SPECTRAL SENSITIVITY OF THE HERRING, *CLUPEA HARENGUS* L.

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INTRODUCTION

Vision in fish has been reviewed by Brett (1957) and Nicol (1963) who mention work on retinal pigments and spectral sensitivity. There appears to be a general distribution of rhodopsin in marine fish with maximum absorption at about 500 mμ, and of porphyropsin in freshwater fish with maxima 20–30 mμ towards the red end of the spectrum. Most spectral sensitivity work had been done on freshwater species using either micro-electrode techniques or behaviour responses such as pseudo-rheotropism and aggregation in different coloured lights.

Very few measurements have been made on the spectral sensitivity of marine fish. Borisov & Protasov (1960), who did not describe their methods in detail, found values of maximum sensitivity at wavelengths from 470–525 mμ in dark-adapted gadoids and other marine species of commercial value. All the species except skate showed a 'Purkinje Shift', the maximum sensitivity when light-adapted ranging from 555–590 mμ. In particular these workers found that the anchovy, *Engraulis encrasicholus*, had a scotopic maximum at 500 mμ and a photopic maximum at 560 mμ. Protasov, Altukhov & Kovaleva (1960) measured the flicker fusion frequency at different light intensities in the anchovy. The fish were placed in a cylinder around which rotated a striped screen. The speed of rotation of this screen was adjusted until the pseudo-rheotropic response disappeared. The flicker fusion frequency gradually decreased with light intensity and there was a sudden drop below 0·01 lux, suggesting a transition to rod vision. Histological examination of the retina showed that pigment migration associated with the transition from cone to rod vision took place at light intensities from 1·0–0·1 lux. Blaxter, Parrish & Dickson (1964) reported that herring, *Clupea harengus*, ceased to avoid stationary nets at light intensities from 0·01–0·0001 photopic lux, depending on the size of the tank and the contrast between the net and the background. They also found that herring ceased to be herded by moving nets at intensities between 0·5 and 0·05 photopic lux. The presence of rods and cones was reported in the retina of the pilchard *Sardina pilchardus* by Vilter (1950) and Baburina (1955) and in the herring by Verheijen (1959). All three authors found a structure, comprising mainly cones, in the ventro-posterior part of the retina.

This paper describes measurements of spectral sensitivity in herring using three behaviour techniques. Clupeids tend to be difficult animals to keep in aquaria, but once established show behaviour patterns in which vision is clearly the dominant sense involved. The data obtained in this experiment had some practical value in relation to measurements of light intensity at sea in studies of fish behaviour. The

threshold light intensity for various behaviour patterns was required and knowledge of the spectral sensitivity curve enabled a light meter to be modified so that its response to different wavelengths of light closely followed that of the species being studied. Thus only light 'useful' to the species was measured.

METHODS

The experiments were carried out on about 20 juvenile herring 9–10 cm. long kept in circulating seawater at temperatures between 6 and 7° C. The tanks were of teak with a glass front and were either single 85 × 75 × 75 cm. (Fig. 1a) or double 170 × 75 × 75 cm. (Fig. 1b). The tanks were illuminated by light from the side. The source was either an aged 6 W. or 36 W. Osram car bulb of known colour temperature in a light-tight housing. The voltage was kept accurately at 12 V. from the mains supply by a fixed and a variable transformer. The light passed through a heat filter inside the housing, through a square cut in the side and a neutral translucent filter outside, and on to another neutral translucent filter in front of the glass window of the tank. The light could be varied in colour and intensity by placing colour and neutral filters in slots in front of the housing or by placing the light source at different distances from the tank.
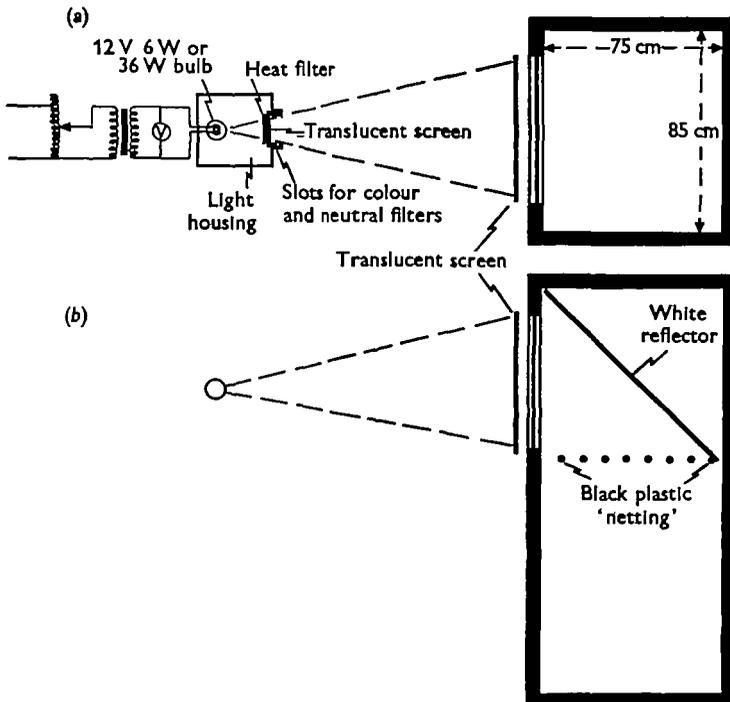

