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BRIGHTNESS DISCRIMINATION IN LARVAE OF PLAICE AND SOLE

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INTRODUCTION

The ability to discriminate changes of brightness should enable a fish to monitor its depth within certain limits in water where there is attenuation of illumination with depth. It should also enable a fish to swim along a 'compass' course using the sun as a reference point provided the fish is sufficiently near the surface for the azimuth of the sun to be detectable (Harden Jones, 1968; Blaxter, 1970). The effectiveness of holding a depth, or remaining within a light preferendum at dusk and dawn, or of holding a compass course, will depend on the sensitivity of the organism to small variations of brightness on either side of the appropriate reference level or direction, vertically or horizontally. With vertical movements the organism has to 'remember' the reference level; with horizontal movements a simultaneous comparison of brightness in different directions is possible.

To date, brightness discrimination in adult fish has been measured by operant-conditioning techniques where an animal is trained to select the brighter (or dimmer) of two backgrounds (Perkins & Wheeler, 1931; Sgonina, 1933), by phototaxis given a choice of sources (Loukashkin & Grant, 1965), by cardiac conditioning (Hester, 1968) and by electroretinogram techniques (Kobayashi, 1962; Protasov, 1964). No work has been done on larval fish, many species of which have incompletely developed eyes with a pure-cone retina until they metamorphose some weeks or months after hatching (Blaxter & Staines, 1970). Although it is unlikely that young larvae have the swimming powers to make effective horizontal sun-compass movements, some species, especially clupeids, are known to change their vertical distribution by day and night (Russell, 1926, 1928; Bridger, 1958; Stevenson, 1962; Ryland, 1964).

The techniques of Blaxter (1968, 1969) for measuring visual thresholds and spectral sensitivity of teleost larvae were adapted for brightness-discrimination experiments, and the results are described in this paper. The principle of the experiments was to present the larvae with two horizontally opposed light sources, using the phototactic response to see how different the two sources had to be in intensity before the larvae selected one or other.

METHODS

The larvae were hatched from eggs and kept in the same way as described in earlier experiments (Blaxter, 1968, 1969). The species used were plaice *Pleuronectes platessa* L. and sole, *Solea solea* (L.). For the experiments the larvae were placed in

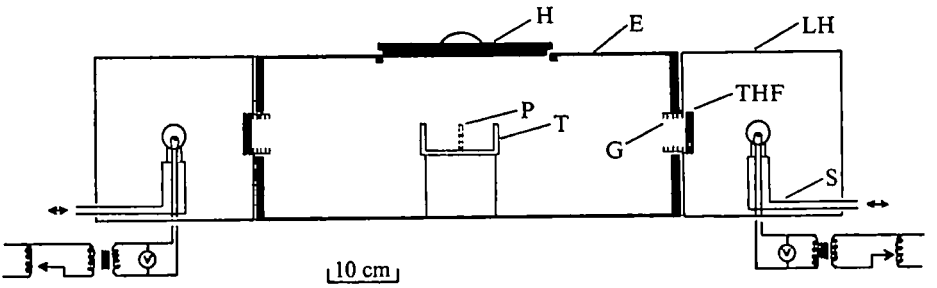

Fig. 1. Diagram of apparatus from the side. E, Experimental box; G, grooves for filters; H, hatch; LH, light housing; P, partition; S, slide for light sources; T, trough; THF, translucent and heat filters.

