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Effect of Colchicine (mechanochemical response) on the melanophores of teleost fish: *Rasbora elanga*

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Abstract
Chromatophores are the neurone-like cells containing pigment granules that are responsible for the brilliant colours of fish, amphibians, reptiles, and cephalopods. Many animals' species are capable of undergoing changes in their colour as an adaptive behavioural response under the control of nervous endocrine systems, mediated by cell surface receptors on the plasma membrane of the chromatophores. Receptors causing pigment aggregation in melanophores have been shown to be linked to the Gi protein, whose activation results in a decrease in intracellular cAMP, while receptors that cause dispersion of the pigment are shown to be liked to Gs protein, whose activation results in an increase in cAMP. The chemical cycle involving a cascade of events such as enzyme activation and various substrate protein (PKA) phosphorylation (dispersion) or dephosphorylation (aggregation), initiates a mechanical cycle for long-range movements of pigment granules depending on polar microtubules and specific motor proteins such as kinesin or kinesin related peptides (dispersion) and dyneins (aggregation) that get bound to the pigment granules. Under investigation *Rasbora elanga* melanophores are endowed with β-adrenergic receptors and thus it is, likely that Ca^{2+} can also affect melanosome movements in melanophores. Among investigations indicating a positive role for microtubules are those using inhibitors of mitosis, for eg., colchicines and related alkaloids. Colchicine in the present study showed inhibitory effect on melanosome movements, in particular those in centripetal direction i.e., including aggregation. Subsequent dispersion of melanophores in PS a phosphodiesterase inhibitor appears to be consistent with respect to centrifugal migration of pigment in the melanophores.

Keywords: melanophores, aggregation, dispersion, chromatophores, phosphorylation, dephosphorylation

Introduction
The colouration of the species is characteristic and it depends not only on the type of chromatophores the animal possess in its skin, but also on the specific distribution of the chromatophores that they have. It is not necessary that all these chromatophoral types are represented in each species and variation with regards to several chromatophore characteristic do exits like their density, differential, distribution the pigment content (qualitatively as well as quantitatively) their sizes, and location in the integument and of course their branching pattern that allows the cells to cover or uncover the underlying cells or tissues, to inhibit or expose the interior of the animal that may also contribute in this expression of overall body tint in the animal. Thus cellular study of this type can bring out specific chromatic patterns morphologically determined in the fish.

In many investigation on different species of fishes the results have suggested that the colour changes in teleosts are normally under the control of both endocrine and nervous systems (Fujii 1969, 1993a, b, 2000; Abbott 1973; Bagnara and Hadley 1973; Fujii and Oshima 1986, 1994) [10, 16, 17, 13, 26, 28, 4]. But in some fish's endocrine are thought to be predominantly responsible for melanophores motility while in others, nervous mechanism seems to dominate. For survival in their habitat fishes require the ability to change their hues and colours rapidly and effectively. Teleost fishes show great variation in mechanism controlling their colour changes. The time taken by them inaccomplishing the changes varies from species to species. The importance of time relation in the study of chromatic responses in teleosts has been realised from the work of Hogbeg (1924) [25]. Number of worker published monographs and review on this. Notable among them are Waring (1963) [41], Fingerman (1963) [7], Fujii (1969, 1993a, b, 2000) [10, 16, 17, 13],
Bagnara and Hadley (1973) and Fujii and Oshima (1986, 1994) [4, 26, 28].

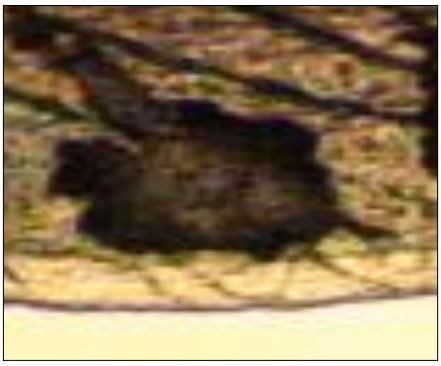
Microtubule based transport plays an important role in routing vesicular organelles with in the cells. Microtubules microfilaments and inter mediate filaments have been implicated to form intra cellular from work for translocation of pigment organelles with in cells. Pigment transport occurs through microtubules and actin filaments transport co-ordination which involves switching between the two cytoskeletal systems. Pigment granule movements in fish melanophores employ motor proteins dynein in kinesin and myosin. The pigment transport in melonophores is regulated by PKA and switching between microtubules and actin filament is controlled through changes in the levels of intracellular CAMP (Radinov *et al* 2003).

