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RESEARCH ARTICLE

STUDY OF LOSE AND GAIN OF MELANOPHORES (MORPHOLOGICAL STUDY) AFTER PROLONGED ADAPTATION IN BLACK AND WHITE BACKGROUND IN FRESH WATER FISH PUNTIUS

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Abstract

The fresh water fish Puntius rapidly changed their color according to their background; palling when the background was white and darkening, when it was black. After the prolonged adaptation of fish in black and white background for 25 days, it was observed that the population of melanophores increases in black background and fish will darken it skin coloration due to the melanophores dispersion all over the body. Darkening of fishes is regulated by the melanocyte stimulating hormons. On the other hand when fishes were kept on white background for 25 days, the number of ofmelanophores may decreases through cell apoptosis and the skin color lighter. This allows them to camouflage themselves by matching their environment.

Introduction:-

Chromatophores are adaptable pigment cells thatcan rapidly change color in response to environmental changes such as light or temperature variations. In fish, these cells are typically 100 micrometers in diameter or larger, located primarily in the dermal layer of the integument, though they can also be found in the epidermal layer. These pigment cells originate from the neural crest and migrate dorso-laterally beneath the ectoderm around the developing embryo (Gilbert1994). The morphology of chromatophores varies has influenced the culture and behavior of various species (Obika,1986). Changes in coloration that result from alterations in the amount of melanin or other pigments within the integument are referred to as morphological" or"quantitative" color changes. These are distinct from "physiological" or "transitory" color changes,which involve the movement and redistribution of existing pigments within chromatophores (Parker, 1948).

Franz (1910) studied color change in flatfish, Pleuronectes platessa, and attracted attention to this area of research. Parker (1948), Waring (1963), Fujii (1969), and Bagnara and Hadley (1973) reviewed various studies on this aspect in differentteleost species. Ahmad (1972) studied this in Phoxinus phoxinus, Jain (1978) in Nandusnandus, Dubey (1991) in Catla catla, Kuruvilla (1991) in Garra gotyla and Mystus bleekeri, and Sugimoto (1993a, b) in Oryzias latipes. They noted changes inthe population and morphology of melanophores, and Sugimoto (1993b) extended these observations to conclude that long-term adaptation to particular background significantly affects both morphological color change and the responsiveness of melanophores to neural and hormonal factors. Ahmad(1974) studied transitory color changes in the European minnow, Phoxinus phoxinus,and concluded that sensitivity to hormonal control develops first in pigment cells, followed by sensitivity to nervous control. Sugimoto et al., (2000) observed apoptosis in skin pigment

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cells ofthe medaka during longterm chromatic adaptation, suggesting that sympathetic innervation plays an important role. They concluded that apoptosis regulates the balance of pigment cells in the skin of medaka fish to adapt to their environment. Recently, Sugimoto et al.,(2005) investigated the influence of longterm chromatic adaptation on pigment cells and striped patterns in zebrafish, Danio rerio. Their findings indicated that morphological responses of superficial chromatophores contribute to effective and rapid background adaptation of the dorsal skin,while prolonged adaptation also affects hypodermal chromatophores inthe flank, altering striped pigment patterns.

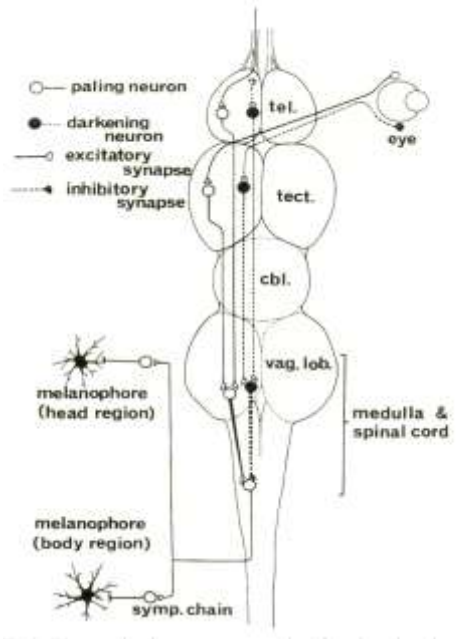


Fig1. Diagram showing nervous connections from lateral eye into the central nervous system and finally to chromatophores in fish (After Iwata and Fukuda, 1973)