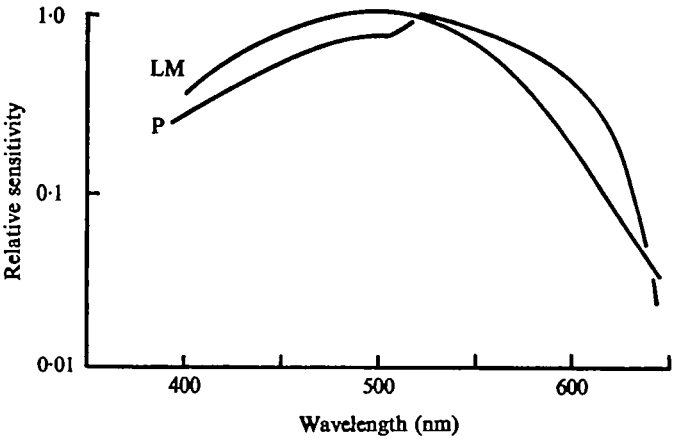

Fig. 2. Graph showing relative sensitivity of the light meter (LM) and the spectral sensitivity of plaice larvae (P) (the larvae were dark-adapted, but since they have a pure-cone retina this is a photopic spectral sensitivity curve).

small clear perspex troughs measuring 10 × 9 × 4 cm high with a centre removable partition. Usually a stock of eight troughs was used one at a time, each containing 30 larvae; after an experiment they were kept in the dark for at least 30 min before being used again. The apparatus (Fig. 1) consisted of an oblong light-proof box with a hatch at the top and a square hole at each end into which two light housings fitted. These housings were light-proof, equipped with slots for filters, and each contained a 36 W, 12 V car headlamp bulb in a socket which could slide within the housing. The bulbs were matched for intensity and colour temperature using an EEL Microphotometer and Colour Temperature Meter. The microphotometer (see Fig. 2) had maximum sensitivity between 450 and 550 nm and was calibrated in metre-candles (1 m-candle in these experiments is approximately equivalent to 0·5 μW/cm^2). The light meter had a spectral response similar to the spectral sensitivity of the larval eye (presumably the photopic curve as the eye is composed only of cones) so that only light 'useful' to the larvae was being measured.

Before an experimental run a reference level was chosen by placing neutral-density filters of equal value in the slots of the two light housings and checking tha

the intensity at the centre of the experimental box was the same in both directions. Small adjustments could be made by sliding the bulbs within their housings. All experiments were carried out in a temperature-controlled dark room. The larvae were held in their troughs for at least 30 min in the dark before use. A trough, without its centre partition, was then placed in the experimental box, the hatch was replaced and the larvae were left for 2 min. After this time the hatch was removed and the centre perspex partition was carefully and slowly inserted into the trough to separate the larvae in the left- and right-hand sides. There was no evidence that water movements caused by the partition influenced the distribution of larvae, and only rarely was a larva trapped under the partition. The larvae could be counted in each half under a dim light and the partition was then removed and the trough and larvae were retained for a later experiment. The score for the experiment was the net number of larvae photopositive (at the brighter end) or photonegative (at the dimmer end). After observing the distribution with equal intensity lights the light at one end was reduced or increased by changing the neutral-density filters or the position of the bulb and the larval distribution was observed using another trough. The minimum change in intensity between experiments was always more than the difference in light-intensity levels at the centre and end of the trough. The need for this put a constraint on the length of the troughs.

Most experiments were carried out with plaice larvae of different ages. The sole larvae were found to be much less responsive to light and in the early post-hatching period they have no functional eyes.

RESULTS

Control experiments, where the two light sources are equal in intensity or where both are switched off, give a measure of the random distribution of the larvae and should approximate to zero. In 50 experiments, each with 30 plaice using equal intensities for the two lights the net score was $-0{\cdot}22$ (S.E. 0·64) and in eight further experiments with the both lights switched off the net score was $+0{\cdot}62$ (S.E. 1·85). In the case of equal stimulation the sign is conventional indicating the larvae were to the right or left of the trough. Neither mean is significantly different from zero. One immediate finding, confirming earlier experiments (Blaxter, 1968, 1969), was that the larvae of both species tended to be photopositive at high levels, and photonegative at low levels, of illumination. In Fig. 3A examples are given of an experiment on plaice at a high and a low level of illumination, clearly demonstrating this effect. Because of the change in sign of the phototaxis, experiments on brightness discrimination at some intermediate light levels (Fig. 3B) were inconclusive, the larvae showing no clear-cut response to light. To arrive at a threshold of brightness discrimination a profile of the points was drawn for experiments at each reference level where there was a clear response to light (e.g. see Fig. 3A) and the threshold was taken as the intensity difference where the profile met the horizontal line demarking the 80% confidence limits for the controls.

