rise in discharge rate was observed (Graph L, Table XI) and this was allowed to continue at a sustained rate for a period of two and one half minutes when an injection of strychnine sulphate was administered to the tissues beneath the neuromasts innervated by nerve Z. No change in discharge rate was observed as a result of the strychnine injection. This observation held true for all remaining experiments performed on nerves Y and Z of the four fish.
DISCUSSION

Electron-microscopical evidence presented by Flock and Wersall (1962) regarding the double innervation to the hair cells of the lateral line organs of *Lota vulgaris*, did not indicate if one of these nerve components was truly efferent or possible recurrent collateral fibers from afferent nerve fibers. In the initial investigation in these experiments an allowance was made for the possibility that the discharge recorded from the proximal end of a severed bundle of nerves supplying a lateral line organ might be antidromic afferent discharges and not discharges of efferent origin. Doubt as to the origin of the discharge was mitigated by the results of the succeeding experiments which indicated that stimulation of surrounding lateral line organs, both contralateral and ipsilateral, had no noticeable effect upon the discharge rate of the proximal portion of the severed nerve. Stimulation of these adjacent neuromasts would presumably produce potentials in any existing contralaterals to other neuromasts. No increased activity was recorded upon stimulation of adjacent neuromasts and therefore it seems probable that there are no contralateral afferents in the lateral line nerve. Activity recorded from the proximal ends of the cut nerves was from efferent fibers innervating the neuromast.

Recently Schmidt (1965) presented evidence for an efferent nervous supply to the lateral line organs of *Necturus maculosus* and for a similar supply to the lateral line organs of a single specimen of *Rana pipiens*. Gorner (1966) has
presented evidence for an efferent nervous supply to the lateral line organs of *Xenopus*, but in neither case is there evidence that the activity recorded was not antidromic afferent activity, and on these grounds it is considered that their evidence remains inconclusive on this point.

In the second part of the experiment it was found that the discharge rate of the distal part of the severed nerve was greater than the rate of the intact nerve. From these results it is suggested that the activity in the efferent fibers to the lateral line organs might have an inhibitory effect upon the activity of the afferent fibers. Thus when the nerve is cut inhibition of the neuromast is released, and the rate of discharge in the afferent fibers increases.

Strychnine is well known to synaptically block post-synaptic inhibitory action (Eccles (1964)). Eccles, Fat and Koketu (1954) employed strychnine sulphate to suppress efferent activity in the spinal cord of a cat, and they found that a dose rate of 0.08 mg. per Kg. of cat, which is a sub-convulsant dose, reduced the inhibitory post synaptic potentials in the spinal cord nerves by more than half. Eccles (1964) noted that greater doses would virtually eliminate the inhibitory post synaptic potential.

It was found by Curtis (1962) from intra cellular recordings from spinal neurones during iontophoretic injections of strychnine, that the strychnine did not depress the activity of the neurone being examined. From this evidence he suggested that the effect of strychnine was confined to
the region of the inhibitory synapse.

Galambos (1956), Fex (1962), and Desmedt (1962) used strychnine sulphate to block the inhibitory action of the olivo-cochlear bundle upon the generation of impulses in the afferent nerve fibers from cochlear hair cells. The action of strychnine upon the efferent fibers, and efferent synapses, of the lateral line organ hair cells is unknown. However, it was thought that if these fibers did inhibit the generation of impulses in afferent fibers innervating the lateral line hair cells, then it would not be unreasonable to consider that an application of strychnine sulphate to the region of the efferent synapses would block this inhibitory action. Experimental results indicated that the application of strychnine to the base of the lateral line organs increased afferent activity. Control experiments performed after these experiments demonstrated that the increased activity associated with the application of strychnine was not a result of an excitatory effect of strychnine on the efferent nerves and that strychnine had no observable effect upon the discharge rate of the afferent fibers. This indirect evidence supported the conclusion that the efferent fibers were inhibitory.

Schmidt (1966) and Gorner (1966) were unable to show any effect of the activity of the efferent nerve fibers upon the activity in afferent nerve fibers innervating the lateral line systems they were investigating. Gorner concluded that his results indicated an independence of efferent nervous activity from afferent nervous activity in the nerve fibers innervating the neuromasts of Xenopus, and considered that
another approach to the problem was required.

Since the blocking action of strychnine is to compete post synaptically with the inhibitory transmitter for receptor sites (Eccles 1964) then the inhibitory transmitter acts upon the post synaptic membrane in some way. There is an increasing amount of evidence, summarized by Eccles (1964) which indicates that the role of the inhibitory transmitter is to maintain the post synaptic membrane at resting potential or slightly below it. Eccles has suggested further, since all the post synaptic inhibitory effects that have been investigated in the spinal cord of the cat are blocked by strychnine, that they are all caused by the same transmitter substance, and the drugs which are believed to act by competitive occupation of the receptor sites would be sterically related to the inhibitory transmitter. He suggested also from evidence presented by Galambos, Fex, and Desmedt, who used strychnine to block the inhibitory effect of the olivo-cochlear bundle upon the afferent cochlear nerve activity, that the same inhibitory transmitter is responsible for inhibition in this case. This argument could, therefore, also be extended to the action of efferent fibers on the lateral line organs. However, to date no transmitter substance has been isolated or identified at the synapses of any hair cell system (Tanaka and Katsuki 1966).
In conclusion it would appear that the neuromasts of the lateral line system are innervated by efferent nerve fibers. The action of these nerves is to inhibit activity in the afferent fibers from the same neuromast. There appears to be no contralateral or ipsilateral interaction between neuromasts via this efferent nerve supply. The action of the lateral line efferents appears to be very similar to that of the efferent fibers innervating the hair cells in the cochlear of the cat.
INTRODUCTION

Desmedt (1962) suggested a psychophysiological role for the olivo-cochlear efferent nervous supply to the cochlear hair cells. One suggestion was that the ability to neurophysiologically suppress some sounds and enhance the perception of others might be under cortical control.

