Colour Discrimination in Three Air-Breathing Teleosts

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Abstract Hue-discriminatory capacity has been tested in three air-breathing teleosts, *Channa punctatus*, *Clarias batrachus* and *Heteropneustes fossilis*. *Channa* with well-developed eyes and efficiency of vision was successfully trained to discriminate red from green, blue, yellow, grey and violet. It also distinguished violet from blue, green and grey as well as green from blue. The colour vision mechanism in this fish is mediated through trichromasy. The mud dwellers *Clarias batrachus* and *Heteropneustes fossilis* with markedly reduced eyes, showed poor colour-discrimination capacity, and perhaps barbels were evolved in these fishes to compensate their poor power of vision.

Despite electro-physiological techniques being largely employed to explore retinal mechanisms for analysing light in terms of its wave length, direct evidence for hue discriminatory capacity of animals is based on behavioural training experiments. The success in behavioural experiments depends on elimination of 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.

Teleosts show differences in the structure of the retina depending on the amount of light incident on the eye (Blaxter, 1970). The term "retinomotor responses" was defined by Ali (1975) as the dispersion, or concentration of the masking pigment with retinal epithelial cells and the expansion and contraction of rods and cones in response to changes in the ambient light. Guma'a (1982) observed in perch (*Perca fluviatilis*) that retinomotor responses commenced after metamorphosis when the rods developed, and the visual acuity improved exponentially with age. Although colour vision in teleosts is well established (Ali, 1975) it is not known whether this ability bears any relationship with the habitat of fishes. The purpose of the present investigation is to know whether the mud dwelling teleosts are as much efficient in colour discrimination as their counterparts inhabiting the water column of the ponds. The murrel *Channa punctatus* is a marginal-feeder having well developed eyes, while *Clarias batrachus* and *Heteropneustes fossilis* are known to live over mud and burrows (Annon, 1962) with greatly reduced eyes. These fishes were subjected to rigorous training and their ability to distinguish various colours was compared.

Materials and methods

Active individuals of *Channa punctatus*, *Clarias batrachus* and *Heteropneustes fossilis*, measuring 12 to 17 cm in length, were procured from the local market. The training experiment was conducted separately for each species. Two groups of fish of each species, were trained separately to feed from glass tubes. Each group consisted of four individuals. Group-I was first conditioned to respond to red as the positive stimulus with green, blue, yellow, grey and violet as the negative. Group-II was trained to discriminate between blue and green, the latter being the positive stimulus.

Narrow glass tubes (0.4 cm diameter) with one end closed, were used as colour stimuli. Ten shades of each colour solution were prepared by mixing dyes in distilled water in various concentrations. Ten dilutions of each of the dyes eosin, light green and potassium dichromate were prepared as 100 to 1,000 mg/*l* in equal steps. Ten dilutions of each of the dyes methyl blue and crystal violet were also prepared in equal steps, but by dissolving 10 to 100 mg/*l*. The glass tubes were filled with different shades of colour solution, which were then used to train the fishes. The spectral transmissions of blue (100 mg/*l*), red (1,000 mg/*l*), green (1,000 mg/*l*), yellow (1,000 mg/*l*) coloured solutions are given in Fig. 1.

About 1 cm long piece of fresh earthworm *Pheretima* sp., was stuck to the closed end of the tube, and it was covered with a thin piece of

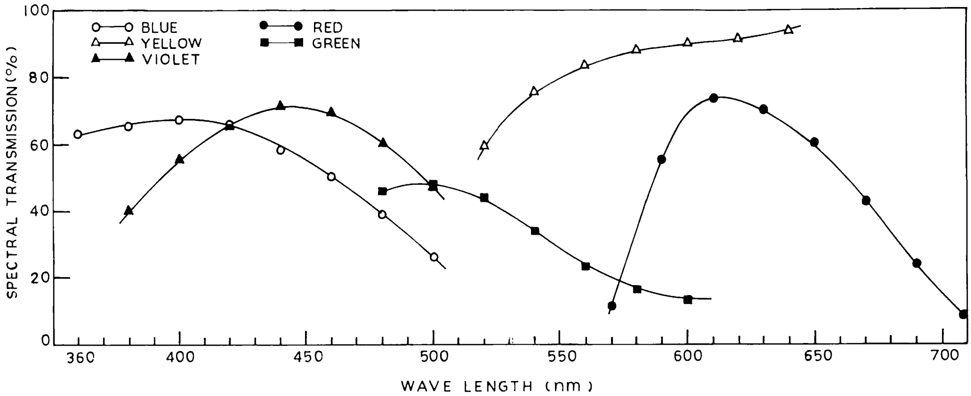

Fig. 1. Spectral transmission curves for various colours.

