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Research Article

EFFECT OF WATER TURBIDITY ON THE PIGMENTATION OF SOME ORNAMENTAL FRESHWATER FISHES

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Abstract

Pigmentation changes in two freshwater ornamental fishes like Polyacanthus fasciatus and Puntius sophore has been studied in different turbid water conditions such as 300-350NTU, 80-100NTU and 4.5-9.75NTU. Total concentration of carotenoids and retinoids from the lipid extracts of scales and liver of both the fishes were measured in HPLC at 450 nm for carotenoids, at 352nm for dehydroretinol and at 326 nm for retinol. It is seen that retinol, dehydroretinol and carotenoid concentration are decreased to a considerable amount in fishes subjected from the high turbid water condition.

Keywords: Carotenoid, Retinol, Dehydroretinol.

INTRODUCTION

Since time immemorial the biologists and aquaculturists are attracted to the bright external coloration of animals, plants and particularly in fishes. This bright external colouration of fishes is due to a compound called carotenoid which provide the red, yellow, orange or green colouration of the fish. It has been found that carotenoid pigments are widespread in fish dwelling in both marine and freshwater habitats. These lipid soluble pigments colour the fish integuments, muscle and gonads of the fish. [1,2,3,4]. But the fishes are unable to synthesise carotenoids and it has to be obtained from food containing these compounds. Carotenoids, play a significant role in the synthesis of vitamin A in fish[1,2,3,4,5]. Vitamin A is a class of lipid molecules comprising of retinol, known as vitamin A1 and dehydroretinol known as vitamin A2 and carotenoids (having both provitamin A – status and others without provitamin A-activities) molecules existing in various organisms belonging to both the animals and the plant kingdoms[6,7]. But the deposition of carotenoids bears a relationship with various compounds and environmental and ecological factors[8,9]. During metabolism, the biogenesis of the carotenoid pigment and its conversion and absorption into various retinoids depend upon several environmental factors such as water quality, monsoon, temperature**,** pH etc.[8,10,11,12]. In the current study, an effort has been made to evaluate the effect of turbidity on the retinoids and carotenoids concentration in the freshwater fishes *Puntius sophore* and *Polyacanthus fasciatus* collected from different turbid water habitat. Turbidity is nothing but a condition of water resulting from the presence of suspended matter in the water[13].The turbid condition of water prevents the direct entry of beams of light. Further the suspended particle of different volume and size or density might have a direct effect on the pigment molecules or on the chromataphores[13].Turbidity moderate intensity affects the concentration of carotenoids as well as retinoids reserves of fish[8]. In most cases, elevated suspended sediments have sub lethal effects. These may include increased fin rot and body abrasion [14,15], paler coloration[16], delayed maturation[17], elevated cough frequency[18] ,elevated microhematocrit (packed red blood cell volume), hemoglobin concentration and red blood cell counts[19,20] and decreased tolerance rates and time to death when exposed to other environmental stressors [16,19,20]. So in this study we investigated the effects of various turbid water conditions on the retinoids and carotenoids reseves of the mentioned fishes which have ornamental as well as commercial value and native to India.

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MATERIALS AND METHODS

Collection of fishes

The fish species selected for this experiment were *Polyacanthus fasciatus* and *Puntius sophore.* The experiments dealing with the study on the effects of turbid water was conducted by collecting the fishes along with turbid water from the river Puthimari (turbidity 300-350 NTU) a small tributary of the Bramahputra river. The river Puthimari was selected as its water remains turbid for more than 2 months during the rainy season. Fishes from river Puthimari was collected and the carotenoids or pigments molecules and the retinoids were measured. Further, water as well as fish was collected from Deepor beel near Guwahati of which water turbidity remains at 80-100 NTU during the same period. Water and fishes for control condition were collected from river Brahmaputra of which water turbidity remains at 4.5-9.75NTU during the same period. Care was taken to collect the samples from the habitats which were affected with high turbidity for about more than 30 days. Collected fishes were kept in glass aquarium for immediate analysis. The fishes were brought out from the aquarium and were euthanized with chloroform. After that, scales and livers of the fishes were dissected out for further extraction of lipids. Deposition of retinol, dehydroretinol and carotenoids concentration was measured through HPLC procedure in all the three water conditions. The period of the experiment was of five years study plan.

Turbidity Measurement Method

Turbidity was measured by using a Secchi Disk in the field and in the laboratory with the help of nephelometer (Digital Nephelo Turbidity Meter 132)[21].

