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Dynamic physiological changes for adaptation to background and mechanisms underlying chromatic strategy for survival in three species of fresh–water Indian minnow, *Puntius*

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Abstract
Animals utilize conspicuous colour and pattern as "aposematic" colouration. Inconspicuous they are conversely exploited as cryptic colouration. For fishes lacking the ability of vocal communication such colours, patterns and their changes represent strategies of the utmost importance for the survival of individuals or of species. For example, protective colour, which constitutes part of the cryptic colouration is useful for avoiding attack by predator, while conspicuous displays function to frighten predators. Therefore, we must understand how the fish reversibly change their chromatic characteristics to survive in their habitat.
The fresh water Indian teleosts, the *Puntius species* were used as the experimental materials Colours of fishes are due to the pigment cells (chromatophores) usually in the dermis of skin tissue, where many varieties of them are located. All the five types of chromatophores namely melanophores (both dermal and epidermal), xanthophores, erythrophores, leucophores and iridophores are represented in *Puntius*. Rapid changes of hues as recorded in these species and chromatic patterns are caused by the motility of these chromatophore species and aggregation of pigment granules in the parikaryon and dispersion throughout the cytoplasm are the characteristics of the cellular motility of an ordinary dendritic chromatophores. These bidirectional movements are the result of perception of the background by the optic and extraoptic (pineal) receptors and are regulated neurally (autonomic nervous system) as well as hormonally (hypothalamus, pituitary) by MSH and MCH. Melatonin appears to have a role in circadian colour changes in the fish. Various neural and hormonal receptors have been characterized along with the pharmacological nature of the chromatic fibres.

Keywords: chromatic strategy, colour change, dispersion, aggregation, melanophores, fish

Introduction
Many pioneers of evolutionary biology, including Wallace and Paulton, spent considerable time discussing animal colouration and describe the types of camouflage that may exist [1, 2] providing key example of natural selection. Early expert were also aware that individual of many species could change colour and Paulton even conducted experiment into the mechanism and function of this [3]. Ever since colour change has been a valuable system to study both the adaptive value of camouflage and the physiological process shaping animal from the diversity [4].
Colour change occurs over multiple timescale cephalopods like cuttle fish can change rapidly in seconds, many fish change in minutes some crabs over a period of hours, caterpillars over days and weeks and certain Arctic animals over months [5, 6]. In many species, a fundamental reason for colour change is that it allows individuals to modify their appearance to provide camouflage turned to the habitate where they live, and to cope with environmental changes occurring over a range of spatial and temporal scales.
Body colour patterns are important for animals because they can function in inter- and intra-species communication and provide camouflage, thermoregulation and protection against solar radiation. In many taxa, colour patterns are caused by large star-shaped pigment containing cells, chromatophores, which are located in the skin. The chromatophores are grouped into subclasses based on the colour of their pigment containing organelles: xanthophores (yellow), erythrophores (red/orange), iridophores (reflective/iridescent), leucophores (white), melanophores (black/brown) and the more rare cyanophores (blue)
Skin colouration of fish is under multi-parametric control and a number of internal and external factors (physical, nutritional, neuro-hormonal) have been known to influence the chromatic state of fish [7]. Some fish can alter their colouration in response to environmental conditions, physiological challenges and stressful stimuli. Changes in skin shade, hue and/or chromatic pattern are due to changes in motile activities of chromatosomes, to the increase and decrease number of chromatophores or to differences in the pigment quantity of the chromatosomes [8-10]. In teleost, it is known that (depending on fish species) physiological colour changes are regulated by the nervous, or hormonal or more commonly by both systems [11-12]. Endocrine control of physiological colour change is mediated by MSH (melanophore stimulating hormone) and MCH (melanophore concentrating hormones). Neural control involves the sympathetic nervous system with a catecholamines more likely norepinephrines, which when at physiological concentrations causes a rapid aggregation of chromatophores [13-14].
The regulation of melanophore activity is complex, with both neural and hormonal control regulating the intracellular levels of cyclic 3-adenosine monophosphate (cAMP), a common intracellular second messenger. The autonomic nervous system activates the secretion of neurohormones, such as norepinephrine from chromaffin tissue, which are delivered by the blood to dermal or epidermal melanophores