Pigment dispersion is activated by an increase in cAMP levels while aggregation occurs when cAMP levels are reduced. Epinephrine binds to a cell surface receptor, which interacts with a G protein. G protein have GTP binding and GTP hydrolysis capabilities. G protein to which epinephrine binds is an inhibitory G protein. When it is activated it inhibits the enzyme adenylate cyclase. Active adenylate cyclase converts APT into cAMP. The cAMP activates cAMP dependent protein Kinase (PKA), cAMP levels drop PKA is inhibited and the pigment granules aggregate. α_1 receptor activation may cause breakdown of phosphate dylinositol in membrane through activation of phospholipase C there by including a change in intracellular Ca^{2+}. Fujii and Fujii (1965) first reported that Ca^{2+} is required

for catecholamine release from the sympathetic nerve terminal in the goby, chosmichthys gulosus. Ca^{2+} and cAMP act in opposition to regulate pigment aggregation and dispersion in melanophores. Epinephrine binds on the cell surface receptor causing influx of Ca^{2+} into the cell from the extracellular space.

Materials and Methods

The Indian fresh water teleost fish *Rasbora elanga* (comman name *Bengala barb*) obtained from Ram sagar reservoir situated in Datia (M.P.) and maintained in an aquarium for at least one week before experiment were used. The fishes were used of either sex with average weight and size. The fresh water teleost fish, the *Rasbora elanga*, with mean overall length of 5-6 cm. and a mean weight of 5 grams respectively were used in the present study. The scale slips used in experiments conducted for this study were isolated from the dorsal trunk region of the animal. The anterior unpigmented part of the scale remains under the glass needle and the posterior pigmentary part remains free for observation. They were plucked and immediately perfused with the physiological saline which had the following composition in mm (Nacl: 12.8, KCl: 2.7, CaCl2:1.8, Glucose, 5.6 and HepesNaOH with pH value 7.4). For each individual experiment 25 melanophores from 5 different scales belonging to different animals were observed. All the experiment was performed at room temperature (20± 240C). The animals were maintained in the laboratory on commercial fish diet. Durring the experiment feeding was cut off. The aquria were cleaned regulary with the removal of faecal material and uneaten food by siphoning process. 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) [24] in amphibian melanophores where 1, representing the maximum aggregation and 5, representing maximum dispersion and 2,3,4 as intermediate stage of aggregation dispersion.



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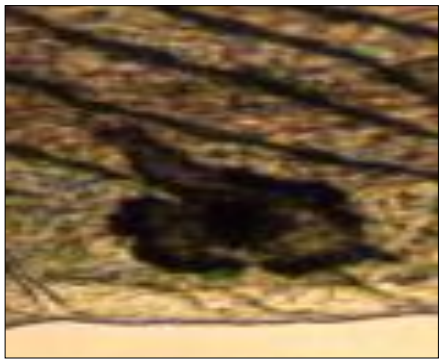

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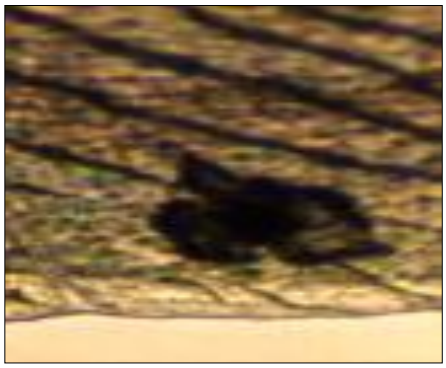

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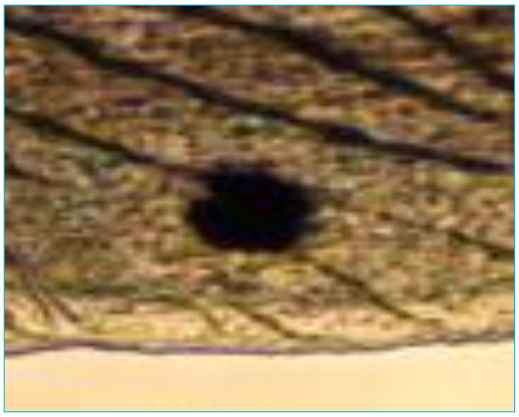

