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RESEARCH ARTICLE

BACKGROUND ADAPTATION IN THE NOCTURNAL AFRICAN CATFISH, CLARIAS GERIEPINUS

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ABSTRACT

The observations on the fascinating aspect of animal behaviour dealing with physiological colour changes in the fish as a result of adaptation to a lighter and a darker background has been taken up to understand the mechanisms relating to this aspect in a nocturnal African catfish, *Clarias geriepinus*.

The rate of colour change were studied in *Clarias geriepinus* (Burchell, 1822). The studies in *Clarias geriepinus* include visual background related transitory colour changes, the nonvisual chromatic responses in the blinded fish on exposure to light and darkness.

The data obtained with regard to the rate of colour changes over two contrasting backgrounds i.e., a lighter (white) and a darker (black) background do point that when the fish are subjected to background reversal (i.e., a black adapted fish is transferred to a white background and vice-versa), they change their colour initially at a faster speed for 10 min. which is followed by a slow and gradual colour change at a later stage i.e., the colour change accomplished is biphasic. Apparently, the nervous system of fish has evolved to allow faster chromatic adaptation than can be achieved via the hormonal system.

The rate of colour changes as a result of adaptation to different backgrounds i.e., white and black do point for a biphasic control, where they appear to be initiated by neural control and completed by simultaneous and synergistic hormonal control in the fish.

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INTRODUCTION

Chameleon is notoriously famous for its colour change abilities with respect to adaptation to the backgrounds they inhabit. Fishes are also known to exhibit such physiological colour changes along with the amphibians, all belonging to the group of poikilothermic vertebrates this physiological colour change, when fish are exposed to various background colours, are relatively fast and reversible while amphibians accomplish the phenomenon relatively slow, where it is largely limited to changes in luminance and appears to function most often in background adaptation (crypsis) and thermo regulation (King *et al*, 1994, Garcia and Sih, 2003, Stegen *et al*, 2004), in reptile, however, most species use colour change for both crypsis and signaling. In sharp contrast in many fish families (at least 24) with display of permanent or seasonal sexual dichromatism (Kodric-Brown, 1998), the rapid colour change (occurring in minutes, seconds or even milliseconds) is typically expressed more by males than females and function in both courtship and contests.

Since the colour changes in fishes were first made the object of experimental study. The pattern of colouration of fishes and the rapid changes of these colour patterns in some species, are due to the presence and cellular activity of pigment containing cells located in the integument. These are specialized skin cells that are commonly referred as chromatophores, and exist as three basic types, melanophores, xanthophores or erythrophores and iridophores. There are great variations among teleost fishes for the time required by the fish for changing its colour from a completely dark stage to a completely pale stage and vice-versa.

The colour change observed in poikilothermic vertebrates, defined as rapid changes in colour caused by intracellular movement of pigment, has intrigued scientists for over 80 yr. This pigment translocation can have wide ranging visual effects, from the dramatic social colour changes in chameleons, to the more subtle background adaptation observed in most fish and amphibians.

A fundamentally similar neuronal circuit form receptor (eye) to effector (chromatophores in the skin) can explain the background-related paling and darkening in the fish under

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study, though it is inadvisable to draw any conclusion regarding relative degree of nervous co-ordination or hormonal influence in the control of colour change in the fish. On comparing the background responses of other species (Neill, 1940; Waring, 1963; Healey, 1967; Khokhar, 1971; Dwivedi, 1972; Jain, 1975; Jain and Bhargava, 1978; Bhatnagar, 1985; Prabhakar, 1988; Garg, 1989; Sharma, 1990 and Handa, 2008), it becomes apparent that though the colour change in the fish are initiated neurally, yet it controls only a relatively limited colour change as reported earlier for *Ictalurus* and *Parasilurus* (Khokhar, 1971), *Clarias batrachus* (Jain and Bhargava, 1978) and *Heteropneustes fossilis* (Bhargava and Jain, 1978) and the subsequent hormonally controlled phase requires a much longer time. It is in sharp contrast to species like, *Phoxinus* (Healey, 1951; Pye, 1964), *Lebistes reticulatus*, *Gasterosteus aculeatus*; *Salmo salar* (Hogben and Landgrebe, 1940; Neill, 1940), where initial neural control covers a large part of the background-related transitory colour change and remaining slow and prolonged phase is taken care of by the hormonal means.

