Colour vision in an Indian Fish *Anabas testudineus* (Cuv.)

BY

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(*With two text-figures*)

Although electro-physiological techniques have largely been employed to explore retinal mechanisms for analysing light in terms of its wave-length direct evidence for hue discriminatory capacity of animals comes mainly from training experiments. This method dates back to 1901 when Zolotnitzky fed fishes on red midge larvae and later successfully lured them with bits of red yarn. Since then experiments have been devised to eliminate luminosity as a factor in colour discrimination either by using finely graded series of coloured papers or by altering the intensity of light transmitted through coloured filters.

Results obtained through behavioural experiments on colour vision are not sufficient, and as such we are still far away from understanding the colour vision system in fish. In our investigation into colour vision the Indian perch *Anabas testudineus* (Cuv.) has been selected for some important reasons. The fish possess accessory breathing organs and can be kept alive in aquarium for a long time without food and without changing water. They can be easily trained to feed from glass tubes. A notable behaviour of the fish is that during monsoon they leave water and move on land (Day 1958).

This fish was, however, successfully trained to discriminate red from green, blue, yellow, grey and violet. It also discriminated violet from blue, green and grey, as well as green from blue. We have, therefore, come to the conclusion that this fish does possess some mechanism for colour vision, and it is mediated through trichromasy.

Methods

Two groups of fish, each consisting of four individuals, were trained separately to feed from glass tubes. The fish, measuring 9 to 11 cm in length, were purchased from the local market. Their sexes could not be determined because of absence of secondary sexual characters.

Group-I was first conditioned to respond to red as the positive stimulus with blue, green, yellow, grey and violet as the negative. It was later conditioned to violet as the positive stimulus with blue, green and grey as the negative. Group-II was trained to discriminate between blue and green, the latter being the positive stimulus.

Glass tubes, used as colour stimuli, were first wrapped with coloured paper and then covered with thin transparent polythene sheet to make them water-proof. Ten shades of every colour tested were selected from homogeneously coloured poster papers.

A small piece, about 1 cm long, of fresh earthworm *Pheretima* sp., was stuck to one end of the tube, and it was covered with a thin piece of black rubber to check direct visual stimulus of food. The negative stimulus had a similar arrangement but was without any food. Once the fish had learnt to associate food with a particular colour, both the tubes were furnished with food, thereby controlling olfactory and gustatory cues.

During each trial a pair of colour stimuli were offered, and if the fish could snap at the right tube directly one positive response was counted. Twenty trials were performed on every daily session. Twenty combinations of colour pairs were selected at random from twenty shades of colours and presented at random with respect to relative position of tubes. The fish were light-adapted by an initial illumination of white light before start of every session.

On the last session of one discrimination test (red versus violet) coloured papers were replaced by spectroscopic filters of similar colour pairs. Glass tubes, covered with white paper, were bathed in the transmitted light. The intensity of light was altered by the use of neutral filters in the light path. Spectral transmission factors of red and violet filters, used in this experiment, are given in Fig. 1.

Results

The fishes were successfully conditioned to respond correctly to coloured papers as well as to light of different wave-lengths focussed on white paper. The average scores, obtained in daily sessions with each of the ten pairs of colours tested, are presented in Table I. Results of the first seven sessions of pre-training with both Group-I and Group-II fishes are not given because the number of trials and responses was not constant during this period.

Filters were used on the 7th session of red (+) versus violet (−). The average score with filters is not much different from that with coloured papers. But the fish were very much confused and almost non-responsive to either of the paired stimuli offered when light-intensities were very low and colours, reflected from tubes, were just recognizable by the human eye.