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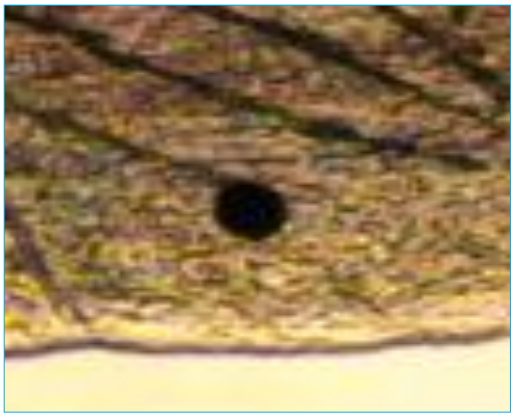

MI = 1

Results

A pigmented structure found in many animals generally in the integument. The term is usually restricted to those structures that brings about changes in colour or brightness. A majority of chromatophores are single cells that are highly branched and contain pigment granules that can disperse or aggregate within the cell. Pigment either is spread out over a large area of the body or is retracted into a small area. The movement of pigment takes place in many chromatophores stimultaneously. So that the effects is a change in the quality of light reflected from the surface of the animals.

Effect of colchicine (Mechanochemical responses)

Colchicine was first isolated in 1820 by the French chemists P.S. pelletier and J. Caventon. In 1833 P.L.Geiger purified an active ingredient, which he named Colchicine. The chemical was later identified as a tricyclic alkaloid, and its pain relieving and anti-inflammatory effects for gout were linked to its ability to bind with tubulin. Colchicine is a medication used for gout. It is a toxic natural product and secondary metabolite, originally extracted from plants of the genus colchicum (*Autumn crocus*, *Colchicum autumnale*, also known as "meadow saffron"). It was used originally to treat rheumatic complaints, especially gout, and still is in use for this purpose today despite dosing issues concerning its toxicity. It is also prescribed for its cathartic and emetic effects. It is also being investigated for its use as an anticanser drug.

Colchicine inhibits microtubule polymerization by binding to

tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively function as a mitotic poison and spindle poison.

The isolated scale preparation were equilibrated in colchicine for 60 min. at 4^0C. The melanophores in the scales had attained full dispersion. Then the epinephrine (10^{-6} M) treated was given to the melanophores, and an unusual aggregation of the pigment was observed. Both at centre as well as the periphery, the pigment aggregated leaving a hollow space in between.