In spite of the fact that the study on the transitory colour change in teleosts has increased several times (*Crenilabrus pavo*, von Frisch, 1912; *Fundulus heteroclitus*, Connolly, 1925; *Ameiurus nebulosus*, Parker, 1934; *Holocentrus ascenciones*, Smith and Smith, 1935; *Parasilurus asotus*, Matsushita, 1938; *Paralichthys dentatus*, *Lophopsetta maculata* and *Pseudopleuronectes americanus*, Osborn, 1939 a, b; *Gasterosteus aculeatus*, Hogben and Landgrebe, 1940; *Anguilla vulgaris*, *Lebistes reticulatus* and *Salmo salar*, Neill, 1940; *Molliesia latipinna*, Pierce, 1941; *Tautoglabrus adpersus*, Hunter and Wasserman, 1941; *Prionotus strigatus*, Lee, 1942; *Oryzias latipes*, Ando, 1960 and many others), the representation of Indian species (*Lepidocephalichthys guntea*, Bhargava and Dwivedi, 1970; *Macrones chryseus*, Jain and Bhargava, unpublished; *Rasbora daniconius*, Dwivedi, 1972; *Nandus nandus*, Jain, (1975).

According to Waring (1963), the melanophore response to light may be divided in two categories, primary (non-visual) and secondary (visual). The non-visual responses do not involve the eyes as receptors and they are coordinated through an extra-ocular reflex, a response involving nervous/endocrine coordination between a stimulus received by a receptor other than the eye and the melanophore or uncoordinated response in which the melanophore or possibly a skin receptor close to it directly respond to light stimulus (Fingerman, 1963; Bentley, 1976). In contrast the visual responses are the result of reception of light by the lateral eyes and usually depend upon the nature of the background, often spoken as black (dark) and white (light) background responses and are coordinated by nerves or hormones or more commonly by both the mechanisms.

The present observation are concerned with the study of rate of colour change mechanism in the fresh water teleost, *Clarias geriepinus* as a result of background response and the colour response of melanophores, the effect of light and darkness on the chromatic response of the blinded fish. The African sharptooth catfish is a large, usually of dark grey or black coloration on the back, fading to a white belly, under normal

background conditions. The region behind the head and anterior to the dorsal fin shows uniform response during the paling or darkening phase of the colour change mechanism and thus this part of the fish has been considered appropriate for the purpose of observation for recording the background responses in the fish.

MATERIALS AND METHODS

Adult *Clarias geriepinus* of either sex were purchased from the local commercial sources (Ladhedi, Gwalior M.P.). They were stocked routinely in transparent glass aquaria (60x30x30cm) for a week at temperature ranging from 21°C to 27°C under natural photoperiodic conditions. The experimental fish ranged from 16 to 22cm in length and were regularly fed on live earthworms. The experiment was performed to study the rate of colour-change mechanism (i.e., rate of paling and the rate of darkening) in the fish as a result of white and black background response under constant illumination.

For studying colour changes of skin to background tones in light the healthy fish from the stock tank placed in natural light condition were taken out and placed in white/ black background with overhead illumination (40 watts). Five fish as an experimental group were weighted and placed for a period of 24 hrs. Over an aquarium (30x10x10 cm) painted black on outer walls and covered at top with black cotton net tied on the outer front of aquaria. To study the rate of paling these black adapted fish from the aquarium were gently transferred to white painted glass troughs. The pre-experimental shade was recorded using Munsell grey series colour standards. The colour changes were recorded at present regular intervals of time until no further change could be noticed for a considerable time period. Then the fish were transferred to a white background in an aquarium of similar size but painted white on outer walls and covered with white cotton net tied similarly as in the previous case and allowed to remain there for a period of 24 hrs. So as to adapt the fish to its maximum on this background.

To study the rate of darkening the white-adapted fish were gently transferred to a black troughs and the same procedures as mentioned before was adapted for taking the observation until no further change in their shade could be noticed for a considerable time period.

Ranging from perfect black to perfect white, the Munsell grey series includes 18 colour standards which are arbitrarily numbered between 1 to 9 with half step resolution between 1.5 to 2.5 and so on 1 represents the maximal darker shade while 9 represents the maximal lighter shade to accommodate most of the changes a fish can undergo using its adaptation to natural backgrounds.

