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Effect of contrasting background colour change in fish: *Rasbora elanga*

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Abstract
Many lower vertebrates are able to adopt the colour of their skin to that of their background. This adaptation is possible because of the ability of these animals to shift pigment in certain special cells in their integument the chromatophores (Parker, 1948; Waring, 1963; Fujii, 1969, 2000; Bagnara and Hadley, 1973). The most common type of chromatophores are the black/brown melanophores, which contain the dark pigment melanin. When the background is lighter in nature, the pigment melanin is made to aggregate and it contributes little to the colour of the animal and the animals look pale but when the background is dark and melanin gets dispersed throughout the cytosol of the cell, it imparts its colour to the animals which look dark in order to blend with the background.

Keywords: Chromatophore, Melanophore, Aggregation, Dispersion

Introduction
Animal species, ranging from insects to different vertebrates, have characteristic body colours and patterns. These are the traits that are under strong selection pressures and colour patterns often evolve as adaptation to environmental surroundings, in which it frequently resulted in cryptic colouration. The sexual selection acts on colour and patterns leading colourful displays and also more diverse colouration patterns (Endler, 1980) [4]. We have numerous examples across the animal kingdom crustaceans (Thurman, 1988) insects (Hinton & Jarman,1972) Cephalopods (Norman, 2000) amphibians (King *et al*., 1994) reptiles (Cooper & Greenberg, 1992) and fishes (Kodric-Brown, 1998) which clearly demonstrate the importance of cryptic colours and patterns as an anti-predator strategy (Ryer *et al*,. 2008, Cook *et al*., 2012). The colouration in fish is labile and can be manipulated at the individual level both by morphological (Sugimoto 2002, Leclercq *et al*. 2010) [13, 10] and physiological colour change (Aspengren *et al*. 2009 a, b) [1]. These chromatic responses are of importance for both camouflage and communication. The ability to undergo colour changes may thus have a suppressive effect on selection by allowing a larger niche.
In poikilothermic vertebrates two levels of physiological (transitory) colour change responses are recognized "Primary responses" in which chromatophores respond directly to incident light (Babak 1910) [2] specially during embryonic and early larval or later stages, though not universal and "secondary responses" to background associated with later larval stages and adult life (Wenckebach, 1886; Babak, 1910; Hogben and Slome, 1931; Bagnara and Hadley, 1973; Fujii, 1969 and 2000) [2, 9, 3, 5, 8]. These latter responses that are the usual chromatic adaptation and they are mediated through the eyes and controlled by the nervous system and/or hormones.

Materials and Methods
The fresh water teleost fish, the *Rasbora elanga,* with mean overall length of 5-8 cm. and a mean weight of 7-10 grams respectively were used in the present study. Fish were procured with the help of a local fisherman from Ramsagar reservoir situated in Datia, Madhya pradesh, India. The fishes were used of either sex with average weight and size. On the day of their arrival to the laboratory, fishes were treated with water containing $KMno_4$ to prevent them from infection. They were stocked routinely in transparent glass aquaria (30x30x60 cm.) for a week at temperature 18-30 ^{0}C under natural photoperiodic condition. They were given a period of a week to get adapted to the given conditions (acclimatization), so as to make them suitable for experimental work.

Procedure

Background-related colour changes (physiological/ transitory or chromomotor colour change)

To study the colour change of skin to background tones in light, the healthy fish from the stock tank were placed in natural light condition, were taken out and placed in white/ black background with overhead illumination. Five fish as experimental groups were placed for a period of 24 hrs in round glass trough (30.5 cm diametter) painted black/white and covered with black/ white cotton net on the open surface of the trough so as to serve as the black/ white background. To study the rate of paling these black adapted fish from the aquarium were gently transferred to white painted glass trough. The pre experimental shade was recorded using Muncell grey series colour standards. The colour change were recorded at regular intervals of time until no further change was noticed for a considerable time period. To study the rate of darkening white adapted fish were gently transferred to a black trough and the same procedures as mentioned above were followed.

Matching the shade of fish: Munsell Grey series Index number

Munsell Grey series has a colour range from perfect black to perfect white, includes 18 colour standards which are arbitrarily numbered between 1 to 9 with half step resolutions in between 1.5 to 2.5 and so on. 1 represents the maximal darker shade while 9 represents the maximal lighter shade. This entire scale in itself is able to take care of the possible paling or darkening that the most fresh water fish species may undergo in natural background condition or if adapted to white or black backgrounds in laboratory under lighting condition.