Solvents and Chemicals

Light petroleum ether (b.p. 40-60^0 C, 60-80^{o}C), L.R. grade was obtained from British Drug Houses (India), Glaxo Laboratories (India) Ltd. The solvent was dried over pure calcium chloride anddistilled twice before used. Diethyl ether wassupplied by Alembic Chemicals Works Co.Pvt.Ltd., Boroda. It was made peroxide free by distilling it over reduced iron. Chloroform was supplied from B.D.H. Chemicals Division, Glaxo Laboratories (India).Absolute ethanol was procured from Bengal Chemical and Pharmaceutical Works Ltd. Other solvents like acetone, acetic anhydride used were obtained from BDH, Laboratory Chemicals Division, Glaxo Laboratories (India)Pvt. Ltd. Different authentic retinoid samples, suchas retinol, dehydroretinol, ß-carotene, were obtained from Hoffman La Roche, Basel, Switzerland, BASF, Germany and Roche Co. Ltd., India.

Extraction of carotenoids and vitamin A

Lipids from the livers and the scales of the fish were extracted through light petroleum (40-60°C) ether extract using anhydrous sodium sulphate[22]. The extraction efficiency was tested by following a parallel method followed after Folch *et al*[23]. 200mg/lit of BHT was added to Folch solution or light petroleum ether which acted as antioxidant. It was found that both light petroleum and Folch solution showed similar extraction efficiency. However, in the present study, light petroleum (40-60°c) is used and retinyl propionate and β -apo-8^1 - carotenoic acid ethyl ester (CAEE) are used as internal standards. The liver oil was extracted with light petroleum until the extract was colourless and gave no colour with $SbCI_3$ reagent. The combined extracts were filtered and the solvent removed by distillation under reduced pressure at 40°C. The last traces of the solvent were removed *in vacuo* and the oil preserved until further used or saponified under reflux for 10 minutes with methanolic solution of KOH (10% wt/ vol.). The extraction of Vitamin A was done in thrice with peroxide-free diethyl ether and after that the etheral extract was freed from alkali, dried over anhydrous sodium sulphate and under reduced pressure the solvent was removed by distillation. The saponicate was either dissolved in known volume of light petroleum ether or in HPLC solvent for estimation.

Estimation

The carotenoids and vitamin A extracts were estimated using HPLC technique in the Analytical Nutrition and Chemistry Division of National Institute of Nutrition (ICMAR) Hyderabad. The extracts were sealed under nitrogen and HPLC analysis was made. The HPLC system included a liquid chromatograph (various model 5000) and integration (No.4270), an inject (Rheodyne model 725) with a 20µl loop and a various wave length detector.

HPLC procedures

HPLC system (waters) with column 300mm x 3.9 mm Nova - Pack C_{18} (4 mm) and a Guard -Pak precolumn module (water 5) were used. Standard carotenoid and retinoids samples (5.0 mg) were dissolved in 100 ml toluene: methanol (1: 1) containing 500 mg BHT (butylatedhydroxy toluene)/litre for producing 50µg/mlstandards. These standard stock solutions are stable and could be preserved at -20°C for 4 months. These were further diluted with the mobile phase to give working standards. HPLC grade solvents were degassed by vacuum filtration prior to use and water double distilled. Both retinoids and carotenoids were separated using HPLC grade solvents, acetonitrile: dichloromethane : methanol: water: propionic acid (71:22:4:2:1, v/v*)* as mobile phase with the flow rate of 1.0 ml/minute in the first 10 minute run, detection of carotenoids pigments was performed at 450 nm and dehydroretinol in 352 and retinol in 326 nm. All the other HPLC procedures were followed after Guillou *et al*[24].

Statistical Analysis

All the data obtained during the period of investigation are statistically analysed after Sokal and Rohlf[25]. The level of significance between two sets of data are calculated according to students t-test. Probability i.e. P value at 5 percent or lower for two sets of data are taken as significant.

RESULTS

The retinoids (retinol, dehydroretinol) and total carotenoids present in liver and scales of fishes collected from different turbid water conditions are presented in the following table. The retinoids and carotenoids were estimated from the liver and scales of the fish through HPLC as described in materials and methods. From the results it has been found that fishes dwelling in a high turbid areas show a lesser amount of pigmentation, whereas fishes collecting from low turbid water condition show greater pigmentation with high amount of retinoids and carotenoid deposition. The mean ±SD values of retinol, dehydroretinol, total liver and scale carotenoid are presented in the Table 1.