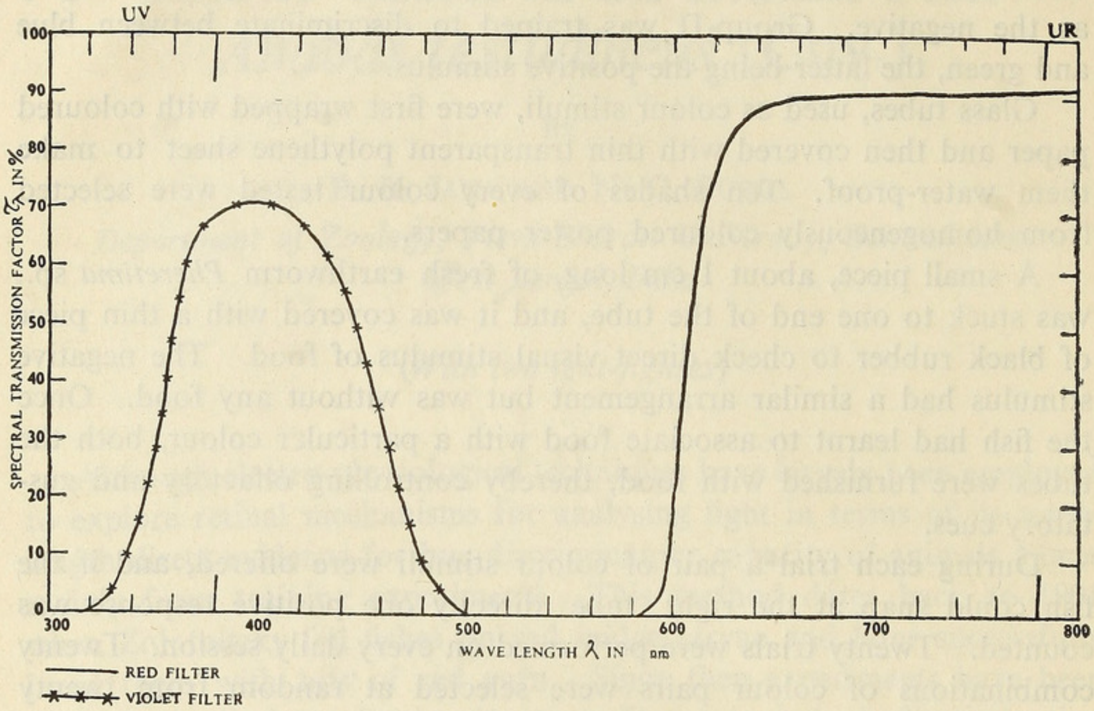

Fig. 1. Spectral transmission factors of red and violet filters.

Every daily session normally includes twenty trials. But on some occasions the fish were reluctant to respond to either of the paired stimuli offered. This happened when they were confused with the paired stimuli as well as towards the end of a session when the fish were relieved of acute hunger. Trials had to be increased in these cases to elicit twenty responses.

After thirty-six days' association with red as well as violet as the positive stimuli Group-I was deconditioned and blue was given as the positive stimulus with respect to red the negative. The gradual increase of positive responses for blue with simultaneous decrease in responses for red was transformed into percentage responses and plotted against days in Fig. 2. The two complimentary curves, the learning curve for blue and the deconditioning curve for red, show that after seven days the fish were deconditioned. Data-set I in Table I shows the average scores of the last seven sessions with blue (+) versus red (−).

Table I shows that the percentage of correct responses for all the colour pairs tested except red and violet varies from 83·35 to 96·85, which is fairly high and needs no further verification. The average scores with red (+) versus violet (−), obtained from six daily sessions excluding the 7th session with filters were statistically analysed by means of a t-test with a view to testing whether red and violet were really indistinguishable to the fish under investigation. Here t was found to be