For the purpose of this experiment the blinding of fish (stocked animals in constant light) was achieved by anaesthetizing them in 1% paraldehyde solution. Both the eyes were removed by making an incision in the skin around the eyeball and then by transecting the optic nerve. After their recovery from the effects of anesthesia these blinded fishes were placed under darkness for a minimum period of 24hrs. The chromatic

response of light of (40w) was studied in the same trough without any physical disturbance to the fish. The initial and subsequent observations were carried out at definite time intervals until the fish attained the equilibrium and there was no more change in the shade. The observations are plotted graphically Fig-.

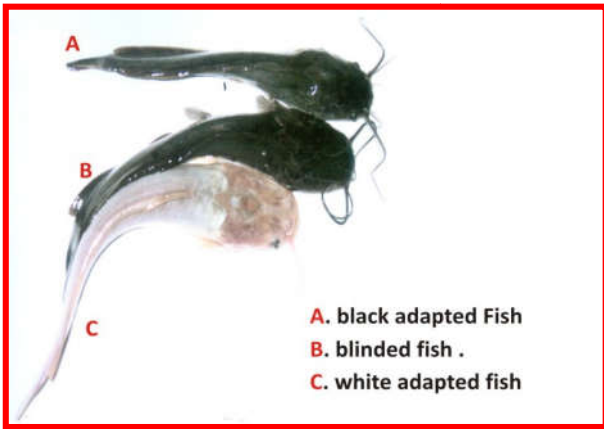


Figure 1

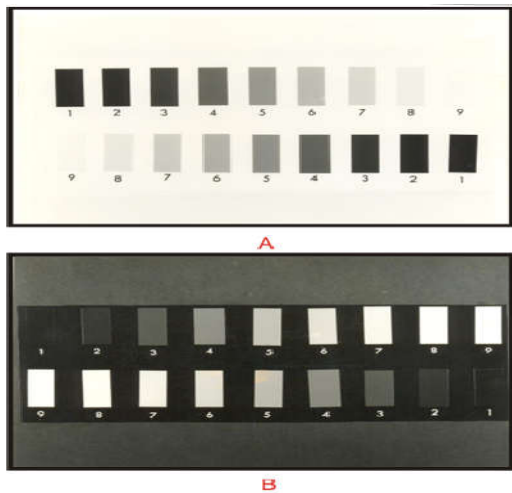


Fig 2 The Munsell Colour Index (MCI) scale as used in measuring the melanophore responses of the fish over two contrasting backgrounds (A) White background (B) Black background.

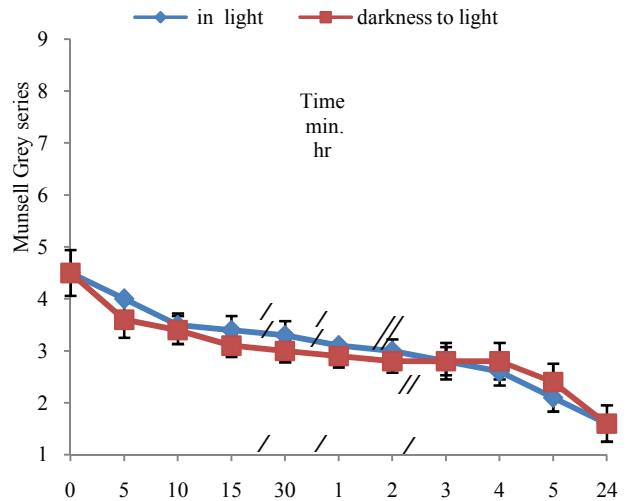


Fig 2 Effect of colour change in blinded fish *Clarias geriepinus* on exposure to light and darkness to light. The vertical lines represent the standard deviation.

RESULTS

From the graphical representation of the data, it is found that as a result of background response the process of colour change, i.e., the rate of paling as well as rate of darkening, is quicker during the first 10 minutes and thereafter it becomes slow and gradual. It is rapid in the first 10 minutes where the fish attained the M.C.I. 1of 4.8 grade. Thereafter, the process of colour change becomes slow and gradual and the fish attains the grade of 6.7 of M.C.I in five hours. When the fish were allowed to remain in the white background overnight they attained the maximum paling of grade 6.9 at 24 hour stage of adaptation.

The rate of darkening is also rapid in first 10 minutes where the grade attained by the fish was 3.7. Then the colour change became slow and gradual and in 5 hr stage of adaptation, the fish attained the grade of 2.8. They further change their shade to a maximum of grade 2.6, when allowed to remain in the same background for a time period of 24 hrs.