Previous behaviour experiments have shown that fish are able to localise a sound source against background noise. In this case the background noise encompasses every acoustic phenomena except those emanating from the sound source. Even the fish's body movements are, under these conditions, counted as background noise.

The previous experiments indicated that the activity of the efferent fibers to the lateral line organs of the goldfish remained unaffected by the stimulation of the other lateral line organs of the same fish by touch, water jet, and air bubbles. These stimuli would not only stimulate the lateral line organs, but would also affect the other organs which are acoustically receptive, such as the ears and the touch receptors. It would appear, therefore, that the activity carried in the efferent fibers to the neuromasts does not originate in neighbouring neuromasts or in other acoustically receptive systems. What then is the origin of the efferent activity to the lateral line organs, and how, if at all, is it related to the level of background noise?
Knowing that the efferent fibers do not appear to be affected by the stimulation of surrounding acoustically sensitive systems, it was decided to investigate the effect of certain types of noise caused by body movements upon the lateral line organ efferents.
EXPERIMENTAL

The general experimental procedures and apparatus used in these experiments have already been described in the previous section. However, in this series of experiments the opercular movements of the fish were monitored. A small hole was made near the outer margin of the operculum and a fine steel hook inserted in this hole. A thread secured the hook to the arm of a mechano-electric transducer mounted above the operculum. A single cored, screened cable connected the transducer to the input of another 122 pre-amplifier which was now used as a single sided pre-amplifier with one of its differential inputs connected to ground. This signal was then fed into the other channel of the tape recorder and the oscilloscope.

In order to investigate the effect of intramuscular injections of tubocurarine chloride upon the discharge rate of the lateral line organ efferent nerves, tubocurarine chloride dissolved in saline was injected directly into the muscles. The tubocurarine chloride was administered to the fish at a dose rate of 0.1 mg. per 100 gms. of fish through a syringe fitted with a 23 gauge hypodermic needle.
RESULTS

The Effect of Opercular Movements Upon the Rate of Discharge from the Lateral-Line Efferent Nerves.

Ten goldfish were used in these experiments and in no case could any relationship be drawn between either the opercular movement rate or the amplitude of movement, with efferent discharges recorded from Y and Z of the buccal branch of the facial nerve (Figure 6). The opercular movements were recorded on the upper trace of the oscilloscope, and the efferent discharges were simultaneously recorded on the lower trace (Figure 9a and 9b). The lack of relationship between opercular movement and efferent discharge held whether the fish was in the water or out of it.

Thus, it would seem that the efferents play no part in suppressing noise produced by the breathing movements. The neuromasts of the head must therefore record the water displacements associated with its own breathing movements. It is of course possible that this information is rejected at a more central level in the nervous system.

The Effect of the General Level of Muscular Activity, and the Intramuscular Injection of Tubercurarine Chloride on the Discharge Rate from the Lateral Line Organ Efferent Nerves.

Three goldfish were used in this experiment and the nerves Y and Z of the buccal branch of the facial nerve
Figure 9

A  The simultaneous recording of opercular movements, upper trace, and bipolar electrode recordings of action potentials in efferent fibers of nerve Y, when the fish was held out of water.

B  The simultaneous recordings of opercular movement, upper trace, and bipolar electrode recordings of action potentials in efferent fibers of nerve Y when the fish was submersed in water.
OPERCULAR MOVEMENTS
ACTION POTENTIALS

100 μV.
1 SEC.

100 μV.
0.2 SEC.

50 μV.
0.2 SEC.

50 μV.
1 SEC.

100 μV.
0.2 SEC.
The application of tubercurarine chloride intramuscularly to the opercular muscles of goldfish, and the changes in efferent activity associated with the changing muscular state of the fish when recorded from nerve Z (Figure 6).

<table>
<thead>
<tr>
<th>Time from beginning of experiment (minutes)</th>
<th>Duration and Type of Muscular Activity</th>
<th>Application of Tubercurarine</th>
<th>Impulses per Second</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Respiration</td>
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<td>19</td>
</tr>
<tr>
<td>1</td>
<td>&quot;</td>
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<td>6</td>
<td>&quot;</td>
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<td>14</td>
</tr>
<tr>
<td>7</td>
<td>Respiration Ceased</td>
<td>Application of Curarine</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
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<td>5</td>
</tr>
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</table>
Diagrammatic representation of the results presented in Table XII.

The application of tubercurarine chloride intramuscularly to the opercular muscles of goldfish, and the changes in efferent activity associated with the changing muscular state of the fish when recorded from nerve Z.