TABLE I

AVERAGE SCORES OBTAINED BY TWO GROUPS OF FISH DURING EXPERIMENT

Days	Group-I								Group-II	Average
	Red (+)					Violet (+)			Blue (+)	Green (+)	
	A Green(−)	B Blue (−)	C Yellow (−)	D Grey (−)	E Violet (−)	F Blue (−)	G Green (−)	H Grey (−)	I Red (−)	J Blue (−)	
1	17·10	19·50	17·25	18·25	13·25	19·25	17·75	18·75	19·25	17·25	17·11
2	16·50	18·25	16·75	18·75	12·75	19·50	19·25	19·25	17·75	19·00	17·67
3	19·25	19·75	18·50	—	9·50	—	—	—	16·75	18·75	17·16
4	19·00	19·00	—	—	11·75	—	—	—	18·00	20·00	17·80
5	18·25	19·00	—	—	10·25	—	—	—	13·25	19·25	16·85
6	19·75	18·75	—	—	8·75	—	—	—	16·75	—	15·10
7	15·25	19·00	—	—	11·75	—	—	—	17·25	—	15·81
Average	17·87	19·03	17·50	18·50	11·11	19·37	18·50	18·50	17·00	18·85	16·78
Percentage	83·35	95·15	87·50	92·50	55·55	96·85	92·50	92·50	85·00	94·25	83·90

equal to 8·96 which is well beyond the 0·1% significance level of t with df 15, the latter being only 3·733. Thus it can be concluded that the

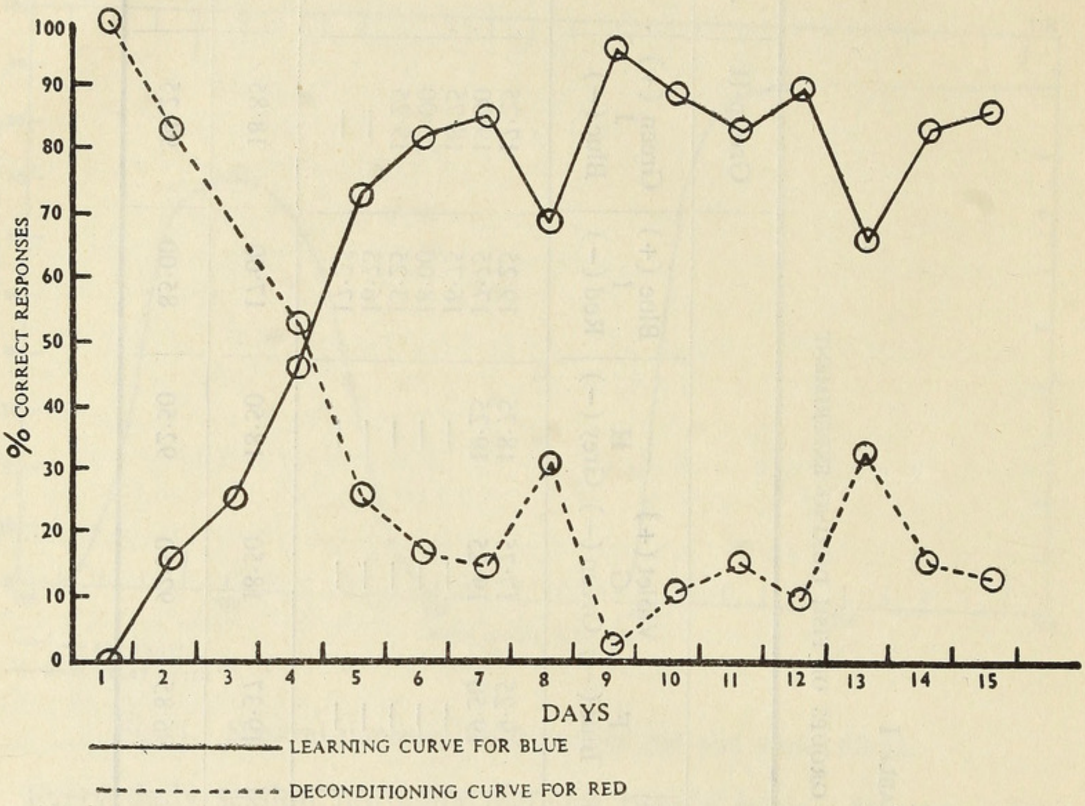

Fig. 2. Percentage responses for blue and red stimuli in relation to days.

fishes can distinguish between red and violet to some extent. The number of times they moved towards red as shown in data-set E in Table I is, on the average, significantly above 10.

When the positive stimulus was shifted from red to violet the fish immediately succeeded in making 92·50 to 96·85% correct responses in the discrimination of violet from blue, green and grey. This indicates that the fish could discriminate red or violet from other colours with almost equal ease, although they had difficulty in discriminating red from violet.