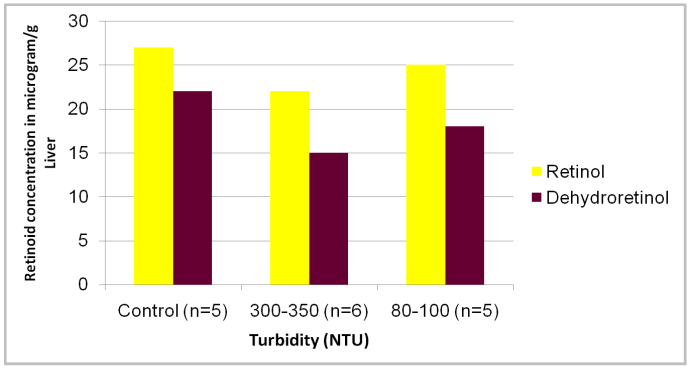

Fig. 1. Effect of turbidity on retinoid concentration of *Polyacanthus fasciatus*

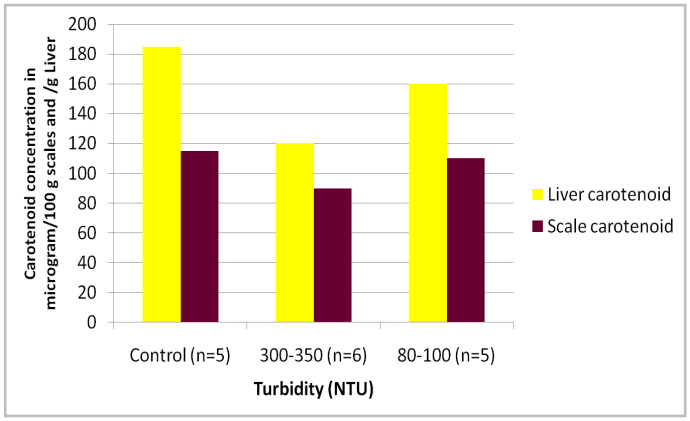

Fig. 2. Effect of of turbidity on carotenoid concentration of *Polyacanthus fasciatus*

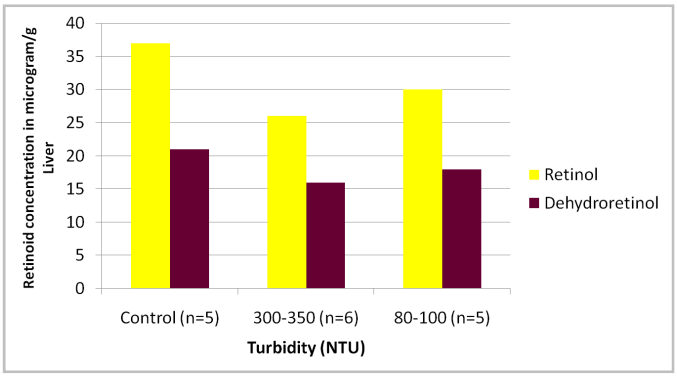

Fig. 3. Effect of turbidity on retinoid concentration of *Puntius sophore*

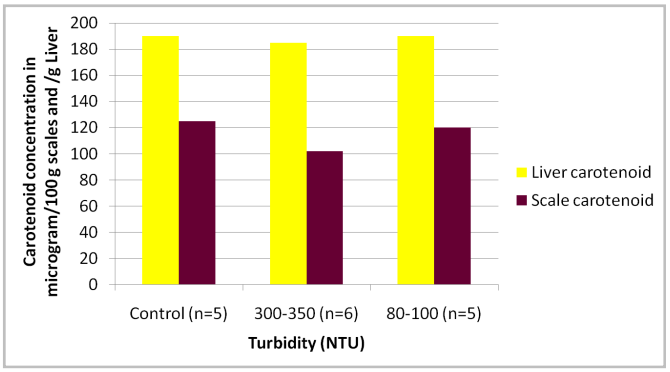

Fig. 4. Effect of turbidity on carotenoid concentration of *Puntius sophore*

Table 1. Effect of different turbidity (NTU) on the concentration of retinoids and carotenoids

Fish size and No	Turbidity (NTU) *	Amount		Total	
		Retinol (µg/g) Liver	Dehydroretinol (µg/g) Liver	Carotenoids (µg/100gm) Liver	Scales
P. fasciatus	Control (n= 5)	27 (± 1.0)	22 (± 0.5)	185 (±1.5)	115 (± 1.5)
(n = 6)	300-350	22 (±1.5)	15 (±2)	120 (±10.5)	90 (±1.5)
(n = 5)	80-100	25 (± 1)	18 (± 0.5)	160 (± 1.5)	110 (±.5)
P. sophore	Control (n= 5)	37 (± 1.0)	21 (± 0.5)	190 (± 0.5)	125 (± 1.5)
(n = 5)	300-350	26 (±2.5)	16 (±1.5)	185 (± 1.5)	102 (± 1.5)
(n = 5)	80-100	30 (±0.5)	18 (±0.5)	190 (± 1.5)	120 (± 0.5)

The results are the mean value of the fish as shown with the brackets and significantly different P<0.05

Size of the fishes :

P.fasciatus =(6±0.5cm)

P. sophore =(5±0.5cm)

*300-350 NTU (Puthimari river Latitude: 26°25'8.98" Longitude: 91°28'32.16".)
80-100 NTU (Deepar beel 26 ° 7' 26" Latitude/90o 38' 49''Longitude).