The activity levels of discharges recorded from nerve Z has been drawn in for normal conditions during regular respiratory movement, after the application of tubercurarine and subsequent cessation of muscular activity, and during violent muscular activity.
RESPIRATORY MOVEMENT
MOVEMENT CEASED
STRUGGLING MOVEMENT

TUBOCURINE CHLORIDE

TIME, MINUTES

IMPULSES PER SECOND

0 2 4 6 8 10 12 14 16 18

TIME, MINUTES
were disclosed for bipolar recording. The operculum was connected to the arm of the mechano-electric transducer by a thread, and the movement of the operculum, and the recordings of efferent activity from nerves Y and Z were displayed on the oscilloscope.

The opercular movements and the efferent activity from nerve Z were observed for six minutes in one particular preparation (Table XII and Graph M). The rate of discharge from the efferents was observed to vary between 15 and 25 impulses per second. At the end of 6 minutes tubercurarine chloride was injected intramuscularly into the opercular muscles at a dose rate of 0.1 mg. of tubercurarine per 100 gms. of fish (D.J. Randall and G. Shelton 1963) and one minute later the opercular movements were observed to cease. The efferent discharge rate fell to 7 impulses per second, and subsequently within three minutes, to three impulses per second.

Thirteen minutes after the start of the experiment, the fish began to show muscular activity. Although its respiratory movement appeared to be weak or non-existent, the fish raised its dorsal fin, extended its pelvic and pectoral fins and attempted to escape from the clamp. Simultaneously with the appearance of increased muscular movement the efferent activity rose from a discharge rate of 7 impulses per second to 25 and subsequently to a maximum of 31 impulses per second. This fell eventually to 24 per second. Unfortunately, due to violent struggling on the part
of the fish it broke loose from the clamp 18 minutes from
the beginning of the experiment and the experiment was
terminated.

In the other two experiments the efferent fibers
responded in the same manner to the application of curarine.
However, it was not possible to produce any muscular activity
from the animal after the curare injection.
DISCUSSION

That the discharge from the efferent fibers innervating the lateral line organs did not change in response to the opercular movements, indicated that either there was no direct central pathway between the respiratory centre and the lateral line nuclei, or if there was the time constant of the transfer of information from the respiratory centre to the lateral line nuclei was too slow for the lateral line nuclei to respond.

Tubercurarine chloride, when injected intraventricularly into the brains of cats produces acute convulsions, and when injected intravenously produces motor paralysis or neuromuscular block (Feldberg 1963). Tubercurarine chloride is a large molecule, and there is little exchange, if any, of this drug between the blood stream and the brain across the blood-brain barrier in cats. Feldberg points out, that if absorption of intraventricular tubercurarine were to occur, the ensuing neuromuscular block would mask the centrally induced convulsions. That this does not happen does not mean that no tubercurarine escapes the brain, but that amounts absorbed by the blood stream are too small to block motor endplates.

It is believed that the results observed in this experiment were not as a result of the action of tubercurarine on the fishes central nervous system, because the levels of tubercurarine used were so small. There was insufficient
tubercurarine injected intramuscularly to completely block all the motor end plates, and since it is believed that tubercurarine actively competes with the transmitter substance at the neuromuscular junction (Del Castillo and Katz 1957) then most of the intramuscularly injected tubercurarine would have bound itself to receptor sites on post synaptic motor end plate membranes, and little would have passed into the blood stream. If the quantity of tubercurarine injected intramuscularly had been very large, then any entering the blood stream and penetrating to the brain would have had no noticeable convulsive effect due to the paralysed state of the fish.

Increased muscular activity is a source of sensory input to the brain through the action of proprioceptors situated in the muscles. This increased afferent activity is also an indication of an increase in efferent activity, as the brain controls other proprioceptors and motor axons to antagonistic muscles. Associated with increased muscular movement is a requirement for increased sensory awareness. For example, in locomotion the speed of locomotion of the fish is partially dependent upon the fishes ability to rapidly orientate itself.

It seems feasible, therefore, to record a rise in efferent activity to the lateral line organs during increased muscular activity since lateral line organs are considered to be at least partially concerned with the orientation of the fish. Conversely it is not surprising to observe a decrease in efferent activity during muscular inactivation.
by the application of a paralyzing drug such as tubercurarine chloride.

The effect of the apparently total blanket inhibitory action of the efferents on the lateral line organs by the increased muscular activity cannot be discussed at this stage, but will be discussed later on in the light of evidence obtained from other fields.
HYDRODYNAMICAL INVESTIGATION

To Investigate Changes in Hydrodynamic Field Around the Heads of Stationary and Swimming Goldfish caused by Respiratory Movements of the Fish.

INTRODUCTION

Apart from being a possible source of noise to the fish, respiratory movements may also change the hydrodynamic field around the fish, also changing the stimulus field around the lateral line organs, on the head of the fish.

An investigation of the hydrodynamic field around the heads of swimming and stationary goldfish was proposed. It was intended to investigate any changes which may take place in the hydrodynamic field during respiration, and to see if there was any indication that fish could use these changes as an aid to directionally locating objects and the near field displacements of sound sources in the water surrounding the fish.
Figure 10

A Schematic diagram of the optics of the system used to observe birefringence in the Hector bentonite solution.

B The arrangement of the fluid polaroscope used in this experiment.