The threshold increment discriminated (ΔI) can be expressed as a percentage of the reference level (I), the fraction $\Delta I/I$ usually being known as the Weber fraction, often expressed as a percentage. In the present experiments I was taken as the intensity of the dimmer of the two lights. For example, if the larvae showed a dis-

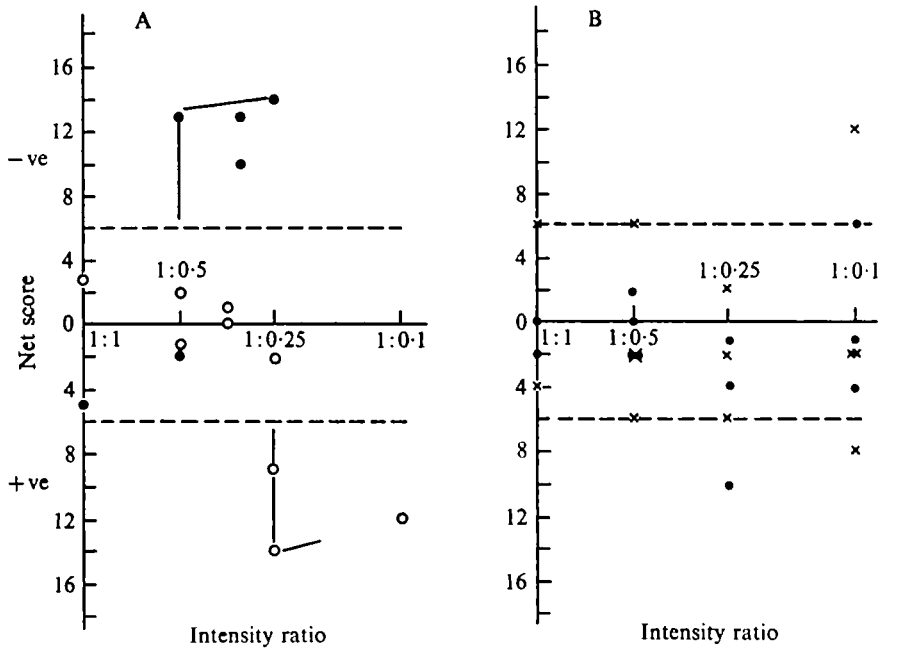

Fig. 3. Graphs showing the net scores (the net number of plaice larvae moving to the brighter light +ve, or dimmer light −ve) plotted against the difference in intensity between the light sources (shown here as a ratio − 1 : 1, they were equal in intensity; 1 : 0·5, the brighter is twice the intensity of the dimmer source; 1 : 0·25, it is four times brighter; 1 : 0·1, it is ten times brighter). In A scores are shown where the reference levels (the brighter light) were 10 m-candles (○) and 0·001 m-candles (●) respectively. Note the strong positive and negative responses. In B the reference levels are 0·1 (●) and 0·01 (×) m-candles, and here the responses are weak and no threshold is obtainable. The horizontal lines show the 80% confidence limits for scores with the two light sources equal in intensity.

tribution significantly different from random when the intensity of one light was 1·0 units and the other 0·25 units, a ratio of 1 : 0·25, then

$$\Delta I/I = \frac{0{\cdot}75}{0{\cdot}25} \times 100 = 300\%.$$

(If the Weber fraction is taken as a percentage of the brighter light, it can never exceed 100%.) Plots of the Weber fraction for different reference levels are given for different species and ages in Fig. 4A, B. The dashed lines show where no threshold was obtainable due to a change in sign of the phototaxis. In general, Weber's Law, that the increment of intensity discriminated is independent of the reference intensity, is shown to be valid for the larvae, as indicated by the horizontal nature of the relationships. The Weber fraction appears to be between 60 and 200%, showing that larvae can discriminate the intensity of a light from a reference light, if it is 60 to 200% brighter.