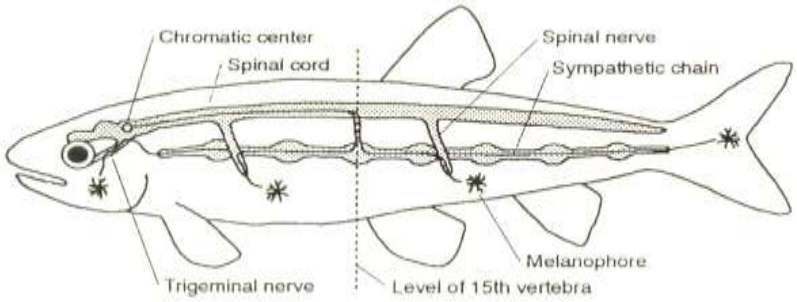


Fig. *2* Diagram showing the chromatic nervous pathways from melanosome-aggregating centre to melanophores in fish originally described in the minnow*P. laevis* by von Frisch (1911).

Material and Methods:-

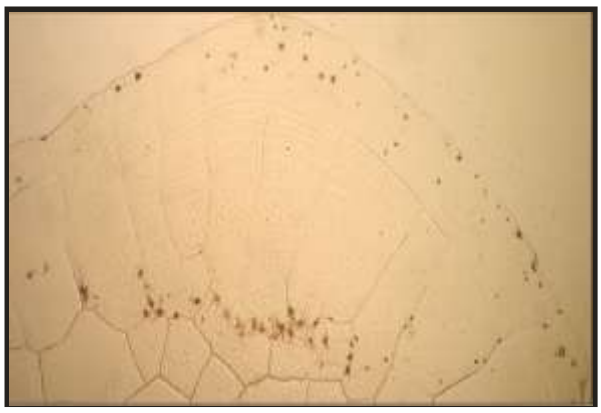
The study utilized the Puntius species, including Puntius sophore (the pool barb), Puntiusconchonius(the rosy barb), and Puntius ticto (the ticto or two spot barb), of both sexes. These fish were collected from Tighra reservoir, approximately 23 km from Gwalior, and Puntiusticto were obtained from Pilua dam, about 40 km from Gwalior. Under routine conditions, fish ofboth sexes were kept in clear glass tanks withan intermediate background created by placing brown paper underneath. Experimental fishwere placed in large round glass troughs (8 liters) with either clear bottoms (light brown) or sides painted with black or white enamel, creating neutral, black, and white backgrounds, respectively. For analyzing scales, melanophore density and melanosome distribution were examined.After the experiment, fish were fixed in Bouin's fluid fortwo days, washed in running water,and then preserved in 70% alcohol. Scaleswere then removed,and melanophores were countedunder light microscope using a veeple inthe eyepiece. The pigmentary area ofthe scaleswas calculated using an occulometer disc.

The experimental protocol involved dividing the entire body surface of the fish intosix sites: AD (Anterior dorsal), MD (Mid dorsal), PD (Posterior dorsal) for the upper half, and AV (Anterior ventral),MV(Mid ventral), PV (Posterior ventral)forthe lower half. Under natural conditions, a gradient in shade exists,from grayish onthe dorsal surface to silvery on the ventral surface.Observations were made on skin samples fromthedorsolateral and latero ventral trunk regions and from the DS component of the fish.Five scales from each site, taken from the third row of dorsal and sixth of ventral sites belonging to the GBS component, andeven scales contributing to dark spots were examined. In total, 37 scale slips per fish were studied. Microscopic observations onmelanophore characteristics were conducted in five fish of each species, and the mean results were recorded. Twenty fish were divided into four groups of five animals each. Group 1 served as the initial control group. Group 2 was placed on a neutral background under natural photoperiod, Group 3 in continuously illuminated white background ,and Group 4 in continuously illuminated black background. The initial and final body shades were recorded using the Munsell grey series. Groups 2-4 were exposed to their respective backgrounds for 25 days with consistent light intensity of 4000 Lux for 24 hours each day at the water surface in the troughs.