Fig. 1. Diagram showing experimental apparatus and tank from above.
(a) Single tank; (b) double tank.

The following colour filters were used (transmission peak in brackets): Ilford colour filters 600 violet (440 mμ), 601 violet (440–450 mμ), 602 blue (470 mμ), 603 blue-green (495 mμ), 604 green (520 mμ), 605 yellow-green (550 mμ), 606 yellow (575 mμ),

608 red (660 mμ), and Wratten filter 72 B orange (600 mμ); Ilford neutral filters of densities from 0·31 to 2·96. All filters were calibrated by a recording spectrophotometer. Experiments were also carried out in white light.

The principle of the experiments was to observe a behaviour pattern dependent on the presence of light and, using different colour filters, to measure the threshold light intensity at which the behaviour disappeared. Before each experiment the fish were dark-adapted for at least 1 hr. The behaviour patterns studied were as follows:

(a) *Phototaxis—using the single tank.* Herring ceased to shoal in low light intensities, but were then found to swim into the glass wall of the tank when a dim light was shone through it. For each colour filter the intensity of light from the side was reduced in gradual steps to the point where no fish were seen swimming into the glass. This was taken to be the threshold for phototaxis. Observations were made by flashing a torch covered with a red filter for about 1 sec. at 1 min. intervals.

(b) *Feeding—using the single tank.* Herring did not feed in darkness. A given number of pieces of fresh squid flesh in cubes of about 4 mm. were dropped into the tank in front of the glass window so that they were silhouetted against the light source. After about 20 sec., the time taken for the food to sink half way down to the bottom, the red torch was flashed and the number of fish feeding was counted. Later the uneaten pieces of food were siphoned off the bottom and counted. Two criteria were used to measure the threshold light intensity for feeding. One was that the fish should first cease to feed as a group—a group being taken as at least four individuals. The other, at a lower light intensity, was when individual fish just ceased to feed. Care was taken, by checking their feeding activity in good light conditions at the end of each experiment, that the fish did not become satiated during an experiment.

(c) *Perception of a barrier—using the double tank.* Here the herring were confined in one half of the double tank before the experiment by a barrier of horizontal and vertical strands of black plastic, about 4 mm. in diameter and 2 cm. apart. The barrier was silhouetted against a sheet of white Perspex placed diagonally across the other half of the tank so as to reflect the light from the light source outside the tank. In high light intensities only a small number of fish would pass through this barrier in a given time, and the light intensity was reduced until the number of fish passing through suddenly increased. This was taken as the threshold. Observations were made by flashing a red torch, long enough to count the fish once each minute during a period of 10 min.

The assessment of the threshold light intensity was somewhat subjective but experiments which were repeated showed good agreement. The difference in light intensity between many fish and no fish performing a behaviour pattern was quite large, being about half a log. unit.

Once a threshold had been established the light intensity was measured underwater at the centre of the glass screen, or in the centre of the barrier in the last experiment, by a photomultiplier tube (type EMI 9524 B) in an underwater case (Craig & Lawrie, 1962). The photomultiplier had been calibrated by the National Physical Laboratory and its response to both light intensity and colour was known. This enabled the relative light intensity to be measured for all colours. The peripheral light intensity tended to be a little lower but the difference was much reduced by the translucent screens and where possible the behaviour pattern was confined to the centre.