From the table, it is observed that the fish species *P.fasciatus* shows its retinol, dehydroretinol, total carotenoids concentration in control condition as 27 (± 1.0) (µg/g), 22(±0.5)µg/g and 185 (±1.5)µg/100g in liver and 115 (±1.5) µg/100g scale carotenoids and at 300-350 NTU turbid condition it is found as 22 (±1.5) µg/g retinol, 15 (±2) µg/g dehydroretinol, 120 (±10.5) µg/100g carotenoids in liver and 90 (±1.5) µg/100g carotenoids in scales. In turbid condition 80-100NTU it is recorded the retinol, dehydroretinol, total carotenoids concentration in liver and scales as 25 (± 1) µg/g, 18(±0.5) µg/g, 160(±1.5) µg/100g and 110(±0.5) µg/100g respectively. Again in case of fish species *P.sophore* it is observed that the total retinol, dehydroretinol, total carotenoids concentration in control condition are as 37 (± 1.0) (µg/g), 21(±0.5)µg/g, 190(±1.5)µg/100g in liver and 125(±1.5))µg/100g in scales. In 300-350NTU turbid condition the retinol, dehydroretinol, total carotenoids concentration in liver and scales are found as 26(±2.5) µg/g, 16 (±1.5) µg/g, 185(±1.5) µg/100g and 102(±1.5) µg/100g and in turbid condition 80-100NTU retinol, dehydroretinol, total carotenoids concentration in liver and scales are found as 30(±0.5) µg/g, 18(±0.5) µg/g, 190(±1.5) µg/100g and 120(±0.5) µg/100g respectively.

DISCUSSION

In the present study we demonstrated that there is a remarkable change found in fish pigmentation collected from various turbid water condition. It has been found that fishes dwelling in a high turbid areas show a lesser amount of pigmentation, whereas clear-zone area fishes show greater amount ofpigmentation with high deposition of carotenoids and retinoids molecules.

This observation is not surprising in view of our recent findings that the retinoid reserves as well as its synthesis are highly dependent on various environmental and nutritional factors[8,11]. These data are in agreement with the finding showing that temperature, salinity, hardness of water, water pH or water turbidity etc. are the most prominent factors of water which highly influenced the lipid content of freshwater organisms[9] and the dynamic interactions between these factors produce the temporal patterns in the lipid[8,9,11]. The concentration of carotenoids which is directly associated with the pigmentation as well as biogenesis of different form of vitamin A, is vulnerable in such a situation[8]. According to Pavlidis *et al.*[26] pigmentation might depend on turbidity and water colour. Previous work indicated that, within *P. nyererei*, populations inhabiting turbid waters exhibit less red colouration in males[27,28] and weaker colour preferences in females compared to cleared water populations. Colour production may be subject to physiological constraints[29,30]. It is found that the red and yellow colouration in *Pundamilia* is carotenoid based and the availability of dietary carotenoids may covary with underwater light intensity[28]. It is reported that Haplochromine cichlids of East Africa constitute a species rich assemblage with extensive variation in male colouration and it is suggested that the variation in underwater light conditions influences the evolution of these colour patterns[28] . From a study it is also found that in lake Victoria, for example, male colours tend to become more distinctive in locations with relatively high water transparency[27,28] and some colour morphs are completely absent in turbid waters[31]. From the present study it has been seen that the high turbid water i.e., at 300-350NTU highly affects on the retinoids and carotenoids concentration of the experimental fishes compared to fishes collected from 80-100NTU turbid water condition. It is also found that fishes collected from 4-4.4NTU turbid water

condition which was taken as control in our experiment show higher amount of carotenoid deposition compared to fishes collected from 80-100NTU turbid water. The carotenoids either failed to be converted into vitamin A, or the vitamin A reserve is directly affected in the liver in such situation. The present experiment derives support from the studies of Goswami, 2003[8] who found that the retinol and dehydroretinol concentration of fish *P.fasciatus* collected from 250-350 NTU turbid water was as 17.0 µg/g and 11.2 µg/g respectively while fishes collected from 120-150 NTU turbid condition the amount of retinol and dehydroretinol concentration were found as 19.5 and 13.2 µg/g respectively. It could be assumed that the high turbidty of water hindered the conversion of carotenoids into vitamin A as well as deposition of the pigments[8].

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