C The arrangement of the optical system used in the experiment.
VERTICAL CIRCULAR POLARIZATION

LIGHT SOURCE

POLARIZER

\[\frac{1}{4}\] WAVE PLATE

FLOW SECTION

\[\frac{1}{4}\] WAVE PLATE

ANALYZER

ASSUMED HORIZONTAL POLARIZATION

COMPONENTS DISPLACED

VERTICAL SUPPORT

CONDENSING LENS

OPTICAL AXIS

TWO QUARTER WAVE PLATE AND ANALYZER

TANK

POLARIZER AND FIRST QUARTER WAVE PLATE

COLLIMATING LENSES

HEAT AND COLOUR FILTERS TO PRODUCE SODIUM BAND ONLY

150 WATT VAPOUR LAMP

35 MM CAMERA WITH TELEPHOTO LENS LOADED WITH PLUS X FILM

VIBRATIONS IN DIRECTION OF PRINCIPAL STRESSES

INLET-12° LONG FLOW TANK CONTAINING 1% HECTORITE SOLUTION

GLASS FIBRE SCREENS

OUTLET

2 1/2" TYGON TUBING

PUMP

COOLING COIL

VARIABLE TRANSFORMER

B

C
Theory of the fluid Polaroscope

The theory for the fluid polaroscope is the same as that for photo-elastic stress analysis in the solid polariscope, in that stress distribution can be determined by finding the magnitude and direction of two principal stresses in a two dimensional model. This is done by passing polarised light through the solid two dimensional model and looking for two types of lines as they appear at the analysing screen. The isochromatic (a single colour, only when a monochromatic light source is used) gives the magnitude of the principal stresses, and the isoclinics give the inclination of the principal stresses.

The model must be constructed of a material which becomes birefringent when stressed. The polarised light when passing through the material is split into two components each of which vibrate in the direction of a principal stress, and each moves through the material at different speeds. In the fluid polaroscope Hector Bentonite is the optically active material.

In the system set out below (Figure 10a), quarter wave plates were used to eliminate the isoclinics so that the isochromatic lines could be more easily observed.

The quarter wave plates split light entering them into two components vibrating at 90° to one another and leaving the quarter wave plates 90° out of phase. This
produces a combined continually rotating, or circularly polarised, light.

When the circularly polarised light passes through the flow section no isoclinics will be formed because the light is none directional, and, therefore, no light will be passed straight through and all the light reaching the second quarter wave plate will have components in the direction of the principal stresses.

The light leaving the second quarter wave plate and entering the analyser is again in two components, 90° to each other and out of phase, these will interact with each other if they are out of phase and produce isochromatic lines. The degree to which the components are out of phase depends upon the amount of birefringence exhibited by the solution, and this depends on the size of the principal stresses within it.

In a fluid the principal stresses set up are the effective sheer stress due to velocity differentials across the direction of flow.

**Apparatus**

The Preparation of the Birefringent Solution. Hector Bentonite.

The preparation of the solution was followed from directions given by the Baroid Division of the National Lead Company who are the manufacturers of Hector Bentonite (McPherson and Nece 1950).

Hectorite is found with 50% calcite and dolomite
as a montmorillomite clay, and the calcite and dolomite must be removed from the hectorite before use.

To remove the calcite and dolomite the clay was dispersed at (3%) in water containing 0.01% tetra sodium pyrophosphate with a high speed stirrer in a large glass tank for about two hours. This was then allowed to stand over night until all of the calcite had settled out. The supernatent suspension was then decanted off and centrifuged to remove particles coarser than approximately 0.5 microns. The solid content in the solution was adjusted to 1% before use.

The Polaroscope

The polaroscope used in this experiment was a long, shallow plexiglass tank (Figure 10b) through which hectorite solution was circulated by an electrically driven, variable speed, rotary pump. At each end of the tank a series of fine glass fiber meshes were placed in a staggered assembly, i.e. different distances separating each mesh screen, to break up turbulent flow as the water entered and left the tank.

In one set of the experiments the optical axis of the polaroscope (Figure 10c) was set so that it was in a vertical plane through the tank, and in the other set of experiments it was arranged to be in a horizontal axis through the walls of the tank.
Experimental Procedure

The fish was placed in the tank and the water velocity through the tank increased until the fish had to swim strongly to maintain position in the tank. The camera was then focused on the fish. The lighting in the tank however was poor, and a compromise was made between the aperture size and the shutter timing which was not optimal.

In another experiment the flow was reduced to zero, and hydrodynamic field around the fish was observed under stationary conditions.
RESULTS

The hydrodynamic field about the heads of seven goldfish 3 - 4" long was studied in both side and top elevations.

The opercular movements of stationary fish, during respiration caused an area of birefringence to be formed in the vicinity of the operculum and this was considered to be caused by the displacement of water. Under close scrutiny, it could be seen that during elevation of the operculum, water was both pushed away (damming effect) and pushed forwards (shearing effect) along the surface of the operculum. Unfortunately no successful photographs were taken of this motion due to the extremely poor lighting conditions. During depression of the operculum water was drawn back in a vortex caused by this movement.

In the successive series of experiments the goldfish were made to swim against a current. The swimming movements of the fish caused vortices to be generated along their bodies. However, the vortices were never noticed to extend farther forwards than the trailing edge of the opercular (Figure 11a).

When the fish swam with their mouths shut, a bow wave was generated about their heads (Figure 11b) however this was seen to be reduced when the fish swam with their mouths open (Figure 11c) as indicated by the decreased area of birefringence surrounding the head of the fish. Also at the commencement of the mouth open phase of respiration a small area of birefringence could be detected ahead of the
Figure 11

Drawings made from photographs taken of fish swimming through the Hector bentonite solution.