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 (positive and negative) were offered, and if the fish snaped at the bait in the right tube directly, one positive response was counted. When the fish attempted to bite the wrong colour tube a negative response was counted. The response was grouped as both positive and negative when the fish reacted to both the tubes offered. The score was considered nil when none of the fishes responded to any of the colour tubes given. Twenty trials were performed in each daily session. There were twenty shades of each colour which were selected and presented to fishes at random. The relative position of two tubes were varied every time they were presented to the fishes. The fishes were light adapted by an initial illumination of white light before the start of a session. All the data obtained in this experiment were subjected to statistical evaluation. A Chi-square test was applied with the proposition in the null hypothesis that the fishes were equally attracted to all four possible combinations of positive, negative, both and zero, and the test of significance was obtained with 3 degrees of freedom. A two way variance analysis was performed to see whether there was any variation in the three test fishes in terms of correct responses towards the positive stimulus in a given paired stimuli. This model also permitted to test whether there was any variation in response on different days.

Results

Among the three test fishes, *Channa* was successfully conditioned to respond correctly to coloured tubes, but not *Clarias* and *Heteropneustes*. The average scores obtained on daily sessions with each of the ten pairs of colour tested in *Channa*, *Clarias* and *Heteropneustes* are presented in Tables 1, 2 and 3, respectively. *Clarias* and *Heteropneustes*, in general, were very much confused and discriminate the pairs poorly even when the coloured solutions were quite bright with highest concentrations, while the *Channa* distinguished the pairs well even when the intensity of colour was very low and just recognizable by the human eye.

The results of the Chi-square (Table 4) suggest that all the fishes showed significant variations in the scores between four classes in all the colour pairs tested. Among 200 trials offered in ten days, the average scores per day in various positive stimuli in *Channa* ranged from 15.4 to 19.2 as against 0 to 2.8 in the negative tube. In *Clarias* and *Heteropneustes* the correct responses in the positive stimulus decreased drastically (8.0 to 9.6) with concurrent increase in other combinations (negative—5.2 to 7.2, both—4 to 5.6, zero—0 to 4.4).

After sixty 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.

Table 1. Average scores obtained by two groups of *Channa punctatus* during the experiment.

Days	Group-I									Group-II
	Red (+) Green (−)	Red (+) Blue (−)	Red (+) Yellow (−)	Red (+) Grey (−)	Red (+) Violet (−)	Violet (+) Blue (−)	Violet (+) Green (−)	Violet (+) Grey (−)	Blue (+) Red (−)	Green (+) Blue (−)
1	20	16	20	20	20	12	16	8	12	14
2	16	20	20	20	16	16	20	12	12	14
3	16	20	20	20	12	20	12	20	16	16
4	20	20	16	20	20	12	16	16	16	16
5	20	12	16	16	16	16	12	16	14	18
6	12	16	16	20	16	20	12	20	16	16
7	16	20	16	20	20	16	16	16	16	18
8	16	20	20	20	20	20	16	16	18	16
9	20	12	20	20	12	16	20	20	16	16
10	12	20	16	16	20	20	20	20	18	16
Average	16.8	17.6	18.0	19.2	17.2	16.8	16.0	16.4	15.4	16.0
Percentage	84	88	90	96	86	84	80	82	77	80

Table 2. Average scores obtained by two groups of *Clarias batrachus* during the experiment.

Days	Group-I									Group-II
	Red (+) Green (−)	Red (+) Blue (−)	Red (+) Yellow (−)	Red (+) Grey (−)	Red (+) Violet (−)	Red (+) Blue (−)	Violet (+) Green (−)	Violet (+) Grey (−)	Blue (+) Red (−)	Green (+) Blue (−)
1	8	12	8	8	8	8	8	8	8	8
2	8	8	12	12	4	8	12	12	8	16
3	12	16	8	8	12	8	8	12	12	12
4	12	8	8	8	8	8	12	8	8	8
5	8	12	8	12	12	8	12	8	8	8
6	8	4	8	8	4	8	4	8	12	8
7	8	8	12	8	12	12	8	8	8	12
8	12	4	12	8	8	8	8	12	12	4
9	8	8	4	12	8	12	8	8	8	12
10	12	12	8	12	8	8	12	8	12	8
Average	9.6	9.2	8.8	9.6	8.4	8.8	9.2	9.2	9.6	9.6
Percentage	48	46	44	48	42	44	46	46	48	48

It was again observed that *Channa* was successfully deconditioned only after seven days but *Clarias* and *Heteropneustes* demonstrated poor results even after prolonged training.