The spectral sensitivity curves for the different behaviour patterns were drawn initially by plotting the logarithm of the reciprocal of the threshold light intensity for each filter against the peak transmission wavelength of that filter. This clearly gave only an approximate curve as it did not take into account adequately the different band widths of the filters and differential sensitivity of the light meter over these band widths.

The first curves obtained were therefore corrected in the following way, as suggested by Dr E. J. Denton (personal communication). It was assumed that the energy distribution reaching the animal, for any one colour filter, was not substantially different from the colour temperature of the lamp, apart from the effect of the colour filter itself. The total energy which should then have been registered by the light meter was estimated from the transmission curve of each filter. Every 10 mμ, over the transmission range of a particular colour filter, the energy output (E_λ) of the lamp at that wavelength, the percentage transmission of the filter (T_λ) and the sensitivity of the light meter (L_λ) were multiplied together. These values were then added to give an estimate of the total energy which should have been registered by the light meter for the colour filter. The relationship (p) between this total energy and the actual value recorded by the meter was a measure of the distance of the source from the meter and of the absorption by any interposed media. Thus for filter x:

$$p_x = \frac{KR_x}{\Sigma E_\lambda T_\lambda L_\lambda d_\lambda},$$

where K is a constant and R the meter reading.

Having found p for each filter, the next step was to correct the first spectral sensitivity curves. Again for every 10 mμ step within the transmission range of a filter the energy output of the lamp (E_λ), the percentage transmission (T_λ) at that wavelength and, this time, the sensitivity of the animal from the first spectral sensitivity curve (S_λ), were multiplied and the values summed for the whole transmission range of the filter. This aggregate value was multiplied by the appropriate value of p for the filter. Thus for filter x the value

$$p_x \Sigma E_\lambda T_\lambda S_\lambda d_\lambda,$$

was compared with similar values for the other filters. If the spectral sensitivity curve had been correctly drawn this value for each filter should have been the same. Where it was not the curve was adjusted slightly (giving different values for S_λ) so that all values agreed with that for filter 603.

In order to check the methods employed the spectral sensitivity of a human subject was also determined. In this case the human subject sat behind the translucent screen in air with small pieces of wood suspended behind the screen to simulate food. His threshold was then determined for each filter using peripheral vision, his head being kept in one position with a fixation point for the eye at the side of the screen.

Retinal pigment was also extracted from dark-adapted herring eyes (not the herring used in the experiments) and its transmission characteristics before and after bleaching were measured on a model DU Beckman Quartz Photoelectric Spectrophotometer by Dr L. R. Fisher, National Institute for Research in Dairying, Reading.

RESULTS

The corrected spectral sensitivity curves for phototaxis, feeding as a group and individually, and for perception of a barrier are shown in Fig. 2a, b. The maximum sensitivity was found in the blue-green, from about 510–520 mμ, with a great decrease in sensitivity at the red end of the spectrum and usually a smaller reduction in sensitivity at the violet end. The curve for the barrier experiment is, however, very flat at the violet end. Comparisons of the curves for the different behaviour patterns show that the herring were most sensitive in perceiving a barrier, by 0·5–2·0 log$_{10}$ units, as compared with phototaxis and feeding. They needed less light to feed singly than as a group and less light still for phototaxis. There are considerable differences in the shape of the curves except those for feeding. The threshold light intensity in white light (2600° K.) for each experiment is given in Table 1.
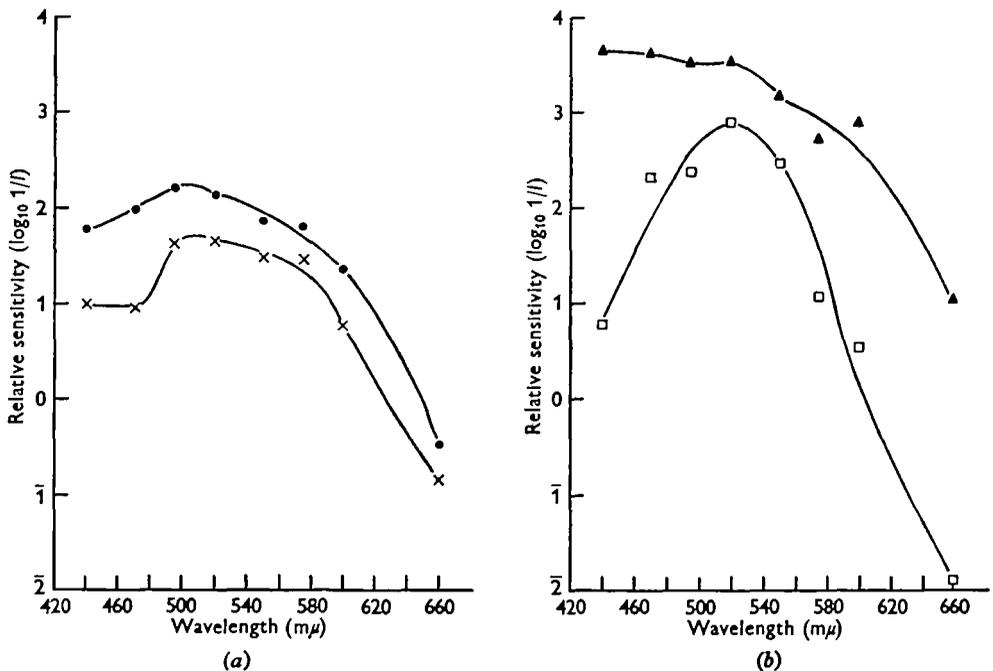