[15]. Norepinephrine binds to G-protein coupled receptors which then activate an intracellular signal cascade, ultimately releasing calcium stores inside the melanophore [16]. Calcium then binds to calmodulin and activates phosphodiesterase which breaks down and thus reduces the intracellular concentration of cAMP. Decreased levels of intracellular cAMP result in melanosome aggregation [17]. A separate pathway has been described through which melanophores are directly innervated by nerves of the sympathetic nervous system [15]. In this case, norepinephrine is stored in the nerve axon terminal, and is secreted after depolarization [18]. Endocrine regulation of melanophores occurs primarily through the pituitary hormone melanocyte-stimulating hormone (MSH) [15]. MSH is secreted from the pituitary gland into the systemic circulation to reach receptors on the surface of melanophores [15]. When MSH binds to a G-protein coupled receptor on melanophore membranes it will activate adenylate cyclase to produce cAMP. Increased levels of intracellular cAMP result in melanosome dispersal. It is important to note that MSH and a second pituitary hormone melanin-concentrating hormone (MCH) have antagonistic effects on melanophores [15]. The endocrine pathway of melanophore regulation therefore does not only control melanosome dispersal, but aggregation as well.

One of the systems for which there exists sufficient information to attempt such a detailed model is transport of black pigment granules (melanosomes) in pigment cells of lower vertebrates (melanophores) [19]. Melanosomes can either aggregate at the cell center (cells appear light) or disperse throughout the cytoplasm (cells appear dark). Melanosome migration is mediated by motor-assisted transport on microtubule and actin filament networks. Movements on microtubules are facilitated by dyneins, which move melanosomes to minus-ends (towards the cell center), and kinesins, which move melanosomes to plus-ends (towards the cell periphery) [19]. Recently, several key components of the signaling pathways that control melanosome distribution have been identified [20-24]. Aggregation or dispersion of melanosomes is initiated by binding of hormones or neurotransmitters to G-protein-coupled receptors. These binding events alter the production of the second messenger, cyclic adenosine monophosphate (cAMP), which controls activities of protein kinases (PKA, PKC) and protein phosphatases (PP2A); these in turn are directly correlated to phosphorylation and dephosphorylation of motor proteins [25-27].

For the analysis of neural regulation of chromatophores, many drugs with established actions have been exploited as fishes suitable for pharmacological studies are readily available, inexpensive and easily kept in large numbers. The purpose of this paper is to study the chromatic strategy for survival in three species of fresh-water Indian minnow, *Puntius.*

Materials and Methods

The fresh water teleost, *Puntius species*, irrespective sex were used as the experimental material. The fish were collected and transported from Tighra reservoir located 23 km from Gwalior (M.P) and they were reared in fresh water aquaria (90 X45X45cm) in our facilities for at least a week for acclimatization. All the experiments were carried out at room temperature between 24^{0}C and 28^{0}C. The scale slips were gently plucked by mean of fine forceps from the dorsal trunk surface of the animal. The isolated scale were immediately immersed in a physiological saline solution which had the following composition in mm (NaCl; 128.3, KCl; 2.8, Glucose; 5.6, $CaCl_2$; 1.8, 0.5M Hepes-NaOH with pH value 7.4).

The effect of drug on the response of certain groups of melanophores were studied with light microscope and were evaluate according to Hogben and Slome (1931) in amphibian melanophores where 1, representing the maximum aggregation and 5, representing maximum dispersion and 2,3,4 as intermediate stage of aggregation dispersion (figure-1).