A Side view of goldfish showing lack of turbulence ahead of the posterior border of the operculum during swimming.

B Enlarged dorsal view of the head of a goldfish swimming with its mouth closed.

C Enlarged dorsal view of the head of a goldfish swimming with its mouth open. An area of negative pressure has been stippled ahead of the fish.
fish. This was thought to indicate the negative pressure caused by inhalation during respiration.
DISCUSSION

The shearing effect caused by the movement of water along the moving operculum of a stationary fish could be considered as a source of noise but respirator movements in resting goldfish are usually quite slow, and it is unlikely that this would reduce the sensitivity of the lateral line organs of the fish to a great extent.

From another point of view the fish could detect changes in the hydrodynamic field caused by the opercular movements as a means to detect stationary objects close to the fish.

Swimming fish alternate between two types of effective flow shapes during their respiratory sequence; namely between bluff bodies and streamlined bodies. Fish physically resemble bluff bodies when their mouths are shut, pushing water ahead of them and setting up a region of positive pressure in the water ahead of them. With their mouths open, and inhaling water and expelling it again from points under the trailing edges of the opercular, fish behave as streamlined bodies. This is because water ahead of the fish which is not able to escape rapidly enough over the surface of the fish during swimming is sucked away by the respiratory pump, thus, avoiding any build up of positive pressure, and increased water resistance ahead of the fish.

One consequence of this streamlining is that fish swimming with their mouths open require less energy for
locomotion than fish with their mouths closed. Fast swimming fish such as the mackerel and the tuna are known to continually swim with their mouths open.

With their mouths closed fish could detect the positions of stationary or vibrating objects ahead of them by measuring changes in configuration of the bow wave caused by displacements set up by the vibrating object, on the one hand, or increased resistance to motion of the fish by virtue of the presence of the rod, on the other. This possibility has already been mentioned by Dijgraaf (1963) and Walters (1966) but they did not mention that this bow wave changed configuration continuously during respiration.

It is also quite possible that the lateral line organs would be potentially more receptive to displacement when the fish have their mouths closed. This is because a large velocity gradient exists across the bow wave so that the velocity of the water layer adjacent to the fishes surface would be close to zero when measured with reference to the fishes velocity. Thus, the lateral line organ cupulae would not be deflected by water displaced by the fishes swimming motion, and so, potentially would be capable of responding to greater water displacement changes than cupulae already displaced.

The water layer adjacent to the surface of the streamlined fish, because of the large velocity gradient of this layer, would be travelling at a velocity similar to that of the fishes swimming velocity when measured with
respect to the fish. Lateral line organs on the fishes head under these conditions would then be deflected an amount proportional to the velocity of swimming. It seems quite possible, therefore, that during the respiratory cycle a fish may alternately detect displacements in the water around it, and measure its own swimming velocity.

The vortices generated down the side of the fishes trunk could be used by the fish for estimating its swimming speed with respect to the water surrounding it. The vortices would cause the lateral line organs to be deflected as they passed down the body, and the quicker the fish swims the faster the vortices would roll down the side of the fish.

Kuiper (1966) gave a theory for the generation of vortices, his main conclusions were that vortices were formed during swimming motion (he had not seen, or heard of evidence, of their formation) and that they always arise from the same point on the fish during swimming, and that they accompanied the fish as it swam. Rosen (1966) supported Kuiper's theory by explaining that he had taken photographs of a fish swimming across a water-milk interphase, and that he had noticed the formation of vortices on the milk surface which accompanied the fish as he swam. He did not produce his photographs to support his statement.

Kuiper's theory and Rosen's statements are not in agreement with Newton's Law of action and reaction, and my findings do not agree with Rosen's. The vortices generated by swimming movements in goldfish are left behind by the
goldfish, since they do, after all, represent water displace­ments, and there is no evidence to show that they are generated from any particular points on the surface of the fish.
GENERAL DISCUSSION

Pumphrey (1950) clearly defined what he considered hearing, the act of hearing, and a sound source to be:-

"The primitive function of hearing is the location of moving objects not in contact with the animal. An animal hears when it behaves as if it has located a moving object, a sound source, not in contact with it. Sound can be defined as any mechanical disturbance which is potentially referable to an external and localised source".

Bearing Pumphrey's definitions in mind, it appears that the fish in the behaviour experiments described previously were responding to sound sources, were localising them, and consequently hearing them. Pumphrey did not state what reference points he was considering when he defined that an animal hears when it locates a moving object, but presumably he was considering movement of the object with respect to the animal. In which case the fish in these experiments can be said to be detecting the position of the stationary rod by responding to acoustical stimulation brought about by the interaction of the fishes bow wave with the rod, and avoiding it.

The behaviour experiments indicated that the lateral line organs were directionally sensitive to the vibrating rod, and to displacements resulting from interaction of the fish's bow wave with the stationary rods. The point of view that the fish's lateral line organs are both directionally
and displacement sensitive has been previously put forward in theoretical terms by Bergeijk (1963), and from experimental evidence derived from investigations of the lateral line microphonics of Fundulas by Harris and Bergeijk (1962). Considering Harris and Bergeijk's results, Pumphrey's definitions and the behaviour experiments presented here, it is concluded that the lateral line organs are implicated as organs of short range hearing.