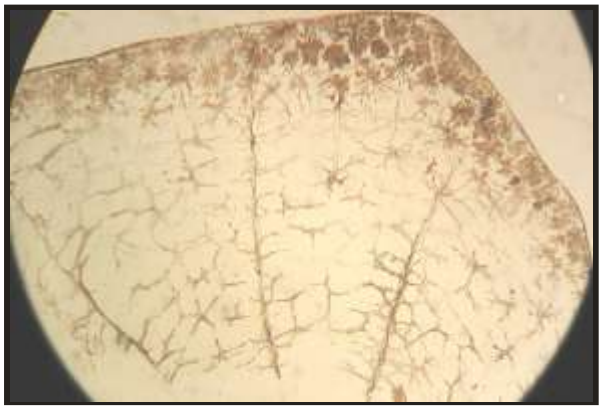
Observation and results Morphological/chromogenic colour change: The tables show the summary of statistical data aboutthe change inthe number of melanophores in all seven body areas (which belong to the GBS and DS parts) of three species of fish, Puntius. In the freshly caught fish (Group I),they looked darker on the outside. The average number of melanophores was 406.11, 484.76, and 466.87 in the dorsal area (AD, MD, PD sites) and 99.13, 74.21, and 59.07 in the ventral area (AV, MV, PV sites) for Puntiusconchonius. In the DS part, the average was 590.46 per 6mm² (Fig3and Table 1). In Group II (fish that stayed in a neutral background for 25 days), they were slightly darker than GroupITheir skin color was more on the darker side. The number of melanophores was higher in DS (429.70) compared to Group I. The average number was 513.97, 538.81, and 529.10 in the dorsal area (AD, MD, PD sites) and 101.11, 80.76, and 62.81 in the ventral area (AV, MV, PV sites) per 6mm². In DS, the number was 665.16 per 6mm² (Fig. 3 and Table 1). In Group III (fish that stayed in a white background for 25 days), they looked much lighter.

Their skin color matched I.N. 8.0 in the Munsell Grey Series. The melanin was much less than in Groups I and II. The average number of melanophores was 145.41, 133.30, and 123.45 in the dorsal area (AD, MD, PD sites) and 20.32, 11.39, and 14.66 in the ventral area (AV, MV, PV sites) per 6mm². In DS, it was 293.25 per 6mm² (Fig. 3 and 4 and Table 1). In Group IV (fish that stayed in a black background for 25 days), they looked much darker. Their skin color matched I.N. 1.0 in the Munsell Grey Series. Melanophores were spread in both new and old cells. The melanin was higher thaninany other group. The number of melanophores was highest among all groups. In the dorsal area, the average was 741.44, 770.49, and 547.80 in the AD, MD, PD sites respectively, and in the ventral area, it was 101.36, 79.27, and 78.36 in AV, MV, PV sites. In DS, it was 759.63 per 6mm² (Fig. 4). In Group III, the number of melanophores was the lowest, and in Group IV, it was the highest in all body parts. The average number of melanophores was higher inthe dorsal areas than in the ventral areas. However, the DS part had more melanophores than the other six areas in all groups.