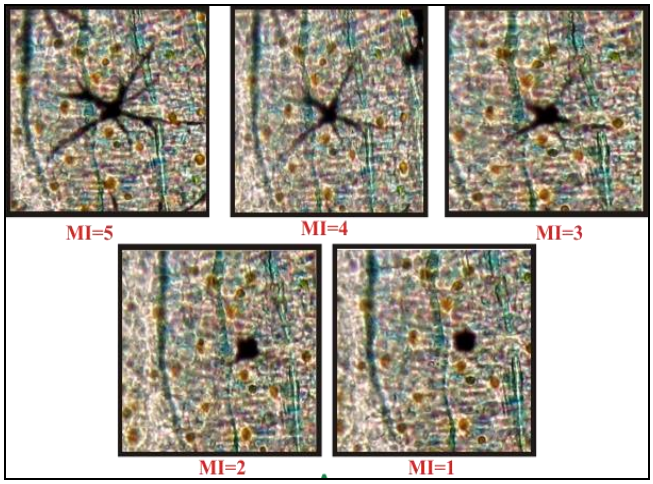

Fig 1: Melanophore indices (5-1) as were used for measurement of melanophore responses in the study.

Observation and results

Four types of chromatophores have generally been Observed, black melanophores, yellow and orange xanthophres, two types of light reflecting iridophores and white leucophores. Erythrophores were occasionally observed in only one species *i.e. Puntius sophore*, where they appear to have a role in nuptial colouration in the fish. It is a kind of recognition colouration which is seen mostly in males of various species of fish during breeding season and is usually restricted to part of the body surface. In *Puntius sophore*, a scarlet red band was noticed extending from the base of the gill to the base of the caudal fin (figure-2).

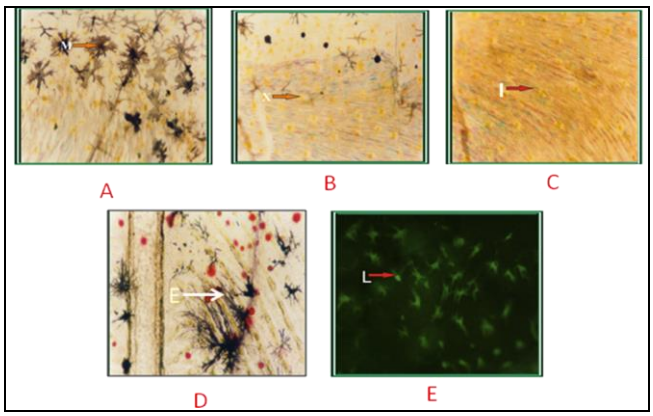

Fig 2: Photomicrograph showing of various types of chromatophores in isolated scale preparation form dorsal region of the fish (Puntius) X 100. Melanophores (M) B. Xanthophores (X) C. Iridophores (I) D. Erythrophores (E) E. Leucophores (L)

The fresh water teleosts, the species of *Puntius* are quite sensitive in their background-related chromatic responses and like many other teleosts, they become dark on a black-background and pale on a white one under overhead illumination.On both white and black backgrounds chromatic responses were completed in two phases. The initial phase was rapid and lasted for 5 min. Further changes as a second phase occurred slowly and gradually until maximal adaptation to the respective background was attained (figure-3).

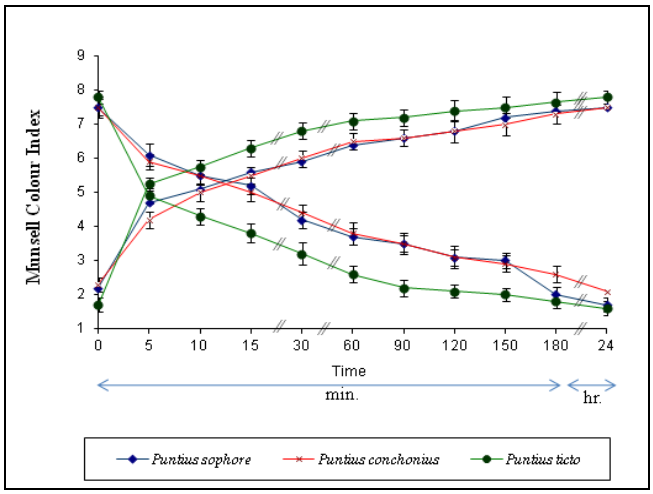

Fig 3: Change in body shade of black-and white-adapted fish as a result of adaptation to white and black background with overhead illumination. The values are expressed as mean (5 animals) ± S.D. (vertical lines) of the mean.