Thus the chromatic response in the fish in these experiments fluctuated between final grades of 6.9 and 2.6 in white and black backgrounds respectively, which may be referred as the maximal range in the chromatic states that a normal intact fish can adapt to. Blinded fish failed to respond to changes in relation to their background. They remained dark on a black as well as a white background in light. After blinding (10 min), the body shade of fish in white background was recorded as grade 4.5. The fish became dark and it was observed as I.N 3.4 at 15 min. The rate of darkening slows down after 1 hr post blinding.

At 5 hr. stage, the darkening exceeded even greater than that recorded in intact fishes with 2.1 I.N. When observed at 24 hr stage, a further enhancement in the darkening (1.6 I.N.) was quite evident. The light exposed fish when placed in darkness-they paled and showed a grade of 4.5.

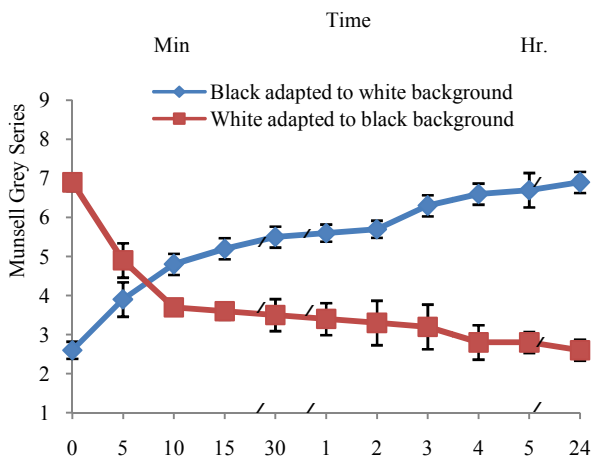


Fig 3 Rate of darkening and paling in white/black adapted fish placed on black and white background respectively. The vertical lines represent the standard deviation.

When the fish kept on darkness overnight were exposed to light, fish further became dark rapidly. Body shade as recorded in 15 min. was about 3.1 in the I.N. Fish adapted to a grade of 2.4 in 5 hr., their maximum darkening within 24 hrs was equivalent to grade 1.6. A comparative study of both the processes indicates that in the first 10 minutes the rate of colour change is more or less the same while in the later phases, the rate of paling of the fish is faster than the rate of darkening and thus enabling the fish to become more easily adapted to a white background than to a black background.

DISCUSSION

The fresh water teleost, *Clarias geriepinus* is quite sensitive in its background response and like many other teleosts it becomes dark on a black background and pale on a white one under overhead illumination in its attempt to blend with the background. The receptors for the adaptation to background are the eyes, since after enucleation the background responses (both black and white) were abolished and likewise the animals attained an intermediate shade. With the results obtained, it is possible to divide the background response (both white and black) of the fish into two phases – the initial faster one and the later as slow, gradual and prolonged. Such a faster chromatic response as has been observed in the initial phase is suggestive of a nervous co-ordination and the subsequent prolonged phase points to a hormonal mechanism working simultaneously.

There is a general agreement that teleost melanophores are innervated by at least the sympathetic pigment-aggregating fibres (Parker, 1948; Waring, 1963; Bagnara and Hadley, 1973; Bentely, 1976). In the present study it is found that the fish is easily adapted to a white background as compared to its adaptation to black background. This suggests the involvement of paling a fibre which initiate and control the initial rapid background response and which is further supplemented by a hormonal mechanism (melanin-concentrating hormone – MCH) during the later phase of background response.

The fish *Clarias geriepinus* belongs to the osteichthys group of fishes in which the nervous system has evolved mechanisms for the regulation of rapid and quickly reversible changes in coloration (Fujii and Oshima, 1986). In vertebrates, the endocrine system is almost universally operative. Until now, however, no reliable data are available about the possible role of nervous system in lower classes of fish including Chondrichthys. So, Fujii (1993) is of the view that presumably, hormonal regulation is exclusively responsible for the motility of their chromatophores. Among vertebrates higher than fishes, incidentally, only a few lizards, including Chameleons, are known to have neurally controlled chromatophores.

Thus in the light of above it may be proposed that the initial rapid paling/darkening in the fish is due to neurally controlled melanophores in this fish under present study. The transitory colour changes as a result of adaptation to a white and black background in the fish are due to differential stimulation of eye whereupon the information of the nature of background is sent to CNS where it is immediately processed and sent to chromatophores via direct nervous connection.