Dijkgraaf (1963) is strongly opposed to the view that the lateral line functions as a short range hearing system. He considers that fish do not use their lateral line systems for locating or even detecting sound sources. In 1935, Von Frisch and Dijkgraaf made the observation, from experiments upon minnows, that their behaviour contradicted the idea of sound as a directional stimulus, and although fish heard very well, location of the sound source with the aid of propagated waves seemed beyond their capabilities. Von Frisch and Stetter (1932) used blinded Phoxinus and conditioned these animals to feed to the sounding of a tuning fork. They then, bilaterally, removed the sacculus and lagena from these fish and demonstrated that the fish failed to respond to frequencies above 150 cps., but still responded to frequencies below this. Elimination of the lateral line organs still did not disturb the responses of the fish.
Parker (1902) used unconditioned Fundulus in his experiments, which clearly reacted to tuning forks of 100-128 cps. respectively. Complete elimination of the lateral line organs did not diminish the sensitivity. But when both the labyrinths were removed, or the eighth nerve had been cut bilaterally, the reactions were abolished, although the lateral lines were left intact.

These experiments and several others quoted by Dijkgraaf failed to recognise the division of sound energy into two forms, the near field displacement effect and the far field pressure effect. There is indication that Von Frisch and Dijkgraaf (1935) were unaware of the presence of near field displacements associated with a sound source. Also their concept of sound being a directional stimulus is fallacious, because at the time that remark was made, they considered sound to consist of propagated pressure waves, which are not vector quantities.

The same comments may also be levelled at Parkes (1902) experiments and Frisch and Stetter (1932). Neither group indicated if the response they elicited from the fish by sounding a tuning fork was directional. Since it is considered that one of the criteria of sound perception is directionality, Frisch and Stetter's and Parkes' experiments must remain inconclusive.

Thus the evidence presented by Dijkgraaf to support his argument against the lateral line organs being sensitive
to low frequency sound is thought to be inconclusive from one point of view, namely, that the acoustic evidence upon which it is based is ill defined.

Another section of Dijkgraaf's (1963) review recognises the significance of the near field effect produced by a sound source, but does not consider the perception of it to constitute hearing. He believes that the lateral line organs are current detectors responding to amplitude, direction and extent of water displacements applied as spatial and temporal patterns to the surface of the animal. He thus does not consider the need to designate the lateral line system as a vibration receptor or even as a short range auditory receptor (Pumphrey 1950) since the adequate stimuli are neither essentially vibratory nor do they include sound in the normal meaning of propagated, rhythmically, repeated pressure waves.

In the first place, there is neurophysiological evidence available to show that both current stimulation (Sand 1937) and vibrational stimulation (Katsuki 1951, 1952) are adequate stimuli to the lateral line organs. There is however, no evidence available at present to indicate that either of these stimuli are biologically adequate. The hydrodynamic experiments indicated that the surfaces of the fish may only rarely be exposed to direct water currents. The water, displaced as a result of locomotion, down the sides of the goldfish in these experiments was in the form of vortices. Even respiratory currents, caused by the
pumping of water from the buccal cavity of the fish caused vortices or turbulence. The effect of the passage of vortices or turbulent water passed a lateral line organ would be to oscillate it irregularly and at low frequency.

Dijkgraaf's concept of the normal meaning of sound as being propagated, rhythmically, repeated pressure waves is an over-simplification. Sound is rarely rhythmic, and under Dijkgraaf's concept, speed and explosive sounds, for example, would be outside the normal meaning of sound.

Secondly his concept of sound as being propagated pressure waves is subjective. Terrestrial animals, in particular, may use the properties of the far field pressure waves to detect and localise a sound source, but this is no justification for considering an organ which responds to the near field displacements as not being an organ of hearing.

It is concluded that arguments which have been presented to exclude the lateral line organs as organs of hearing are basically unacceptable on the grounds that they fail to recognise the near field effect as an acoustical stimulus. On these grounds it is thought that there are, at present, no justifiable reasons for not accepting the lateral line organs as organs of short range hearing.

The acoustic field to which the lateral line organs respond displays greater contrast than the field to which the ears respond. This is because the displacements of the near field effect of sound sources attenuate very rapidly
with distance from the sound source, unlike the self propagating pressure waves of the far field. This discreetness of the near field would tend to reduce the chance of interaction between the near fields of adjacent sound sources, and thus presents a sharper more contrasting field to the lateral line organs, than the acoustic field of the associated pressure waves would do to the ears.

Another feature which contributes to simplifying interpretation of the sensory field of the lateral line organs is the definite polarization of the displacements. The lateral line organs have a polarised sensitivity to water displacements, which makes them sensitive only to displacements along one axis. This gives fish a facility for analysing the direction of these displacements without the necessity of resorting to complicated central mechanisms, a point which has previously been made.

Thus fish would be able, if they used this facility to reduce the displacements in the acoustic field of their lateral line systems into directional components. There is behavioural evidence, which has been previously discussed, to illustrate the ability of fish to differentiate between vertical and horizontal displacements.

Although the acoustic field of the displacement effects from sound sources presents a contrasting stimulus to the lateral line organs, the problem of background noise is omnipresent. Large amorphous displacements from water currents, surface waves, and general turbulence contribute to the major source of noise to the sensory field of the
lateral line organs and, it is considered necessary to propose the existence of a mechanism which suppresses this effect. Behavioural evidence is available to demonstrate that fish are able to detect displacements from a sound source against displacements of greater magnitude contributed by background noise.