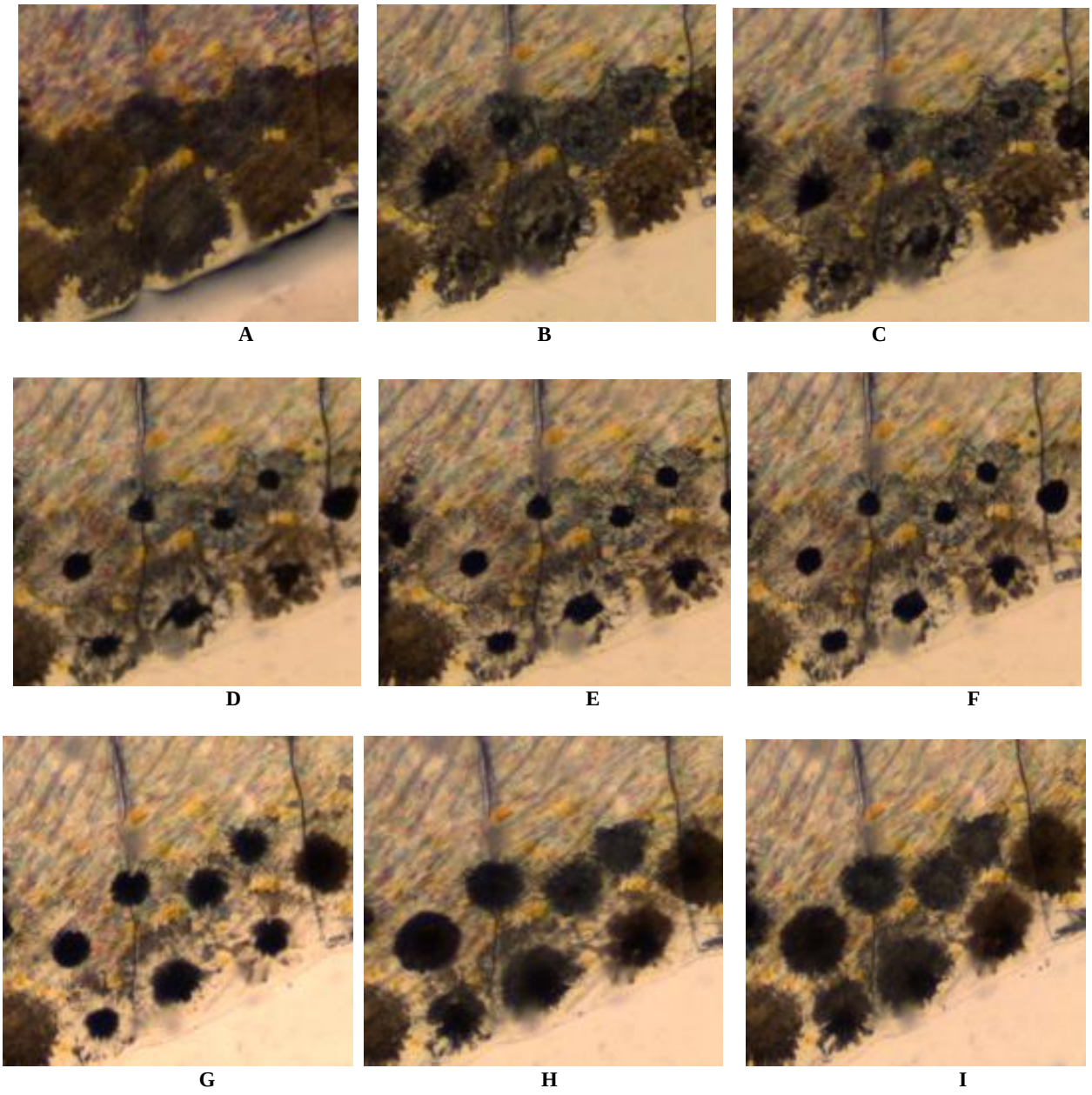

Fig 1: photomicrographs showing effect of colchicines (a microtubule inhibitor) on melanophores in isolated scale preparation × 100.

A. Equilibrated in colchicine at 4^0C (60 min), melanosomes get completely dispersed in the melanophores,
B. 1 min, (C) 2 min, (D) 3 min, (E) 4 min, (F) 5 min after the application of adrenaline (10^{-6} M) melanophores are unusually aggregated the melanosomes appear to stuck in periphery whole some show centripetal movement & accumulate in the cell centre confirming the disruption of microtubules. (G) 15 min (H) 35 min (I) 45 min after they were perfused in PS, respectively.

Discussion

The migratory movements of pigmentary organelle within the melanophores of teleosts makes them capable of undergoing rapid or even instantaneous chromomotor colour changes that are predominantly controlled by the autonomic nervous system (Bagnara and Hadley 1973; Fujii and Oshima 1986) [4, 18]. The pigment aggregation activity of melanophore have been shown to be controlled by sympathetic postganglionic fibres where peripheral transmission is proved to be adrenergic and receptors involved are demonstrated to be alpha adrenergic (Grove 1969 a; Reed and Finnin 1972; Fernando and Grove 1974 a; Fujii and Miyashita 1975; Fujii *et al*. 1980; Anderson *et al*. 1984; Kumazawa and Fujii 1984; Kasukawa *et al*. 1986; Patil and Jain 1989b; Nagaishi and Oshima 1989; Zhong and Minnemann 1999) [9, 39, 22, 15, 20, 2, 28, 26, 35, 32, 43].

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore

colchicine effectively function as a mitotic poison and spindle poison. The isolated scale preparation were equilibrated in colchicine for 60 min. at 4^0C. The melanophores in the scales had attained full dispersion. Then the epinephrine (10^{-6} M) treated was given to the melanophores, and an unusual aggregation of the pigment was observed. Both at centre as well as the periphery, the pigment aggregated leaving a hollow space in between.