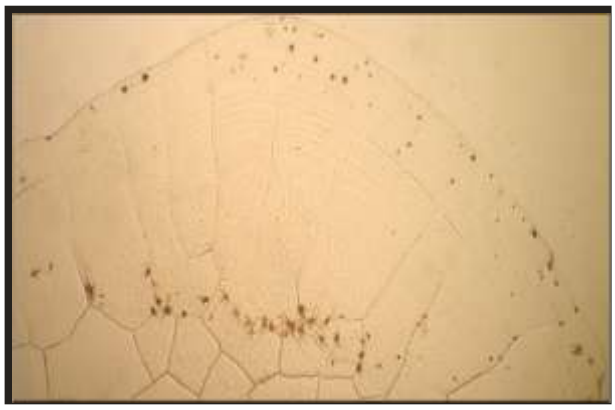
However, the DS part had more melanophores than the other six areas in all groups. The highest number was in Group IV (Fig. 3-5 and Table 1).



At the starting stage of the experiment (Pre-experiment fish)



After 25 days of adaptation to normal background



After 25 days of adaptation to white background

After 25 days of adaptation to black background

Fig.*4*: Photomicrograph showing the melanophore population in a scale belonging to GBS area in fish, *Puntius conchonius*

Fish No.	Region	Sites	Group-I		Group-II		Group-III		Group-IV	
			A.M.P./ fish	C.A.P./ fish	A.M.P./ fish	C.A.P./fish	A.M.P./ fish	C.A.P./fish	A.M.P./ fish	C.A.P./fish
1	D O R S A L	**AD**	242.4	400.00	339.6	600.06	58.8	98.98	365.2	610.36
2			238.2	397.00	232.0	400.00	146.4	153.70	386.4	679.86
3			170.6	281.51	340.0	500.00	122.0	183.70	396.6	879.94
4			295.2	492.00	326.6	580.00	135.0	142.04	437.0	722.72
5			278.8	460.06	300.8	489.79	179.0	148.64	501.8	814.33
Mean			**245.04**	**406.11**	**307.8**	**513.97**	**128.24**	**145.41**	**417.4**	**741.44**
S.D			**48.09**	**80.51**	**45.26**	**79.90**	**44.19**	**30.46**	**30.46**	**107.02**
Gain/Loss %						+ **26.55%**		**-64.19%**		+ **82.57 %**
1		**MD**	271.0	505.59	353.4	493.57	46.4	74.35	419.0	671.47
2			262.8	503.44	347.4	510.88	80.2	157.87	513.6	753.07
3			246.0	492.00	369.0	559.09	72.4	109.36	479.6	740.74
4			275.4	519.62	356.4	509.27	97.6	159.47	524.4	907.20
5			229.0	403.16	333.0	621.26	103.6	165.49	505.0	780.00
Mean			**256.84**	**484.76**	**351.84**	**538.81**	**80.04**	**133.30**	**488.32**	**770.49**
S.D			**19.19**	**46.66**	**13.15**	**52.22**	**22.65**	**39.91**	**42.13**	**86.27**
Gain/Loss %						+ **11.14%**		**-72.50%**		+ **58.94**

										%
1			223.6	361.81	271.4	488.12	58.2	92.67	275.2	468.02
2			255.8	491.92	315.8	498.10	87.0	138.97	338.4	523.83
3		**PD**	234.6	465.47	289.8	561.62	131.60	160.23	356.0	626.76
4			275.0	513.05	304.0	522.33	54.8	55.68	306.0	520.42
5			285.2	502.11	317.6	575.36	106.8	169.71	366.0	600.00
Mean			**254.84**	**466.87**	**299.72**	**529.10**	**87.68**	**123.45**	**328.32**	**547.80**
S.D			**26.05**	**61.32**	**19.34**	**38.35**	**32.57**	**48.15**	**37.44**	**64.51**
Gain/Loss %						**+ 13.2%.**		**-73.55%**		**+ 17.33 %**
1			57.0	103.26	46.4	90.98	14.8	31.48	60.8	127.73
2			38.2	69.20	47.75	64.09	8.2	16.53	43.6	78.33
3		**A V**	41.0	77.35	69.4	110.50	9.2	16.54	59.4	73.49
4			70.6	131.71	72.2	133.45	5.8	16.56	61.8	110.35
5	**V**		63.0	114.13	58.4	106.56	6.0	20.40	58.0	116.93
Mean	**E**		**53.96**	**99.13**	**58.83**	**101.11**	**8.92**	**20.32**	**56.72**	**101.36**
S.D			**14**	**25.85**	**11.91**	**25.67**	**3.65**	**6.46**	**7.47**	**24.11**
Gain/Loss %	**N**					**+1.9%**		**-79.50%**		**+ 2.24 %**
1	**T**		40.2	54.03	59.6	98.67	3.6	13.13	47.0	82.74
2			53.4	89.81	48.4	78.31	4.2	11.80	58.4	85.38
3	**R**	**M V**	43.4	67.31	43.0	61.60	3.2	10.22	44.6	61.60
										101.6