Muncell grey series Index number Standard quality of light reflectance as selected for present study

1. 1.5 % (Maximum darkening)
2. 3.0 %
3. 6.5 %
4. 12.1 %
5. 19.1 %
6. 30.3 %
7. 44.3 %
8. 57.5 %
9. 72.5 % (maximum paling)

Colour standards prepared by exposing bromide photographic paper for different period of time under constant illumination of controlled light intensity were obtained from late prof. R. Fujii, Dept. of Biology, Toho University, Chiba (Japan). Thus nine rectangular strips were cut and mounted on a wooden platter (27× 10× 0.7 cm.) in a linear fashion starting from index 1 to 9. In a row below to this similarly nine strips with a reverse order i. e. from 9 to 1 were mounted for convenience of matching the fish. Two such wooden boards were used one was painted black and other white with synthetic enamel paints before the strips were mounted on.

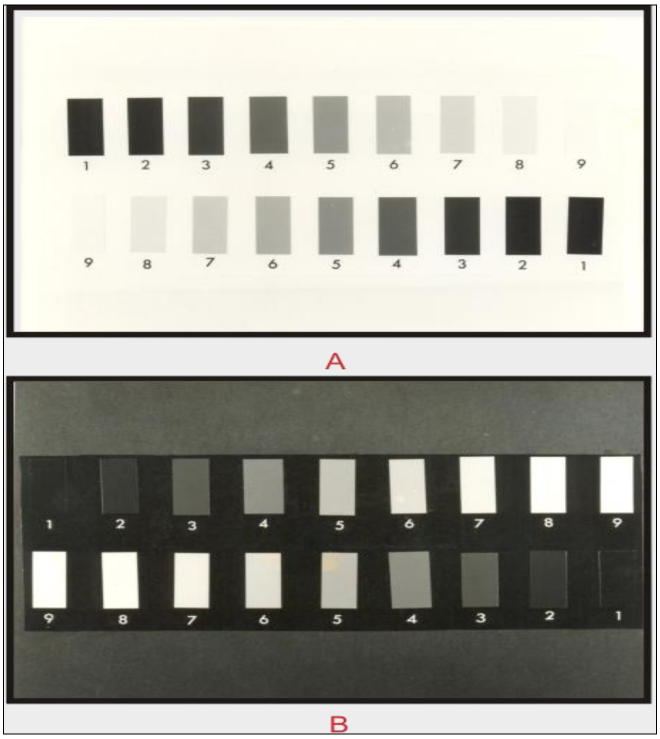

Fig 1: Munsell Grey Series Index number

Results

Rate of paling: The rate of paling in the fish (24 hr black-adapted fish) was quite rapid initially for first 5 min of adaptation where the fish having grade 3.1 (Munsell Colour Index) attained the paling equivalent to the grade of 5 in 15 min. The maximum Paling achieved by fish was 7.3 when they were allowed to remain in the white background for 24 hr.

Rate of darkening: The rate of darkening in the fish (24 hr white-adapted fish) was quite rapid initially similar to the rate of paling in the fish. It was rapid for first 15 min., in which body shade was recorded as 6.1. Subsequently, the rate of response was observed to be quite slow and gradual and after 3 hr the grade attained was 4.2 in the scale. The maximum darkening of fish equivalent to grade 3.1 was recorded on the next day at a stage of 24 hr of adaptation.

On both white and black background chromatic responses appear to be completed in two phases. The initial phase was rapid and lasted for 15 min. Further changes as a second phase occurred slowly and gradually until maximal adaptation to the respective backgrounds was attained. The shade varies between grade 3.1 to 7.3 and thus these grades may be treated as minimum and maximum for a normal fish

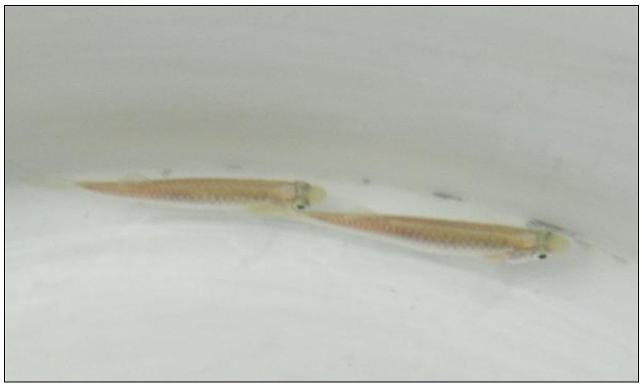

Fig 2: White adapted fish over a white background

Fig 3: Black adapted fish transfer to a white background.