The colour change so recorded in the fish under study significantly varies with species like, *Fundulus* (Parker and Brower, 1937), *Molliensia latipinna* (Pierce, 1941) and *Macropodus opercularis* (Umrath and Walcher, 1951) in which the rapid colour changes in response to white/ black illuminated background are mediated entirely through the nervous system and hormones have little role to play. It also significantly varies with some other species like *Anguilla anguilla* (Neill, 1940) and *Amphipnous cuchia* (Bhargava and Shrivasa, 1983), where control of colour change is predominantly hormonal while neural control is of little importance. It thus appears to occupy a position between *Phoxinus*, *Lebistes*, *Gasterosclous* and *Salmo* on one hand and *Anguilla* and *Amphipnous cuchia* on the other.

The background-related colour changes are abolished on blinding the animal and likewise the fish attain a darker shade (I.N. 1.6). Such an effect of blinding on pigmentation also has been reported for various other teleosts that have been discussed earlier for time relations pertaining to background-related chromatic responses. As the slow, gradual and prolonged phase of colour change is likely to be dependent on hormonal regulation of melanophores in the fish and as the melanophores are responsive to α -MSH (Fig.14), the darkening in the intact fish appear to result in the release of pars intermedia from a central inhibitory control resulting in an uncontrolled secretion of MSH.

In the present work, it was found that the darkening in the blinded fish (1.6 grade of M.C.I.) achieved after exposure to light, is of greater extent and beyond to that in otherwise intact completely black-adapted fish (2.6 Grade of M.C.I.), in response to an illuminated black background. This may indicate a role for pineal in a non-visual chromatic response probably through further inhibition of the paling centre.

In *Clarias geriepinus*, the melanophores cover or uncover the pineal area in response to changes in light as well as the colour of the background. In this respect this catfish resembles such fishes as *Sphyrna barracuda*, *Strongylura rotata*, *Hemiramphus besiliences* and *Synodus synodus* (Breeder and Rasquin, 1950), *Heteropneustes fossilis* (Bhargava and Jain, 1978), *Clarias batrachus* (Garg, 1989). However, it differs with species such as *Astynax nexicanus*, *Ameiurus nebulosus*, *Haemulon milanurum* and *Thalassoma bifasciatum* (Breeder and Rasquin, 1950) where the pineal areas are permanently covered, the tissues are apparently two opaque to permit entry of light.

Have made a comparative survey of the type of sympathetic neuro-melanophore transmission in catfishes (order Siluriformes). 3 variants in this regard have been presented. The first one holds that adrenergic post-ganglionic fibres of the sympathetic system control the rapid melanin aggregating responses of integumental melanophores. It is an orthodox kind of regulation reported in large variety of teleosts (cf. Fujii and Oshima, 1986). The second type was characterized as the cholinergic one and reported so far for only two species of siluroids i.e., the Japanese common catfish *Parasilurus asotus* (Fujii nad Miyashita, 1976) and the translucent glass catfish, *Kryptopterus bicirrhii* (Fujii *et al.*, 1982). The third type being

described by them was the one in which adrenergically controlled melanophores were shown to possess extra cholinceptors (Corydoras paleatus, Mystus vittatus, Pimelodella pictus etc.

The work in India with regard to fish chromatophores and colour change includes aspects like integumentary colour pattern (Bhargava and Jain, 1974; Dwivedi, 1978) transitory colour change (Jain and Bhargava, 1978), quantitative colour change (Dwivedi, 1976; Sharma, 1990, Circadian colour change (Bhargava and Jain, 1981), effect of pollutants on melanophores and colour change and effects of other automatic drugs on various fresh water fish species that have been investigated from India include Nandus nandus (Jain, 1976), Rasbora daniconius (Dwivedi, 1978), Heteroneustes fossilis (Bhargava and Jain, 1978; Gupta, 1992; Rajiv and Bhargava, 1990; Shrivastava, 1991; Rajiv, 1989), Labeo rohita (Jain and Patil, 1990, 1992), Punitius conchonus (Khare, 1990), Punitus sophore (Dwivedi, 1995), Cyprinus carpio (Bhatnagar, 1985; Chowdhery, 2003; Gulzar, 2011), Trichogaster trichopterus (Handa, 2007) and Clarias geripinus (Singh, 2008), Balantiocheilos melanopterus (Rather, 2009), Mystus vittatus (Gupta, 2009) and Catla catla. Lokhande (2003), however, has characterized the adrenoceptors on the melanophores of this species. In a preliminary report, we have presented the results of some pharmacological agents concerning nervous regulation of melanophores in the fish with some interpretation on the adrenergic peripheral transmission to melanophores along with the implication of microtubules in the translocation of the pigment and a possible role for cAMP in the signal transduction processes in regulation of melanophores in the fish.