4	**A**		44.0	89.32	47.6	79.06	2.6	10.20	60.6	7
5			50.0	70.59	48.6	86.17	6.6	11.62	40.8	64.96
Mean	**L**		**46.2**	**74.21**	**49.44**	**80.76**	**4.04**	**11.39**	**50.28**	**79.27**
S.D			**5.36**	**15.32**	**6.12**	**13.47**	**1.54**	**1.22**	**8.73**	**16.43**
Gain/Loss %						**+ 8.82%**		**-84.65%**		**+ 6.81 %**
			33.0	54.00	35.0	60.67	8.2	18.94	55.2	95.50
			27.8	46.48	28.6	55.42	5.2	13.74	47.0	78.33
		PV	32.0	48.33	31.4	53.95	2.4	12.86	48.8	73.49
			45.4	84.70	43.2	81.78	4.8	15.54	42.8	65.84
			34.4	61.87	34.6	62.23	5.0	12.24	51.6	78.65
Mean			**34.52**	**59.07**	**34.56**	**62.81**	**5.12**	**14.66**	**49.08**	**78.36**
S.D			**6.56**	**15.52**	**5.48**	**11.15**	**2.06**	**2.69**	**4.68**	**10.00**
Gain/Loss %						**+ 32.65**		**-75.18%**		**+ 32.66 %**
1	**C**		1249	546.18	1301	560.35	449.58	195.71	**1769**	741.17
2	**A**		1428	600.00	1563	737.39	457.00	195.84	**1807**	730.58
3	**U**	**DS**	1314	602.27	1519	608.41	875.00	373.13	**1923**	683.92
4	**D**		1590	633.87	1737	722.73	750.00	343.63	**1898**	825.80
5	**A**		1330	570.00	1595	696.96	852.00	357.97	**2020**	816.70
	L									
Mean			**1382.2**	**590.46**	**1543**	**665.16**	**676.71**	**293.25**	**1862.4**	**759.63**
S.D			**118.65**	**33.51**	**141.38**	**77.05**	**187.23**	**89.59**	**89.89**	**60.31**
Gain/Loss %						**+ 12.62%**		**- 50.32%**		**+ 28.65 %**

Table 1: Showing quantitative changes in the population of melanophores in different sites of the fish, Puntiusconchonius exposed to illuminated neutral, white and black backgrounds. The figures represented the number of melanophores calculated per 6mm2. Group-I, II, III and IV represent initial freshly caught fish (Control) and those exposed to neutral, white and black backgrounds for a period of 25 days. A.M.P/fish represents the average melanophore population in 5 scales/fish. C.A.P/fish represents the calculated average population of melanophores in 6 mm2 area in 5 scales/fish, + represents % gain and – represents % loss of melanophores.