Table 1A: Rate of paling in black-adapted fish on being placed over a white background.

Time	Minutes					Hours				
Fish no.	**0**	**5**	**10**	**15**	**30**	**1**	**2**	**3**	**5**	**24**
1	3	4	4.5	5	5.5	6	6	6.5	6.5	7
2	3.5	4	4	4.5	5	5.5	5.5	6	6.5	7
3	3	3.5	3.5	4	5	5.5	6	6.5	7	7.5
4	3	3.5	4	4.5	5	6	6	6.5	7	7.5
5	3.5	3.5	4	4.5	5	5.5	6	6.5	7	7.5
Mean	3.2	3.7	4	4.5	5.1	5.7	5.9	6.4	6.8	7.3
SD	0.24	0.24	0.31	0.31	0.2	0.24	0.2	0.2	0.24	0.24

Table 1B: Rate of darking in white adapted fish on being placed over a black background.

Time	Minutes					Hours				
Fish no.	**0**	**5**	**10**	**15**	**30**	**1**	**2**	**3**	**5**	**24**
1	7	6.5	6	6	5.5	5	4.5	4.5	3.5	3
2	7	6.5	6.5	6	5	5	4.5	4.5	4	3.5
3	7.5	7	6.5	6.5	6	5.5	4	4	4	3.5
4	7	7	6.5	6	5.5	5	4.5	4	3.5	2.5
5	7.5	7	6.5	6	5.5	5	4	4	3.5	3
Mean	7.2	6.8	6.4	6.1	5.5	5.1	4.3	4.2	3.7	3.1
SD	0.24	0.24	0.2	0.2	0.31	0.2	0.24	0.24	0.24	0.37

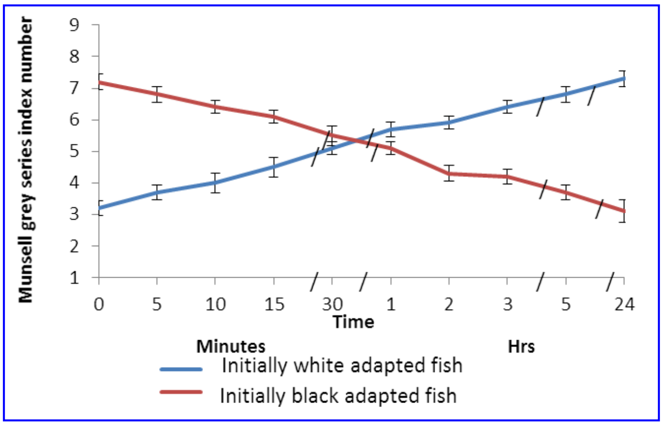

Fig 4: Change in body shade of black and white adapted fish as a result of adaptation to white and black background with overhead illumination.

Discussion

Among the poikilothermic vertebrates, the frogs and reptiles usually change colour as an adaptation to the background, in the timeframe of an hour or more while some fish can adjust in minutes (Kelman *et al*., 2006; Rhodes & Schlupp, 2012). It is partly due to neurohumoral regulation of fish chromatophores (where full range of mechanisms of control of chromatophores is realized), whereas the frogs and reptiles use hormones for this purpose (Sumner, 1940; Fujii, 2000) [14, 8], but there are also differences at the intracellular level where chromatophores in fish show smaller, better co-ordinated and higher speed of the pigment organelles than the frogs or reptiles (aspergren *et al*., 2009 a, b) [1]. Researchers (Oshima and Fujii, 1987; Nagaishi and Oshima, 2006; Mathger *et al.*, 2003) focusing their studies on bright coloured reflecting iridophors or leucophores could record that colour change in some fish possessing these can cells occur in or even within seconds. According to Kasai and Oshima (2006) these cells may also be regulated by light directly, very rapid colour changes have been observed for several fishes (Ramchandran *et al*.1996; Kodric-Brown, 1998; Mathger *et al*., 2003) but actual time measurements or data on mechanisms are often unreported. In fishes control of visual (secondary) chromatic responses are co-ordinated either entirely neurally, entirely hormonally or through an interplay of these agencies in various proportions. In majority of fishes, the chromatic changes as are sponse to different backgrounds are elicated due to the combined effects of hormone(s) elaborated from hypothalamus or pituitary and/or released from the pituitary gland and the neurohumor (s) released at the adrenergic neuro-chromatophore juctions.

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