Table 1 shows that the percentage of correct responses in *Channa*, for all the colour pairs tested, varied from 77 to 96%, which is fairly high and needs no further statistical verification. This suggests that *Channa* could discriminate red from green, blue, yellow, grey and violet as well as violet from blue, green and grey with almost equal case. The correct responses were almost similar whether both the tubes were furnished with food or not. In the cases of *Clarias* and *Heteropneustes* the percentage of correct responses remained always below 50%, ranging between 38 and 48% which showed that these fishes had difficulty in distinguishing various colour pairs. This result was also confirmed with the arrangement of both colour tubes provided with and without food.

It has been indicated in the variance analysis (Table 5) that the fishes under investigation showed no significant difference in responses on

Table 3. Average scores obtained by two groups of *Heteropneustes fossilis* during the experiment.

Days	Group-I									Group-II
	Red (+) Green (−)	Red (+) Blue (−)	Red (+) Yellow (−)	Red (+) Grey (−)	Red (+) Violet (−)	Red (+) Blue (−)	Violet (+) Green (−)	Violet (+) Grey (−)	Blue (+) Red (−)	Green (+) Blue (−)
1	8	8	4	4	12	8	8	8	8	12
2	16	8	12	8	12	8	12	12	4	8
3	12	12	8	8	4	12	8	8	4	4
4	8	8	4	4	4	12	8	8	8	12
5	4	16	4	12	12	12	8	8	8	8
6	12	8	8	12	8	8	12	8	8	8
7	4	8	4	8	8	4	12	12	12	8
8	8	12	8	12	4	12	8	12	12	8
9	8	4	12	8	12	8	8	8	8	8
10	12	8	12	12	16	12	8	12	8	8
Average	9.2	9.2	7.6	8.8	9.2	9.6	9.2	9.6	8.0	8.4
Percentage	46	46	38	44	46	48	46	48	40	42

Table 4. Results of the Chi-square (based on 3 degrees of freedom).

Serial numbers	Colour pairs	Values of Chi-square		
		Channa	*Clarias*	*Heteropneustes*
1	Red (+), Green (−)	372.80*	89.92*	78.40*
2	Red (+), Blue (−)	427.84*	87.36*	60.48*
3	Red (+), Yellow (−)	451.52*	80.96*	37.44*
4	Red (+), Grey (−)	538.56*	88.0*	77.12*
5	Red (+), Violet (−)	398.40*	54.08*	80.32*
6	Violet (+), Blue (−)	375.36*	74.56*	97.60*
7	Violet (+), Green (−)	330.56*	80.32*	74.56*
8	Violet (+), Grey (−)	354.88*	87.36*	82.24*
9	Blue (+), Red (−)	405.82*	93.76*	41.92*
10	Green (+), Blue (−)	398.98*	91.20*	40.0*

* Significant at 1% level.

different days in all the colour pairs offered ($F_{9,18} \leq 1.26$; $P > 0.05$) with the exception of blue (+) versus red (−) combination where the variance ratio ($F_{9,18} = 2.59$) was just significant ($P < 0.05$). The test fishes, on the other side, showed significant differences in the scores in each colour pair ($F_{2,18} \geq 13.81$, $P < 0.01$). The three species were then compared with respect to the average score obtained in each paired stimuli arrangement by means of a critical difference (C.D) test. It is fairly concluded that the scores in *Channa* were significantly different ($P < 0.02$) from the scores obtained with *Clarias* or *Heteropneustes*. The differences in the scores between the latter two were always less than CD values at 5% and hence, not found significant ($P > 0.05$).

Discussion

The results of the present study clearly reveal that colour discriminating capacity varied greatly in these fishes, and that their colour vision system is closely associated with their habitual mode of life. *Channa*, a dominantly marginal feeder with well developed eyes, could discriminate various colour pairs while the bottom feeders *Clarias* and *Heteropneustes* could not do so. It has been stated that most bony fishes with a good number of cone cells in the retina have some form of colour vision but sensitivity may

Table 5. Analysis of variance.