The sole function of chromatophores (except the iridophores) is pigment aggregation in the centre of the cell or dispersion throughtout the cytoplasm. This alternative transport of pigment allows the animal to effect the desired colour changes. The transport of pigment occurs in response to extracellular cues such as the neurotransmitters and/or hormones. Microtubules that radiate from the cell centre towards the periphery of the dendritic processes, play an active role as reviewed by Obika (1986) and Fujii (1993b) [13]. A sliding force between the surface of chromatophores and the microtubules would cause the movement of chromatophores in a centripetal or centrifugal direction. Cytoplasmic dynein and kinesin, the two motor proteins have been shown to be involved in the centripetal (retrograde) and centrifugal (orthograde) translocation of pigment, respectively. More recently microfilaments have also been implicated to play a role in uniform distribution of chromatophores in association with the established role of microtubules. Myosin V here has been shown to act as the motor protein (Langford 1995). Both these components and the bidirectional movement of pigment in melanophores is depicted in the context of melanophores (which are predominantly found in species that change their colour in a series of black- grey- pale, such as the one under present study), when the melanin granules are dispersed throughout the cell the fish appears dark and when they are aggregated towards the centre of the cell the fish appears pale. However the change in colour, shade hue as well as pattern in the fish at a particular time is the net effect of changes in all its chromatophore population at the given point of time exhibiting, macroscopically the combined effect in response to particular stimuli received/ perceived by the animal. For the transport of pigment in the cytosol of melanophores the role of cytoskeletal molecular motors is quite evident (Radionov *et al* 1998; Aspengren *et al* 2006) [37, 3]. Making use of drugs that disrupt the microtubules and the actin filaments a role for these cell organelle in the bidirectional transport of melanosomes can be assessed.

Perceived chiefly by light receptors (either ocular or extraocular), cues from the outside are integrated into efferent signals in the CNS to adjust the chromatophores. Both, the physiolocal as well as morphological colour changes usually involve an adaptation to an illuminated background, mediated through the eyes, brain and either a hormonal or nervous control systems and more commonly by both types of controls. Studies on the role of eyes (which are required) have shown that the determining factor is the background's albedo, only the ventral area of the retina is stimulated by light coming from directly above, resulting in skin darkening. On a white background of high albido, both ventral as well as dorsal area of the retina are stimulated resulting in skin paling. Dramatic proof of the validity of this concept was provided by experiments in Killifish (*Fundulus)*, where in rotation of the eyes actually reversed the normal responses (Waring, 1963) [41]. The account by Kinosita (1963) [27], where electrophoretic force was considered to act on the individual granule for its movement in the teleost, Oryzias as supported by measurements with microelectrods, found general validity. According to this theory the anterior of the melanophores is electronegative to the exterior as in neurons and most other cells, the potential difference in the dispersion phase being between 20 and 37 mv about half that of a large axon. In this phase the cell body region has the 37 mv value and the tips of the branches 20 mv, and since the melanosomes themselves carry a negative charge, they move out towards the tips. The aggregation transmitter induces a reversal of the internal potential gradiant and then the granules aggregate in the centre of the cyton.

Fujii and Novales (1969) [42], were of the opinion that it is the local depolarization of the membrane by neural or hormonal mediators which is responsible for the difference in transmembrane potential between base and tip of each branch of the melanophores. They also supported the 2nd theory of chromatosome movement which involves the now familiar cytoplasmic microtubule as this organelle has been reported in chromatophores from various species. Current researches are diverted on the elucidation of the mechanism of intracellular transduction of chemical energy into movement of pigment granules. The orientation of microtubules in pigment cells such as melanophores resembles closely that seen in fibroblast cells, with microtubules plus ends located that in the periphery (Mc Niven *et al*. 1984, Mc Niven and porter 1986) [29, 30]. Rogers *et al* (1997) [39] are of the opinion that aggregation of pigment granules towards the cell cntre probably occurs by a cytoplasmic dynein-dependent process while their dispersal is driven by conventional kinesin or a KRP (kinesin-related peptide). Nielson *et al*. (1996) [33] have actually detected the presence of both of these motor protein on the pigment granules in the cultured melanophores of Atlantic cod through their immunofluorescence observation.