The cochlear and the lateral line system are both confronted with a common acoustical problem, namely the selection of specific sounds from a background of noise. Both the lateral line, from results obtained in the previously described behaviour experiments, and the ears (Bekesy 1960) appear to be capable of overcoming this problem. A brief examination of the method in which mammals overcome the noise problems may, therefore, throw some light on the possible mechanisms used by fish.

Bekesy's proposal was that the nervous system possessed an ability to funnel particular bits of information while rejecting or attenuating other information. His arguments, when applied to the auditory system produced a concept of the acoustic field as one where a particular sound, the one the observer wished to hear was centralised in the field, and the effect of surrounding acoustic stimuli was attenuated. This concept of the acoustic field is analogous with the visual field perceived by the optic system.
Pfalz (1962) drew support for Bekesy's funneling theory from neurophysiological experiments he performed on mammalian auditory systems. He found the discharge in secondary neurones in the cochlear nuclei to be maximal at an optimum frequency, but the neurones were found not to respond to other frequency ranges. Pfalz realised that his evidence in collaboration with experimental evidence obtained by Fex (1962), from investigations performed on the mammalian olivo-cochlear bundle, indicated the existence of a mechanism for sharpening sensation. In this case it was loudness. There is evidence to indicate that a mechanism for neural funneling exists even in the lowest levels of the auditory system.

If a background noise attenuating mechanism operating along similar lines to the olivo-cochlear bundle serves the lateral line organs of fishes then it is expected that such a mechanism would share some of the fundamental characteristics of the olivo-cochlear bundle. Peripheral evidence of the existence of the olivo-cochlear bundle is indicated by the efferent inhibitory innervation of the cochlear hair cells which is sensitive to acoustic stimulation of both the ipsilateral and contralateral ears.

Results of neurophysiological investigations of the innervation of the anterior lateral line system in the goldfish demonstrated the existence of efferent innervation to single organs. This innervation, whilst being inhibitory,
could not be shown to be acoustically sensitive. The interaction between afferent and efferent nerves innervating a single neuromast however, was not investigated. If the activities of the afferent and efferent fibers innervating a single lateral line organ can be shown to be interdependent, and that this interdependence can be shown to be the most peripheral part of a noise attenuating system, then it must be assumed that if such an attenuating system existed the most peripheral part of the mechanism is operative on the activities of second order lateral line neurones B (Figure 12a). This criterium has been put forward since it is assumed that by virtue of its nature, a noise attenuating system must be acoustically sensitive. However, efferent fibers do not appear to be acoustically sensitive, and since these are proposed, as a minimum, hypothesis, to be operative at first order neurones A (Figure 12a), the attenuating system is proposed to operate at higher sensory levels than these neurones. On the other hand it is possible that the activity of the efferents in fishes are dependent of the afferent activity, in which case a noise attenuating system could be operative at first order neurones. The first possibility of a noise attenuating system E(Figure 12a) acting at second order neurones has been shown.

An attenuating system at this level, if it followed the pattern of action of the olivo-cochlear bundle, would
consist of inhibitory neurones E which would attenuate activity resulting from noise in second order neurones B. The activity in E would of necessity be sensitive to acoustic stimulation, and the reception of this would be through afferent activity from other neuromasts. However, the detection of noise by the lateral line organs is dependent upon the orientation of the organ. The displacements caused by noise have components in all planes, and these components will have different values from each other. Thus the acoustic information to E would have to be contributed by a population of lateral line organs orientated in the same plane as the lateral line organs under consideration.

Although the efferent anterior lateral line nerves were not demonstrated to be acoustically sensitive, they were found to be sensitive to general muscular activity in the body. The significance of this dependence is not at present fully realised, and it was suggested earlier that the dependence may be correlated with an increased response rate of the fish to stimulation by water displacements. However, a consideration of the central connections of the anterior lateral line nerves has lead to a suggestion for the role of the efferents.

The anterior lateral line nerve enters the brain and divides into dorsal and ventral roots, and the ventral root especially becomes closely associated with the vestibular nerve (Kappers, Hubers and Crosby 1955). While
Figure 12

A A model constructed to show the arrangement of the proposed noise attenuating system acting upon second order lateral line neurones in the medulla of a goldfish.
A is a first order lateral line neurone.
B is a second order lateral line neurone.
C is an inhibitory efferent neurone.
D is a motor centre neurone.
E is a noise attenuating inhibitory neurone.

B A model illustrating the possible role of the lateral line system as a part of a mechanism for the reflex control of swimming.
N is a lateral line organ
M is a Mauthner neurone innervating a motor neurone.
T is a trunk myotome on the contralateral side of the tail of the fish.
E is an inhibitory efferent neurone innervated by a proprioceptive neurone.
the smaller dorsal roots terminate mainly in the cerebellum
and posterior lateral line lobes of both the ipsilateral
and contralateral sides of the brain, many of the ventral
root fibers terminate in the anterior lateral line lobe,
and some thick fibers closely accompany the vestibular nerve.
These terminate together in the region of the nucleus
tangentialis and the Mauthner Neurones. Their thickness
and direct connection with the motor tegmentum of the
medulla oblongata lead Kapper, Hubers and Crosby to suggest
that centrally the ventral lateral line roots were concerned
with the establishment of reflex pathways to motor centres.