On 15 min perfusion with PS, the melanophores in freshly isolated scale preparations achieve the state of full dispersion (M.I=5) in all the 3 species studied. When Physiological solution was substituted with K^+- rich saline, a quick aggregation of the pigment was recorded and the state of full aggregation (M.I=1) of the pigment was achieved within 5 min (figure-4).

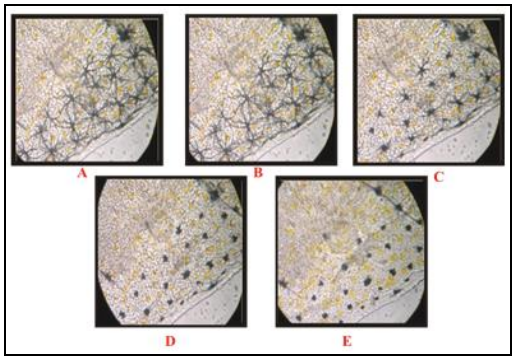

Fig 4: Typical serial photomicrographs showing responses of a group of innervated melanophores in an isolated scale preparation of the fish, *Puntius conchonius* to K^+-rich saline.

Adrenomimetic drugs such as epinephrine (E), norepinephrine (NE), dopamine (DO), phenylepinephrine (PE), clonidine (CL), isoproneline (ISO) were all capable of inducing concentration-related melanosome aggregation within the melanophores. These results clearly demonstrate the pharmacological nature of the fibres controlling the melanophores and the colour changes in the fish, the chromatic fibres belong to the sympathetic system. Yohimbine (selective alpha-2 antagonist) completely blocks the pigment aggregating effect of NE in *in vitro* responses. This supports the presence of α_2-adrenoceptors on the melanophores. Prazosin (selective alpha-1 antagonist), however failed to block such an aggregating response of NE at the concentration tested (figure-5).

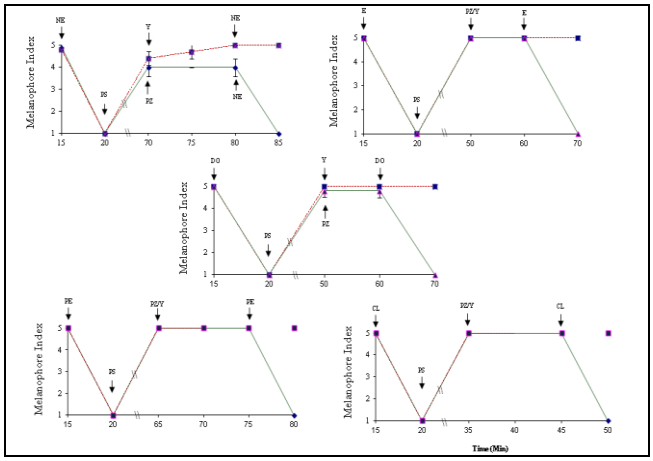

Fig 5: Effects of Prazosin (PZ) (10^{-4}M), an α_1 adrenergic antagonist and Yohimbine (10^{-4} M), an α_2 adrenergic antagonist on melanosome-aggregating effect of NE (10^{-7}M), E (10^{-6} M), DO (10^{-6}M), CL (10^{-4}M) and PE (10^{-5} M) in PS-equilibrated (15 min) melanophores of the fish, *Puntius sophore*. Values = Mean ± S.D, N= 5

For the characterization of cellular receptors mediating melanosome dispersion, the effects of certain beta-agonists have also been tried. The E aggregated melanophores when treated with ISO (nonselective β-adrenergic agonist), completely dispersed the melanosomes within melanophores. However when such melanophores were treated with Propranolol (nonselective β-antagonist), the dispersion response of the agonist was abolished. The observations thus reveal that the melanosome dispersion within *Puntius* melanophores may be mediated through the beta-adrenergic receptors (figure-6).