Sources of variation	df	Variance ratios (F)									
		Red (+) Green (−)	Red (+) Yellow (−)	Red (+) Blue (−)	Red (+) Grey (−)	Red (+) Violet (−)	Violet (+) Blue (−)	Violet (+) Green (−)	Violet (+) Grey (−)	Blue (+) Red (−)	Green (+) Blue (−)
Days	9	0.28	1.17	1.26	0.57	0.80	0.76	1.29	1.02	2.59*	0.43
Fishes	2	13.82**	44.42**	21.00**	50.46**	17.68**	26.0**	23.43**	20.77**	43.92**	22.18**
Error	18										
Total	29										

* Significant at 5% level.
** Significant at 1% level.

vary from species to species and also with the age of the individual (Norman and Greenwood, 1975). Poor hue discriminatory capacity in *Clarias* and *Heteropneustes* might be explained due to the fact that these fishes live over muddy bottoms of the swamps as omnivorous bottom scroungers (Lowe-McConnell, 1975), and obviously they are adapted to poor photic conditions of muddy waters and burrows. Barbels are perhaps developed in these fishes as a compensatory measure to cope with the situation. It is significant that eyes are well developed in *Channa* occupying a larger proportion (1:24) of the total body, while in *Clarias* and *Heteropneustes* eyes are much reduced in size occupying an area of 1:40 and 1:45, respectively.

Since *Channa* could recognize red, green and blue and also discriminate them from each other their vision appears to be trichromatic. Jana and Sukul (1972) observed that the Indian perch *Anabas testudineus* possessed trichromatic vision. Trichromasy has also been shown in *Phoxinus laevis* (Hamburger, 1926) and in goldfish (Marks, 1963; Muntz and Cronly-Dilton, 1966). It has been reported that *Phoxinus* could not distinguish red from yellow and purple, and the colour circle is closed for this fish (Frisch, 1925; Wolfe, 1925; Hamburger, 1926).

Unlike *Anabas* which showed preference for red as compared to blue (Jana and Sukul, 1972), *Channa* showed no preference for any particular colour in the present study. The perch *Anabas* showed very poor discrimination between red and violet (Jana and Sukul, 1972) but *Channa punctatus* could recognize red as a distinct hue and distinguished it from violet without any difficulty. This shows that visible spectrum is not narrowed towards the short wave end in *Channa*. That violet and even ultraviolet at 313–233 μm are visible to sticklebacks was reported by Merker (1934). But he suspected conversion of ultraviolet into visible light through fluorescence of water.

Literature cited

Ali, M. A. 1975. Vision in Fishes. Vol. 1. NATO Advanced Study Institute Series. Plenum Press, N.Y.

Anon, 1962. The wealth of India. IV. Fish and Fisheries. Council of Scientific and Industrial Research, New Delhi.

Blaxter, J. H. S. 1970. Light: fishes. Pages 213–320 *in* O. Kinne, ed. Marine Ecology Vol. 1, part 1 (environmental factors). Wiley—Interscience, London.

Frisch, K. Vom. 1925. Farbensinn der Fische und Duplitzitatstheorie. Z. Vergleich. Physiol., 2: 393–452.

Guma'a, S. A. 1982. Retinal development and retinomotor responses in perch, *Perca fluviatilis* L. J. Fish Biol. 20: 611–618.

Hamburger, V. 1926. Versuche uber Komplementar Farben bei Ellritzen (*Phoxinus laevis*). Z. Vergleich. Physiol., 4: 286–304.

Jana, B. B. and N. C. Sukul. 1972. Colour vision in an Indian fish *Anabas testudineus* (Cuv.). J. Bombay Nat. Hist. Soc., 69: 10–16.

Lowe-McConnell, R. H. 1975. Fish communities in tropical freshwaters. Longman, London.

Marks, W. B. 1963. Difference spectra of the visual pigments in single goldfish cones. Doctoral Dissertation, Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Maryland.

Merker, E. 1934. Die Sichtbarkeit ultravioletten Lichtes. Biol. Rev. Bd., 9: 49–78.

Muntz, W, R, A. and J. R. Cronly-Dilton. 1966.

Colour discrimination in goldfish. Anim. Behav., 14: 351–355.
Norman, J. R. and P. H. Greenwood. 1975. A history of fishes. Ernest Benn Limited, London.
Wolfe, H. 1925. Das Farbenunterscheidungsvermögen der Ellritzen. Z. Vergleich. Physiol., 3: 279–329.

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空気呼吸の硬骨魚3種における色彩識別能力

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色調の識別能力が空気呼吸の硬骨魚3種，*Channa punctatus*, *Clarias batrachus* および *Heteropneustes fossilis* において試験された．よく発達し，能率のよい眼をもつ *Channa* が赤を緑，青，黄，灰色および紫から識別するようにうまく訓練された．*Channa* はまた，紫を青，緑および灰色から，同じく緑を青から区別した．この魚の色覚機構は三原色から成りたっている．底生魚である *Clarias batrachus* および *Heteropneustes fossilis* は大変小さい眼をもっており，非常に貧弱な色彩識別能力を示した．そして多分，これらの魚では視覚の能力低下を補うために，ひげが現われてきたのである．