Fig. 2. Showing the relationship between relative sensitivity (log$_{10}$ 1/threshold intensity) and wavelength, (a) Feeding as a group, × — × ; singly, ●—●. (b) Phototaxis, □—□ ; perceiving a barrier, ▲—▲.

Table 1. *Threshold light intensities for white light*

Behaviour pattern	Perception of barrier	Phototaxis	Feeding singly	Feeding as a group
Threshold light intensity for white light in photopic lux	2·9 × 10^{-4}	1·8 × 10^{-2}	7·0 × 10^{-2}	3·6 × 10^{-2}

The corrected curve for the human subject is shown in Fig. 3, compared with the average curve obtained by Wald (1945) adjusted to the same value at 495 mμ. The threshold light intensity in white light for the human subject in the present series of experiments was 3·4 × 10^{-7} photopic lux.

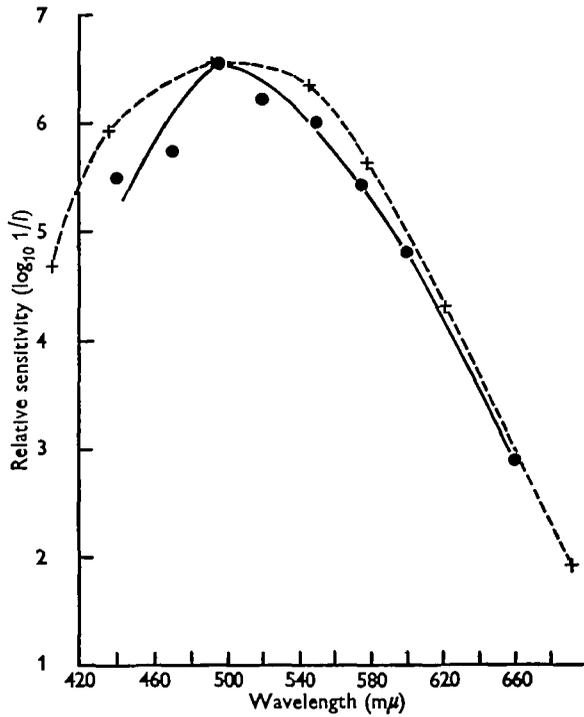The difference curve for the retinal pigment extracted from dark-adapted herring

Fig. 3. Showing the relationship between relative sensitivity and wavelength. Human subject in these experiments, ●——●; mean result for 22 human subjects, Wald, (1945) + --- +.
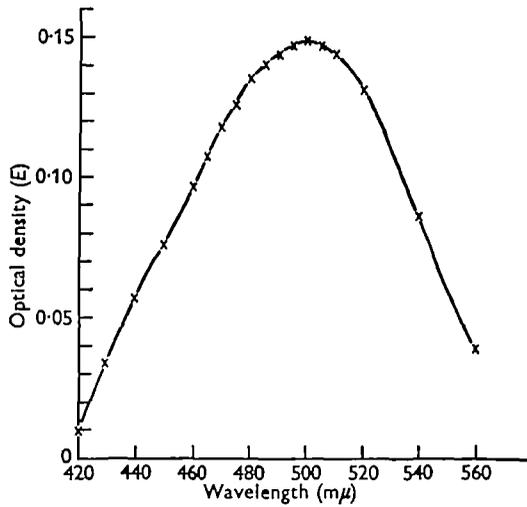

Fig. 4. Absorption characteristics, shown as optical density E

$$\left(\log_{10} \frac{\text{intensity transmitted after bleaching}}{\text{intensity transmitted before bleaching}} \right),$$

of retinal pigment extracted from dark-adapted herring eyes.

eyes is shown in Fig. 4. It shows a clear maximum of absorption at 500 mμ, as found for rhodopsin in other marine fish.