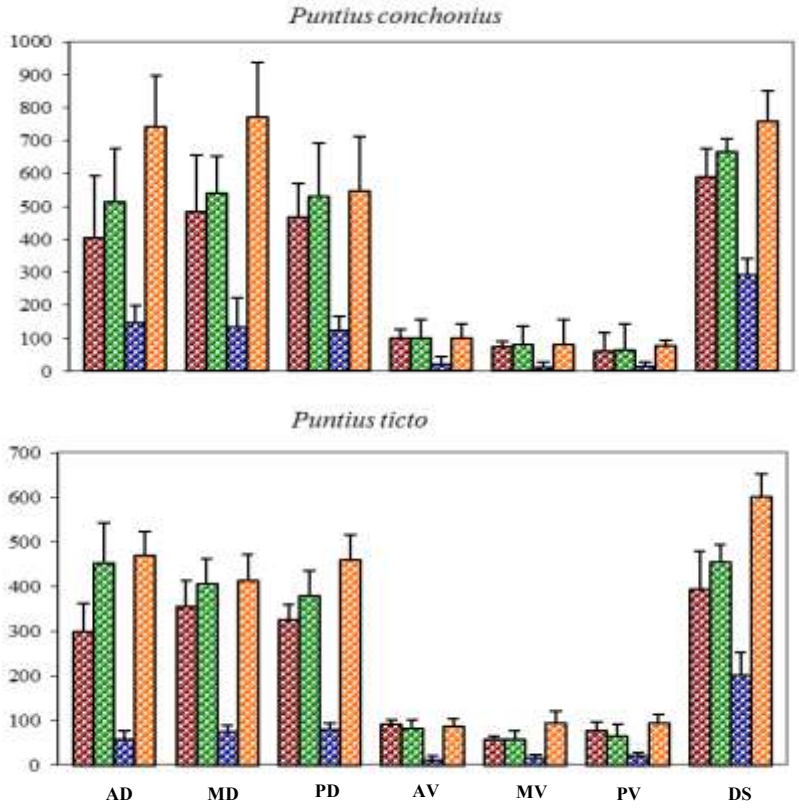


Fig. 3: Diagrammatic representation of quantitative changes in the melanophores of the fish belonging to different sites and the experimental groups (I to IV). The duration of experiment was 25 days. The mean melanophore population shown here is deduced for an unit area of 6 mm^2.
Group I (Initial control) Group II (Exposed to neutral-background)
Group III (Exposed to white-background)
Group IV (Exposed to black-background)

Discussion:-

The consequences touching on this look at of transitory or physiological shade alternate within the fish due to historical past reaction do advise that co-ordination of color change manner appears to be not best anxious (to initiate the responses) however also humoral (to complement the responses) running synergistically. In elasmobranch fishes, in which melanophores are aneuronic like amphibians and most reptiles, melanosome dispersion and consequent darkening due to edition to a black-heritage has been shown to be controlled by means of MSH launched from the pituitary gland (Parker, 1948 and Waring, 1963). adaptation to white- history were explained with the aid of inhibition of launch of MSH. The melanophores in these corporations of vertebrates anticipate the so referred to as punctate country in their unstimulated or resting nation whereupon the melanosomes are aggregated perinuclearly within the

melanophores. for that reason each dispersion (darkening in fish) and aggregation (paling in fish) of melanophores can properly be defined by means of a position of unmarried hormone i.e., MSH. Sherbrooke et al., (1988) had without a doubt stated that in any unihormonal version of melanophore manage the unstimulated state of the melanosomes have to dictate what the moves of hormonal stimulus can be, aggregated melanosomes will become dispersed as is the effect of MSH in most of these poikilotherms.

The simplest exception seems to be teleost melanophores, which become stellate (melanosomes dispersed in to the dendritic approaches of the cellular) in the absence of stimuli i.e., the resting state. Puntius species aren't any exception and as such MCH has been found in these species to have a totally strong action in aggregating the dispersed melanosomes. The receptors for the version to background are unequivocally the eyes, as bilateral blinding actually abolishes the speedy, history-related responses and also fish acquire a darker colour no matter the heritage. three sorts of alteration in pigmentation of the pores and skin as a result of prolonged variation of the animal main to chromogenic or morphological colour changes have been recorded (Bagnara and Hadley, 1973). the first is the exchange in the net amount of melanin within the epidermis, that's normally depending on the synthesis of pigment in epidermal melanophores.