The data-sets B and I in Table I were statistically analysed by the analysis of variance technique to see whether there was any variation in response on different days and in different fishes. The analysis showed no variation, but revealed some kind of systematic error in case of the 5th day observations of the data-set I. Statistical analysis was carried out for both the original data and the data obtained by applying the $\sin^{-1}\sqrt{P}$ transformations. The results are very similar whether the transformation is applied or not.

The two data-sets B and I were also compared in order to see whether there was any colour preference which might have given rise to significant difference between the overall means in the two data-sets. The statistic is

$$t = \frac{\hat{c}_1 - \hat{c}_2}{\{ [s.e. \text{ of } \hat{c}_1]^2 + [s.e. \text{ of } \hat{c}_2]^2 \}^{\frac{1}{2}}}$$

where $\hat{c}_1$ = general means of data-set B,

$\hat{c}_2$ = general means of data-set I

and the degree of freedom for t is the sum of the error df's of the two experiments. The results are given in Table II, which shows significance

TABLE II

RESULTS OF 't' TEST OBTAINED FROM OVERALL MEANS OF DATA-SETS B AND I

Item	Original Data		Transformed Data (Degree)	
	Including 5th day observations of data-set I	Excluding 5th day observations of data-set I	Including 5th day observations of data-set I	Excluding 5th day obervations of data-set I
(1)	(2)	(3)	(4)	(5)
$\hat{c}_1$	18·964	18·964	79·639	79·631
$\hat{c}_2$	17·000	17·625	69·316	71·777
s.e.$\hat{c}_1$	0·1873	0·1873	1·5763	1·5763
s.e.$\hat{c}_2$	0·1841	0·2149	1·1173	1·3093
t	7·47	4·69	5·34	3·83
df	36	33	36	33
Theoretical value of 't' at 0.1% level	3·59	3·62	3·59	3·62

of observed t at 0.1% level in any case. The table also shows that $\hat{c}_1 > \hat{c}_2$ in any case. Hence it can be concluded that preference for red colour is greater than that for blue.

DISCUSSION

In our investigation the fish *Anabas testudineus* discriminated between hues on their qualitative basis and not on their quantitative basis. This is confirmed by the use of wide range of coloured papers. It is quite unlikely the fish would be able to remember all the ten shades of a colour used as a positive stimulus. Moreover, brightness-discrimination in fish has been found to be extremely poor as is evident from the work of Reeves (1919). Reeves showed that *Semotilus* could not discriminate intensities differing in 1 : 4 ratio. This ratio for *Lepomis* is 1 : 2.

Since the fish could recognize red, green and blue, and also discriminated them from each other their vision appears to be trichromatic. Trichromasy has also been shown in *Phoxinus laevis* (Hamburger 1926) and also in goldfish (Marks 1963, Muntz & Cronly-Dilton 1966).

The fish *Anabas* showed very poor discrimination between red and violet although the colours are widest apart in the wave-length scale of the visible spectrum. Reports are there that *Phoxinus* could not discriminate red from yellow and purple, and the colour circle is closed for this fish (Frisch 1925, Wolfe 1925 and Hamburger 1926). But *A. testudineus* could recognize violet as a distinct hue and discriminated it from blue, green and grey without any difficulty. This shows that the visible spectrum is not narrowed towards the short wave end in *Anabas*. That violet and even ultraviolet at 313-253 $m\mu$ are visible to sticklebacks was reported by Merker (1934). But he suspected conversion of ultraviolet into visible light through the fluorescence of water.

Preference for red as compared to blue in these fishes might have resulted from long association of the fish with red as the positive stimulus. But the learning curve for blue in Fig. 2 did not show any significant rise with days after the 7th day. A probable explanation for this behaviour of fish is that since water and ocular media absorb and disperse most of the light of shorter wave-lengths it is quite likely that light of longer wave-lengths may reach retina in greater quantities and consequently stimulate the photoreceptors to a greater extent.

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