It is long known that chromatophores of certain fishes which responed to stimuli with rapid pigment migration contain a well-developed cytoplasmic microtubule system radiating from central areas of the cell (Bikle *et al*.1966; Green 1968; Fujii novels 1969; Schliwa and Bereiter- Hann 1973; Murphy and Tylney 1974; Obika *et al*.1979) [5, 21, 10, 40, 31, 34] and the migration of their pigment granules I impaired by treatment with antimitotic reagents (Wikswo and Novales 1969; Schliwa and Bereiter- Hann 1973) [42, 40]. Like microtubule actin filaments have also been implicated to have a role in melanosome movement. Both types of cytoskeletal elements are polar structure with distinct plus end minus end, which allow directed movement along the track. The actin filaments are proposed to be utilized for achievement of uniform distribution of melanosomes in the dispersed state (Rogars and Gelfand 1998) [38]. A model for melanosome dispersion was proposed. Melanosome initially move to the periphery along radial microtubules and then continue their movement along randomly oriented actin filaments.

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References

1. Abbott FS. Endocrine regulation of pigmentation in fish. *Amer*. Zool. 1973; 13:885-894.

2. Andersson RG, Karlsson JO, Grundstrom N. Adrenergic nerves and the alpha 2-adrenoceptor system regulating melanosome aggregation within fish melanophores. *Acta Physiol Scand.* 1984; 121:173-179.
3. Aspengren S, Hedberg D, Wallin M. Melanophores**:** A model system for Neuronal Transport and Exocytosis? J. Neuroscience. Res. 2007; 85:2591-2600.
4. Bagnara JT, Hadley ME. Chromatophores and colour change – The comparative physiology of animal pigmentation, Prentice Hall, Inc. Englewood Cliffs, New Jersey. 1973, 1-191.
5. Bikle D, Tilney LG, Porter KR. Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus*. *Protoplasm,* 1966; 61:322-345.
6. Dwivedi DK. Studies on color change mechanisms in freshwater fish. *Ph.D Thesis. Univ. Saugor, Sagar,* 1976.
7. Fingerman M. The control of chromatophores. *Pergamon Press. Oxford,* 1963.
8. Fingerman M, Rao KR. Action of biogenic amines on crustacean chromatophores. III. Antagonism by lysergic acid diethylamide of the effect of serotonin on colour changes in the fiddler crabs. Uca pugilator. *Comp. Gen. Pharmacol*. 1970; 1:341-348.
9. Fernando MM, Grove DJ. Melanophore aggregation in the plaice (*Pleuronectes platessa* (*L*.) I. Changes in *in vitro* sensitivity to sympathomimetic amines. *Comp. Biochem. Physiol.* 1974a; 48:723-732.
10. Fujii R. Chromatophores and Pigments in "Fish Physiology" Vol. III, *Hoar, W. S. and Randall, D*. J; Eds. Acad. press, New York. 1969; 3:307-353.
11. Fujii R. Colouration and chromatophores in Evans, D. H. "The physiology of fish". *Boca Raton, FL: CRC Press.* 1993, 535-562.
12. Fujii R. Cytophysiology of fish chromatophores, Int. Rev. Cytol. 1993b; 143:191-255.
13. Fujii R. The regulation of motile activity in fish chromatophores. Pig. Cell Res. 2000; 13:300-319.
14. Fujii R, Kasukawa H, Miyaji K, Oshima N. Mechanism of skin colouration and its changes in blue-green damsel fish, *Chromis viridis. Zool. Sci*. 1989; 6:477-486.
15. Fujii R, Miyashita Y. Receptor mechanisms in fish chromatophores I- Alpha nature of adrenoceptors mediating melanosome aggregation in guppy melanophores. *Comp. Biochem. Physiol*. 1975; 51C:171-178.
16. Fujii R, Novales RR. The nervous mechanism controlling pigment aggregation in *Fundulus* melanophores. *Comp. Biochem. Physiol*. 1969a; 29:109-124.
17. Fujii R, Novales RR. Cellular aspect of the control of physiological colour change in fishes. *Am. Zoologist*. 1969b; 9:453-463.
18. Fujii R, Oshima N. Control of Chromatophore movements in teleost fishes. Zool. Sci. 1986; 3:13-47.