The website online for other two adjustments is the epidermis and they specifically have an effect on the quantity of melanin in dermal melanophores and inside the variety of those cells. usually in fishes, melanosomes have now not been said to be transferred to other varieties of cells such as epidermal cells. some teleostean species such as Poecilia, Fundulus, Lebistes, Gambusia (Parker, 1948;), Phoxinus(Ahmad, 1972),Fundulus (Bagnara and Hadley, 1973), Salmo, (Baker and Ball, 1975), Rasbora (Dwivedi, 1976), Nandus (Jain, 1978), Oreochromis (van Eys and Bonga, 1981, Sugimoto, 2002), Catla (Dubey, 1991), Channa (Chandelkar, 1992),Oryzias (Sugimoto, 1993) and Danio (Sugimoto, 2002 and Sugimoto et al., 2005) had been suggested to show off an boom and a decrease in the quantity and the dimensions of melanophores as well as in the amount of melanin when they had been adapted to illuminated black and white backgrounds, respectively.

The general inference drawn from these findings become that during extended adaptation to historical past, the morphological colour alternate is triggered by way of the physiological colour trade which occurs in response to neural and/or hormonal regulatory device and the morphological steps follow, being controlled through hormonal ideas which includes α-MSH and MCH. The neural tactics having have an impact on on morphological coloration modifications, but can't be ruled out. The use of chemically sympathectomised fish Sugimoto (1993), tested the increase within the variety of melanophores over a white heritage but noted that the size of melanophores was unchanged. He further discovered that denervation had no direct outcomes on black-tailored medaka. So he concluded that neural processes play a function in changing the range of melanophores. With appreciate to alternate in length of melanophores, he recommended the position for α-MSH mainly, the titre of which within the blood has in advance been shown to vary with the colour of the heritage (Baker et al., 1984; Jenks et al., 1977; Wilson and Morgan, 1979). Ahmad (1972) defined his consequences on Phoxinus by using assuming the presence of a pigment-dispersing and pigment-aggregating neurotransmitter. He proposed that the dispersing transmitter, in addition to its feature of pigment dispersion, now not best promotes production of melanophores but also restricts their loss and the aggregating neurotransmitter in addition to its function of pigment aggregation, no longer simplest favours loss of melanophores but additionally limits their formation. He as a result supported the concept of dineuronic control of melanophores. In our examine on Puntius species which have been located for 25 days inside the box with a skinny layer of sand making the historical past greyish in color (impartial history), the average average % growth inside the melanophore population in a fish is handiest 17.17, 15.26 and 19.21%, respectively in Puntiussophore, Puntiusconchoinius and Puntiusticto as compared to the growth in wide variety of melanophores in these fish on an illuminated black-history (being 52.05, 32.seventy four and 38% respectively.

The fish on a everyday historical past gets neither a great black- historical past stimulus nor a real white-heritage stimulus. as an alternative, a gray-historical past stimulus (due to the sandy bottom) facilitates the fish in preserving the melanophores as semi-dispersed through slightly reducing the amount of aggregating neurohumoral transmitter on the nerve finishing and the attention of MCH within the blood. those two conditions appear to favour a mild increase of melanophore population in the course of an extended- time period variation to the impartial heritage as compared to a better boom of melanophore populace on a black-background. Inside the studies offered right here the long time white-background stimulus by using retaining the fish for 25 days, results in an expanded awareness of aggregating neurohumoral transmitter and an increased concentration of MCH within the region of the melanophores. This lengthy-term physiological condition inhibits formation of latest melanophores (melanogenesis) and favours the loss of melanophores (apoptosis) and as such the fish suffers an standard slow loss up to fifty eight.four, 71.four and seventy three.4% (Fig 26-28) respectively in Puntiussophore, Puntiusconchonius and Puntiusticto in their melanophore

populace. On an illuminated black-heritage for 25 days the fish becomes darkish hastily because of dispersion of pigment granules within the melanophores.