DISCUSSION

The irregularity of some points on the curves is probably, at least in part, due to the limitations of the technique. The use of behaviour patterns meant that repeat control experiments could give differences in sensitivity at any particular wavelength of up to 0·5 log$_{10}$ unit. In many cases, however, controls showed much smaller differences or no differences at all. The tendency is for the curves to show the sensitivity of the more sensitive fish, because of the definition of threshold decided upon. The difference in shape of the curves is presumably a sign of differences in reaction to colours, depending on the behaviour pattern, for these techniques may give only 'subjective', rather than absolute, spectral sensitivity curves.

The lack of agreement between the maximum spectral sensitivity (510–520 mμ) and the maximum of absorption of the retinal pigment (500 mμ) may be due to differential absorption of light in the eye by other pigments. Other workers have also found a lack of exact correspondence in such comparisons, e.g. in *Xenopus laevis* (Denton & Pirenne, 1954). The reasonable agreement between the curve for the human eye of one subject, compared with that found by Wald for 22 subjects, suggests there is no serious limitation in the technique.

The differences in overall sensitivity in the different experiments, for instance the higher sensitivity to barriers, and in some cases the relatively high sensitivity to violet light, may be explained by the different nature of the behaviour patterns. Seeking food and feeding are more positive forms of behaviour than avoiding a net. The threshold for perception of a barrier (2·9 × 10^{-4} lux) in white light is of similar order to but somewhat lower than that found previously for a small tank (Blaxter *et al.* 1964). The thresholds of light intensity for feeding (ranging from 0·036–0·007 in white light) may be compared with the somewhat similar values for salmon (Brett & Groot, 1963), of 0·001–0·0001 lux. Jones (1956) found that minnows changed from 'dark' to visual feeding at a light intensity between 0·0007 and 0·00007 lux. It would appear that these species are at least ten times more sensitive than herring from the point of view of feeding and visual perception. With light from the side, and maximum contrast between what is to be seen and the background, and by nature of the criterion chosen for defining the thresholds, all the threshold values found in the present experiments will tend to be the lowest obtainable.

For measurements of light intensity in the sea, in studies of fish behaviour, the use of a Chance-Pilkington O Gr 3 green glass filter in conjunction with an EM 1 type 9524 B photomultiplier tube will give a light meter with a spectral response similar to that of the herring eye when dark-adapted (using the curves for feeding).

So far it has not been possible to obtain high enough light intensities or to develop a technique to determine whether herring show a 'Purkinje Shift'. This is to be expected from previous work and from the structure of the retina. The feeding experiments are being extended to dark-adapted gadoid species and flatfish. It would be interesting to test the reaction of fish to ultra-violet light in view of the fairly high sensitivity found at short wavelengths. A first step would be to measure the transmission of ultra-violet by the lens of fish—Wald (1945) found that the lens was the

main cause of lack of sensitivity to ultra-violet in the human eye. Nicol (1963) cited reports of varying degrees of opacity to ultra-violet light in the lens of fish from different habitats.

SUMMARY

1. Herring (*Clupea harengus*) require light for feeding and avoiding nets. The threshold light intensity for these behaviour patterns and also for phototaxis, using different colours of light, was measured and used to obtain spectral sensitivity curves.

2. The maximum sensitivity of dark-adapted herring lay between 510 and 520 mμ. The threshold light intensity, using white light, varied from $3 \cdot 6 \times 10^{-2}$ to $2 \cdot 9 \times 10^{-4}$ photopic lux, depending on the behaviour being studied.

3. A parallel experiment on a dark-adapted human subject gave a maximum sensitivity at 500 mμ and a threshold light intensity, using white light, of $3 \cdot 4 \times 10^{-7}$ photopic lux.

4. Pigment extracted from dark-adapted eyes of herring had a maximum absorption at 500 mμ.

5. Suggestions are made for measurement of underwater light intensity in studies on fish behaviour.

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