The lack of results in the middle range are probably due to a behavioural 'accident' because of the change in phototactic sign. It seems unlikely that the larvae are unable to discriminate different intensities at these intermediate reference levels.

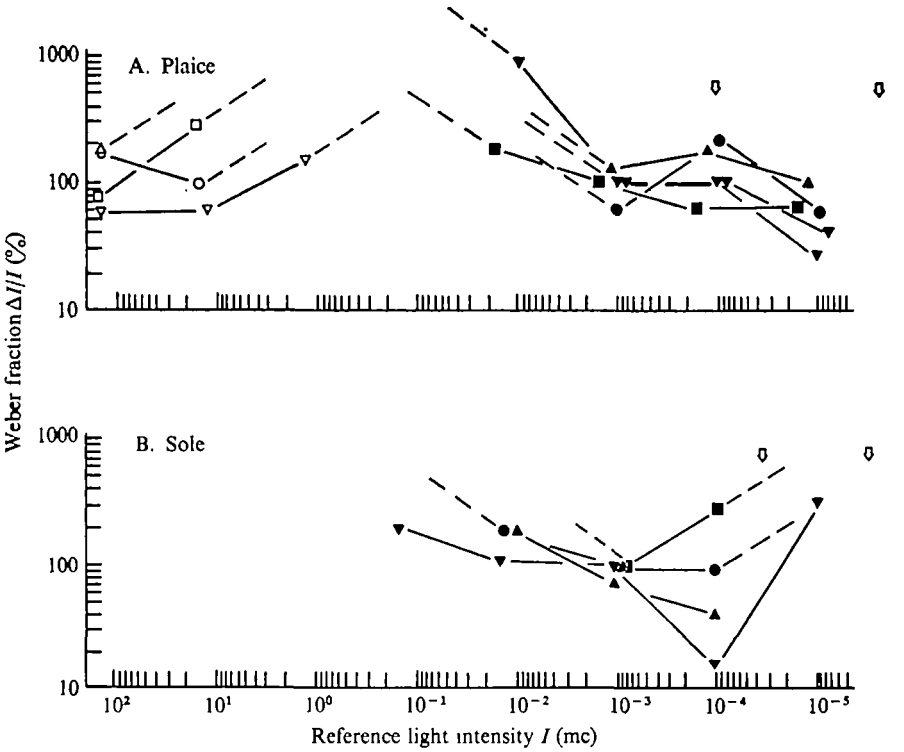

Fig. 4. Graphs showing the intensity discriminated, ΔI, expressed as a percentage of the intensity I, the dimmer of the two sources. This is the Weber fraction, $\Delta I/I \times 100$. A, Plaice larvae: △, 1 day old; □, 6 days old; ▽, 14 days old; ○, 30 days old. The open symbols denote a photopositive response, the filled circles a photonegative response. B, Sole larvae; there was no photopositive response; the symbols for the photonegative response denote: ▲, 19 days old; ■, 21 days old; ▼, 26 days old; ●, 32 days old. The results for the 2 years work are pooled. The vertical arrows denote the range of thresholds for vision from Blaxter (1969).

DISCUSSION

The Weber fractions obtained for older fish by other workers vary widely depending on the species, level of adaptation to light and on the technique employed. The ERG method gives values below 1% for capelin *Mallotus villosus* and the elasmobranch *Holorhinus tobijei* (see Protasov, 1964; Kobayashi, 1962), while Muntz & Northmore (1970) reported 7 to 8% for the rudd *Scardinius erythrophthalmus*. Weber fractions between 10 and 100% are much more common using both the ERG and training techniques (Perkins & Wheeler, 1931; Sgonina, 1933; Loukashkin & Grant, 1965). Some values, even of 320 to 1400%, were found by Kobayashi (1962). In particular, Hester's (1968) experiments showed how brightness discrimination varied with the size of the fish, position of the 'target' on the retina and the water temperature.