For the genus under present investigation i.e., Clarias only five reports on these aspects are available for the species- Clarias batrachus. According to one (Jain and Bhargava, 1978) the transitory background-related colour changes in the fish seem to be regulated both by nerves and hormone (s) from the pituitary, as concluded by time relations shown by the fish as a result of adaptation to black/white background. In another report on this fish, Gurjar (1980) has stated that hypophysectomy results in paling of the fish thereby providing evidence for the role of MSH in its background-related chromatic response. In the third report on the fish (Garg, 1989), nerve to melanophore transmission has been shown to be mediated by post-junctional adrenoceptors of alpha type (more specifically α_2 subtype) and the synthetic salmonid melanin concentrating hormone (MCH) has been shown to induce significant paling when administered intraperitoneally to the black-adapted Clarias batrachus. However, in the fourth report (Patil, 1991) evidence has been presented for existence of a melanophore- aggregating principle in the pituitary of this fish, as the pituitary extract was found to produce paling when administered to another catfish, the Heteropneustes fossilis. In the 5th report on the fish working on control of melanosome movements, Ovais (1994) confirmed the role of adrenergic receptors in melanosome aggregation as pointed by earlier worker (Garg, 1989). He further indicated the presence of cholinceptors of both types i.e., nicotinic as well as muscarinic with a role in dispersion of melanophores, thus putting the fish, Clarias batrachus in the 3rd category of catfish (described above), where adrenergically controlled

melanophores have been shown to possess extra cholinceptors.

CONCLUSION

In conclusion it may be suggested that the mechanism of colour change in Clarias geriepinus involves both the nervous (pigment-aggregating fibres) and hormonal (at least a melanophore- aggregating hormone) systems working synergistically during the entire process. In this respect the chromatic response in Clarias geriepinus resembles with that of daniconius (Dwivedi, 1976) and Nandus nandus (Jain, 1978 a, b). The rate of colour change in the fish Clarias geriepinus has been studied as a response from black to white background and vice-versa, the effects of light and darkness on the chromatic response of the blinded fish. The Munsell grey series on the lines of Derived Ostwald Index (DOI) proposed by Healy, 1967; was used as a standard measure for these experiments. Observations show that the fish is quite sensitive to background responses. The rate of colour changes as a result of adaptation to different backgrounds i.e., white and black do point for a biphasic control, where they appear to be initiated by neural control and completed by simultaneous hormonal control in the fish. The neural control seems to be by sympathetic division of the autonomic nervous system where in post ganglionic fibres controlling melanophores are adrenergic in nature. The eyes and pineal in the fish are concerned with visual (secondary) and non-visual (primary) chromatic responses in the fish. Experiments with blinded fish do point for a role of pineal in nocturnal blanching and the circadian colour changes in the fish. Melatonin, which is known to be secreted from the pineal, is able to pale the blinded fish and darkness in itself is able to induce the same paling in the fish. The later phase of background related darkening and paling responses, seem to be depend upon the hormonal control as here the responses are slow, gradual and prolonged.

Acknowledgements

Foremost, I would like to express my sincere gratitude to my Supervisor Prof. A. K. Jain, School of Studies in Zoology, Jiwaji University, Gwalior, for the constant encouragement, critical guidance throughout the period of study and making valuable suggestions in order to give this report its present shape. My thanks are due to the U.G.C., New Delhi for various laboratory and physical facilities extended to this department by awarding DRS I & II (SAP) Programmes. The limits of colour changes displayed by intact and blinded African catfish, Clarias Geriepinus. On the top is a normal intact catfish that has been darkened by adaptation to an illuminated black background. The fish in the centre is a white-adapted fish that has been blinded (optic enucleation under anaesthesia) and kept over an illuminated white background. Removal of eyes causes moderate darkening on being exposed to light. The fish attains the maximum degree of blackness greater than intact black-adapted fish as can be compared here. The fish at the bottom in one that has been paled by adaptation to an illuminated white background. The brain can easily be viewed from the top due to increased transparency induced by aggregation of overlying integumentary melanophores.

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