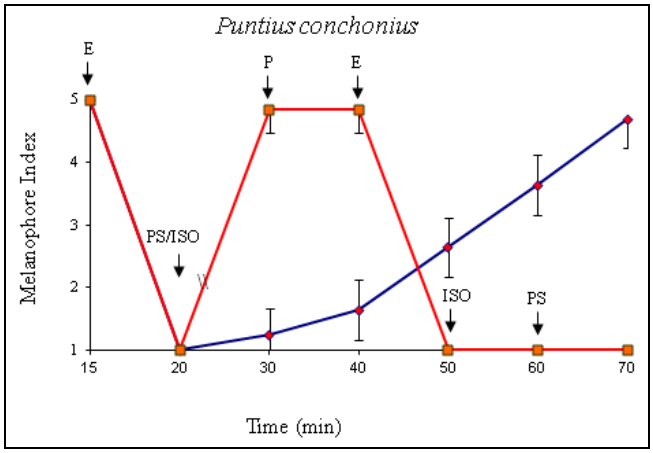

Fig 6: Melanosome dispersion response of *Puntius* Physiological saline equilibrated melanophore to ISO (10^{-7}M) and the blockade of this response by P (10^{-4}M). The melanophores were aggregated by pretreatment with E (10^{-6}M).

Discussion

The contrast in appearance between aggregated melanophores and dispersed melanophores is what we perceive as a lightening or darkening of the skin. A high

percentage of dispersed melanophores results in a dark body coloration. Melanophores are thus capable of causing dramatic changes in coloration, which is critical in vertebrates as a mode of crypsis, a signal to mates, or a warning to predators (Evans, 1993) [17]. Because of the importance of these behaviors, numerous studies have examined how melanophores are controlled. Rapid colour changes in animals, including fishes are due to the motile activity of chromatophores found in the skin. Perceived chiefly by light receptors (either ocular or extraocular), cues from the outside are integrated into efferent signals in the central nervous system to adjust the chromatophores. In fishes, both hormonal and neural systems are operating (Fujii, 1969 and Fujii and Oshima, 1986) [11, 28].

Melanophore-stimulating hormone (MSH), release from the hypophyseal intermediate lobe disperses melanosomes within the melanophores in species of *Puntius*. We also found that action is mediated through MSH-specific receptors on the target cell membrane. The pineal principle, melatonin, was also shown to affect the pigment cells, i.e, through its specific receptors, it aggregates chromatosomes leading to the blanching of the skin. Evidence indicates that melatonin is responsible for circadian color changes and for the formation of integumental colour patterns. Melanin-concentrating hormone (MCH), which originates in the hypothalamus and is secreted from the posterior lobe, has also been proven to aggregate chromatosomes through its specific receptors. The effect was brought on by the mediation of beta-adrenoceptors. Epinephrine from adrenal chromaffin cells may be responsible for the physiological darkening reaction. We presume that this system is involved in the excitement darkening which can sometimes be observed. In any case, hormonal means may have evolved to control chromatophore motility slowly, since the process inevitably includes the gradual increase or decrease of their titers in the blood.

Neural regulation, on the other hand, takes a role in adapting fish more rapidly to the environement. It further functions in regulation chromatophores more differentially among portions of the skin to form colour patterns. The innervation to the chromatophores is autonomic. Our conclusion is, however, that only the sympathetic division of the system is involved in controlling the cells. Peripheral neuro-transmission is usually adrenergic, and the transmitter is naturally nor-epinephrine. Being mediated by alpha-adrenoceptors, the transmitter produces an aggregation of chromatosomes in varieties of chromatophores.

Along may be liberated from the nerve-terminals. Converted into adenosine by relevant ecto-enzymes, co-transmitter may function to counteract the effect of the true one, thus enabling fish to change their hue very quickly. Some other bioactive substances including a few neuropeptides and prostaglandins have been shown to influence the cells, although further work is needed to establish their roles in regulating chromatophore movement. That so many receptor species co-exist on a cell is rather understandable, since the delicate changes in hues and patterns are of utmost importance for animals in the strategy to survive. The many input signals are convergently transduced at the membrane into simpler intracellular phenomena including changes in cyclic AMP and/or Ca^{2+} levels, which lead to the cellular motility [28, 29].

The sophisticated chromatic properties observed in fish provide protection from predator, advertisement territoriality and assist in both survival and intraspecific communication. Skin colour patterns are believed to be an effective comprise between the conspicuous colouration necessary for visual communication and the cryptic colouration essential for predator avoidance.

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