How far can results obtained on larvae be extrapolated to conditions in the sea? Strictly, the experiments deal with simultaneous comparison of light intensity which might be used by organisms to detect the azimuth of the sun. The distribution of light intensity in various places below the surface can be shown by vector diagrams as in Fig. 5. The vector diagrams tend to be most asymmetrical near the surface and when they are in the plane of the sun rays after refraction at the water surface.

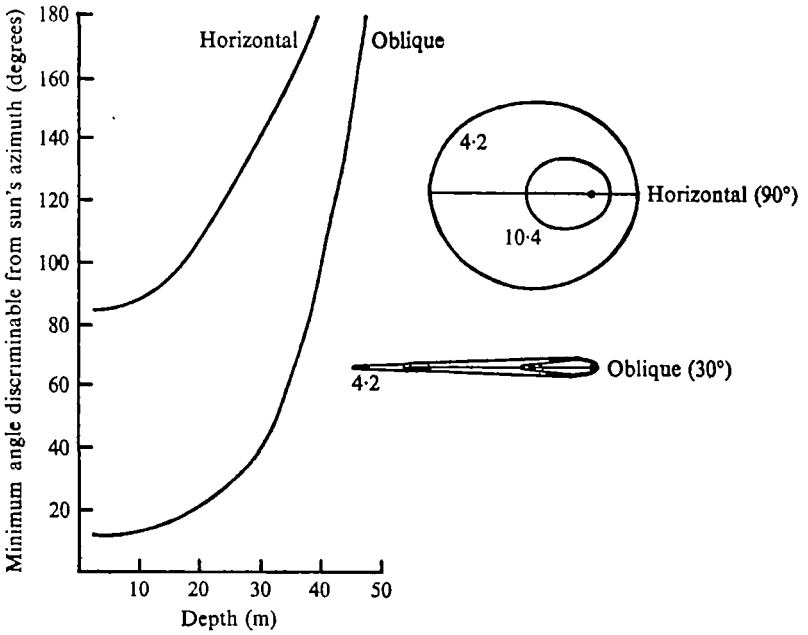

Fig. 5. Graph showing the minimum angle (from the sun's azimuth) which might be discriminated by fish larvae at different depths using a horizontal and oblique (30° to the vertical) line of sight. The insets are the light vector diagrams at 4·2 m and 10·4 m looking horizontally and obliquely (30° to the vertical). The lower vector diagram is $\frac{1}{100}$ of the scale of the upper. All results are based on Tyler's data for Lake Pend Oreille.

This is shown by the work of Sasaki *et al.* (1958) and especially by Tyler (1960). As there is a tendency for light to become increasingly vertical with increasing depth, the vector diagrams in the oblique and horizontal planes become more symmetrical and only the vertical vectors show substantial asymmetry. The most complete data are those of Tyler for Lake Pend Oreille in Idaho, and these data will be used for want of adequate information in coastal regions of the sea where fish larvae are often found. Following the type of argument put forward by Harden Jones (1968) it is possible to calculate the accuracy of detecting the direction of the sun at various depths based on the present experimental results. These show an average ability to discriminate a light when it is 100% brighter than a reference light. It is possible to calculate from Tyler's results on angular distribution of light the angle between the sun's azimuth under water (where the light intensity is maximal) and a direction where the light is reduced by half this maximal level. These angles are plotted in Fig. 5 for lines of sight in the horizontal plane and also obliquely at an angle of about 30° where lines of sight are in the same plane as the sun's rays, at least near the surface. It can be seen that the larvae, in the light environment of Lake Pend Oreille, could only detect the azimuth of the sun to an accuracy of 85° if they were looking horizontally and even then only near the surface. However, if they looked obliquely upwards they could detect the sun's azimuth to an accuracy of 12° near the surface. The possibility of determining the sun's azimuth is very rapidly lost at increasing depths.