The black-heritage stimulus appears to paintings as an inhibiting aspect to the functioning of those corporations which lead to pigment aggregation. consequently the production of neurohumoral transmitter of an aggregating nature is both stopped or reduced on the nerve terminals innervating the melanophores. simultaneously the stimulus obtained through the retina and in turn attaining the hypothalamus and the pars intermedia via the optic nerve either stops or reduces the production and launch of MCH, consequently leading to the maximal dispersion of melanophores and thereby darkening within the fish. This long-term physiological situation inhibits loss and favours formation of melanophores inducing a minimal antagonism to melanogenesis and as such the fish experiences an increase ranging between 32.7 to 52% in their pigmentation (melanophore population).These experiments sincerely indicate a particular dating among transitory and quantitative shade modifications in respect of their manage mechanism i.e the stimulus and the biochemical retailers which generally tend to aggregate the pigment granules (transitory shade alternate) additionally cause a decrease within the melanophore population (quantitative color change) and the inhibition of stimulus and biochemical agents that bring about dispersion of melanin granule (transitory color exchange) appears to be accountable for an increase inside the quantity of melanophores (quantitative coloration alternate).

The end result on experiments on teleost, Puntius are basically in conformity with the observations of in advance people which include Odiorne (1948), Lerner and Case (1959), Jain (1978), Dubey (1991) Kuruvilla (1991) and Sugimoto (1993) in extraordinary teleosts. The facts accordingly shows that the boom in melanophore populace on a extended illumination black-heritage is the result of a probable inhibition of the energetic pigment-aggregating mechanism i.e a test at the manufacturing of aggregating neurohumoral transmitter and melanin-concentrating hormone in place of the functioning of any form of dispersing mechanism. The results acquired within the present study appear to substantiate that there exists a mononeuronic (pigment-aggregating nerve fibres) nervous manage and a single hormonal manipulate (melanin-concentrating hormone-MCH) inside the co-ordinating mechanisms of the chromatic gadget of the 3 species of Puntius studied. Those observations for that reason guide the life of the pigment aggregating fibres only and a melanin aggregating hormone (MCH) controlling the quantitative color alternate mechanism in the fish.

Ahmad (1972) explained his experimental results concerning “quantitative” colour adjustments in Phoxinus with the aid of assuming the presence of a pigment-dispersing and a pigment-aggregating neurotransmitter, which he believed to be released from two one of a kind sets of chromatic fibresfavouring the dineuronic control of melanophores. With demonstration by means of Kumazawa and Fujii (1984) of concurrent release of NE as the most important transmitter and purines (ATP) as the co-transmitter, that reverses quickly the pigment-aggregating impact of potassium triggered NE (the transmitter) from adrenergic melanosome-aggregating nerve in Tilapia, the participation of two neurotransmitters to give an explanation for the consequences obtained by using Ahmad with a unmarried mononeuronic manipulate via sympathetic pigment-aggregating nerve fibers can now properly be understood.

Consequently, the consequences received in research on Puntius species here in their very last clarification are in settlement with the conclusion reached earlier through Kumazawa and Fujii (1984) in that there exists a mononeuronic (pigment-aggregating nerve fibres) apprehensive manipulate and a unmarried hormonal manipulate-MCH) inside the co-ordinating mechanisms of the color changes in these fishes. Conclusion: for the duration of prolonged version to a white history, fish lose melanophores via a process known as apoptosis (programmed cell loss of life). This mobile death frequently takes place in the pores and skin. while the precise vicinity of the loss can vary by means of species, it usually influences the melanophore populace throughout the skin, main to a paler overall appearance. In fish, lengthy-time period publicity to a white heritage triggers a lower in melanophore density due to apoptosis, as discovered in species like medaka, tilapia, and zebra fish. This technique of programmed cell loss of life results in the slow removal of melanophores.

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