19. Fujii R, Oshima N. Factor's influencing motile activities of fish chromatophores. In: Gilles R. *Advances in Comp. Environ. Physiol. Berlin: Springer-Verlag.* 1994; 20:1-54.
20. Fujii R, Jain AK, Miyashita Y. Note on the nervous control of melanophores of the Loach, *Misgurnus anguillicaudatus*. J. Lib. Arts. Sci. Sapporo Med. Coll. 1980; 21:61-73.
21. Green L. Mechanism of movements of granules of *Fundulus heteroclitus. Proc. Natl. Acad. Sci. U.S.* 1968; 59:1179-1186.
22. Grove DJ. The effect of adrenergic drugs on melanophores of the minnow, *Phoxinus phoxinus* (L.) *Comp. Biochem. Physiol*. 1969a; 28:37-54.
23. Grove DJ. Melanophore dispersion in the minnow, *Phoxinus phoxinus* (L.). *Comp. Biochem. Physiol*. 1969b; 28:55-65.
24. Hogben L, Slome D. The pigmentary system.VI. The dual character of the endocrine coordination in amphibian colour change. Proc. Roy. Soc. B. 1931; 108:10-53.
25. Hogben LT. The pigmentary effectors system. Ohver and Boyd, Edinburgh, 1924.
26. Kasukawa H, Oshima N, Fujii. A Comparative survey of type of sympathetic neurotransmitter melanophores in catfish, Comp. Biochem. Physiol. 1986; 85C:115-120.
27. Kinosita H, Ueda K. Physiological studies of the fish melanophore - Concentration and dispersion response elicited by electric stimulation. J. Fac. Sci. Univ. Tokyo. Sect. Zool. 1970; 223:185-187**.**
28. Kumazawa T, Oshima N, Fujii R, Miyashita Y. Release of ATP from adrenergic nerve controlling pigment aggregation in tilapia melanophores. Comp. Biochem. Physiol. 1984; 78C:1-4.
29. McNiven MA, Wang M, Porter KR. Microtubule organizing center in erythrophores; its 3-dimensional structure and behavior during pigment motion. *Cell.* 1984; 37:753-765.
30. McNiven MA, Porter KR. Microtubule polarity confers direction of pigment transport in chromatophores. J. Cell. Biol. 1986; 103:1547-1555.
31. Murphy DB, Tilney LG. The role of microtubules in the movement of pigment granules in teleost melanophores. J. Cell. Biol. 1974; 61:757-779.
32. Nagiashi H, Oshima N. Control of the pigment migration in melanophores in the dorsal and ventral skin of the upside-down catfish. *Comp. Biochem. Physiol.* 1989; 93C:67-71.
33. Nilsson HM, Rutberg M, Wallin M. Localization of kinesin and cytoplasmic dynein in cultured melaophores from Atlantic cod, *Godus morhua*. *Cell Motil Cytoskeleton*. 1996; 33:183-196.
34. Obika M, Turner WA, Negishi Jr S, Menter DG Lo, SJ. Taylor JD, Tchen TT. The mechanism of pigment migration in fish chromatophores. *Pigment. Cell*. 1979; 4:64-71.
35. Patil S, Jain AK. The sympathetic neuro-melanophore transmission in a fresh-water Indian major carp, *Labeo rohita* (Ham). Ind. J. Physiol. Pharmacol. 1989; 33(2):101-106.
36. Reed PL, Finnin BC. Pigmentation: Its genesis and biologic control. *Appleton-century-crofts*, New York, 1972.
37. Rodinov VI, Hope AJ, Svitkina TM, Borisy GG. Functional co-ordination of microtubule-based and actin-based motility in melanophores. *Curr. Biol*. 1998; 8:165-168.
38. Rogers SI, Gelfand VI. Myosine cooperates with microtubule motor during organelle transport in melanophores. *Curr. Biol*. 1998; 8:161-164.
39. Rogers SL, Tint LS, Fanapour PC, Gelfand. Regulated bidirectional motility of melanophore pigment granules along microtubules *in vitro*. *Proc. Natl. Acad. Sci.* USA. 1997; 94:3720-3725.
40. Schliwa M, Bereiter-Hann J. Pigment movements in fish melanophores: morphological and physiological studies. II. Cell shape and microtubules. *Z. Zellforsch.* 1973; 147:107-125.

41. Waring H. Colour change mechanism of cold blooded vertebrates. *Acad. Press. New York*, 1963.
42. Wikswo MA, Novales RR. The effect of colchicines on migration of pigment granules in the melanophores of *Fundulus heteroclitus*. *Biol. Bull.* 1969; 137:228-237.
43. Zhong H, Minneman KP. A1-adrenoceptor sub-types. Eur. J. Pharmacol. 1999; 375:261- 276.