In terms of vertical movement it is difficult to explain any adaptive value in the

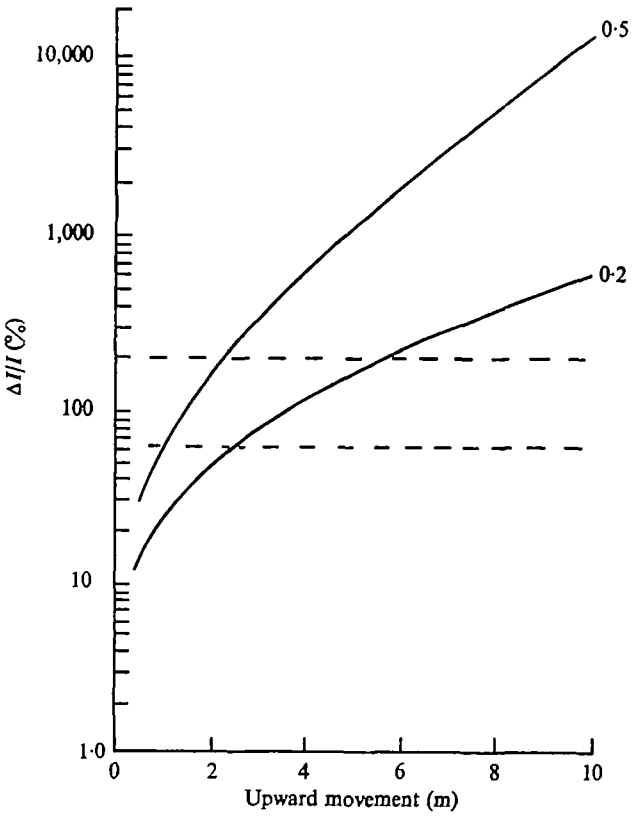

Fig. 6. Graph showing the percentage increment of brightness (ΔI) experienced by larvae moving up different distances from a depth where the reference light intensity is I. The increments for two types of coastal sea water are shown (where the irradiance attenuation coefficient is 0·5 and 0·2 – see text). The horizontal dashed lines show the range of Weber fractions ($\Delta I/I$ as %) found in these experiments.

phototaxis. A positive phototaxis at high light intensities with a reversal of sign at lower light intensities means that the larvae will tend to move towards the surface if they are in the upper layers and to move further down if they are already deep. If larvae are to monitor their movements in the vertical plane sequential comparisons of intensity are required which are much more difficult to measure experimentally. If the present results are applicable to vertical movements, the sensitivity of larvae to depth changes using light can be calculated. This will depend mainly on the characteristics of the water; where attenuation is great in turbid water there will be greater changes of light intensity for a given vertical excursion. In Fig. 6 is given the increment of brightness experienced for vertical movements upwards in different types of inshore sea water. The types of sea water considered were Jerlov's (1968) coastal types 3 and 7, which should cover the main range of turbidity experienced by fish larvae. The irradiance attenuation coefficients used to calculate changes in brightness with depth were taken for the wavelength 525 nm (the centre of the spectral sensitivity curve – see Fig. 2) from Jerlov's table XX. It can be seen that larvae *may* be able to detect a change of depth if they move up a distance of the order of 1–6 m, depending on the type of water.

SUMMARY

The brightness discrimination of larvae of plaice and sole was tested by subjecting them to a choice of moving phototactically to one of two horizontally opposed light sources.

At higher light levels, above 10^0–10^{-1} m-candles, they moved to the brighter of the two lights; below 10^{-1}–10^{-2} m-candles they moved to the dimmer.

Brightness discrimination, expressed as the increment (ΔI) discriminated as a percentage of the dimmer light (I) (the Weber fraction), was of the order of 60–200%.

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