Stephanelli (1951) presented evidence which
collaborated with the anatomical evidence of Kapper, Hubers
and Crosby. He showed in Xenopus, during a study of
metamorphosis, that the Mauthner apparatus can only exist
when both the sensory systems of the lateral line and the
motor system of the tail were present. The absence or slight
development of only one of these systems was found to be
sufficient to allow involution or none differentiation of
the Mauthner Neurones.

He concluded also that a difference in the rate of
differentiation shown by the Mauthner apparatus in teleosts,
urodeles, and anurans was a further proof that its function
was related to the activity of swimming by movements of the
tail and was directly connected with the lateral line so
as to establish a reflex activity involving the organ.
The discovery of the sensitivity of the efferent anterior lateral line fibers to muscular activity and the collaborating evidence described above has led to the formulation of a model (Figure 12b).

The Mauthner neurones are two conspicuous neurones in the medulla oblongata which receive their input principally from motor and vestibular centres. They innervate the motor neurones controlling contraction of contralateral trunk myotomes by means of a giant axon which courses down the contralateral side of the spinal cord. Stimulation of the vestibular XIII nerve on one side of the fish leads to an increase in the excitability of the ipsilateral Mauthner neurones, inhibition of the contralateral Mauthner neurone and contraction of the contralateral trunk myotomes (Retzlaff 1954) (Furukawa and Fusshpan 1963). Because the anterior, ventral, lateral line root fibers are in close association with the vestibular nerves, they have been assumed to innervate the ipsilateral Mauthner neurones in the same manner.

In the model (Figure 12b) stimulation of the lateral line organ N leads to an increased activity in the Mauthner Cell, M, and an increased rate of contraction of the trunk myotome muscles T. This model, although not shown, is considered to exist on both sides of the fish, so that increased activity in the anterior lateral line organs would result in an increased rate of swimming. This system by itself constitutes a positive feedback system, since \( \text{...} \)
as the fish swims so the lateral line becomes stimulated by water displaced down the sides of its body, and this, in turn raises the activity of the Mauthner neurones. Consequently the trunk myotomes contract more strongly and the fish swims faster.

The inhibitory efferent fiber E is shown in the model (Figure 12b) to be innervated by proprioceptors in the trunk myotomes. Only one intercalated neurone is shown, but this is to be taken as the minimum hypothesis since no doubt if this feedback system does exist, it would be more complex than this. Contraction of the contralateral trunk myotomes lead to inhibition of the ipsilateral anterior lateral line organs. The stronger the contraction of the muscles, the greater the inhibition.

These two systems, the one tending to increase the activity of the trunk myotomes, and the other suppressing the activity of the anterior lateral line organs, if they acted together, would lead to a regulation of swimming velocity.

There is some behavioural evidence (Dijkgraaf 1963) to support the idea that lateral line organs do play a part, under certain conditions, in regulating swimming velocity.

Dijkgraaf observed that minnows deprived of their lateral line organs had difficulty in maintaining regulated swimming velocity, but were quite able to maintain their positions in large applied water currents where, he believed, tactile sensation and vision were responsible. He also noticed that minnows kept in an aquarium would swim against
the small current produced by water streaming into the tank through the inlet pipe, however if deprived of their lateral line organs the minnows failed to make this response. From this evidence he concluded that fish use their lateral line organs when orientating in irregular currents, and that they also contribute in some way to the regulation of swimming velocity.

Thus, it is concluded that experimental evidence obtained for the role of the efferent nervous supply to the anterior lateral line organs suggests a motor reflex role for the efferents rather than as part of a noise attenuating mechanism.
SUMMARY

1. On theoretical grounds the lateral line organs were implicated as the acoustically sensitive system which might be used by fish if they were capable of directionally hearing low frequency sound. It was considered that fish might possibly locate sound sources by detecting the near field displacements associated with them.

2. Both blind goldfish and blind cave fish demonstrated an ability to use their lateral line organs to detect water displacements associated with, and hence detect, low frequency sound sources and stationary rods.

3. Blind cave fish were able to locate a sound source against a background noise of constant frequency and amplitude. A physiological noise suppressing mechanism, similar to that found in the mammalian cochlear was suggested to be associated with the lateral line system.

4. An efferent nervous supply was found to innervate the anterior lateral line organs of the goldfish. By the application of a convulsant drug, strychnine sulphate, to the lateral line organs, it was possible to demonstrate the inhibitory nature of these efferent fibers.

5. The discharge rate of the efferent fibers was found to be unaffected by stimulation of other lateral line organs, touch receptors or ears, and to be insensitive to respiratory movements. It was shown, however, to respond to general increased body muscular
activity by an increase in discharge rate.

6. The hydrodynamic field about the heads of swimming goldfish were investigated. The fish were observed to alternate between two flow shapes during respiration. They formed bluff bodies when they had their mouths closed and generated a bow wave, and became streamlined when their mouths were open. This changing configuration lead to the proposal that the anterior lateral line organs functioned both as velocity detectors and near field displacement detectors.

7. It was concluded, firstly, from evidence presented here, and recent collaborating evidence that lateral line organs are a short range hearing system. Secondly, it was thought that if a neurophysiologically active noise suppressing mechanism was associated with the lateral line system, it was confined centrally to the first order lateral line neurones. Finally, it was suggested that fibers might be part of a system controlling muscular movement, and involving Mauthner neurones. A model was proposed.
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