

# Variations in Skin Pigmentation in Freshwater Fishes in the Exposure of Diverse Varieties of Industrial and Mill Effluents

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## Abstract

A skin biopsies are the very important tool to introspect between the whole organism and its cellular physiology. Many recent advances in the field of pollution and behavioral ecology is obtained through experimentation on skin biopsies. In the present study done on fish *Cyprinus carpio* and *mystus vittatus* captured from the most worstly polluted sites of river Aami in Gorakhpur, Uttar Pradesh, which receives tons and tons of heavy metal and toxic xenobiotics from several industries surrounding the river. The results thus obtained, had shown a clear picture that fishes depending on the level of pollutants surrounding them in aqueous medium have got a darker melanophores in turn, a darker skin pigmentation in comparison to control group of fishes.

**Keywords:** Biopsy, Melanophores, Melanin, Pigmentation, Pollutants

## 1. Introduction

Melanophores are specialized cell derived from the neural crest that contains membrane bound vesicles called melanosome. Membranes are filled with melanin a dark non fluorescent pigment that plays a principal role in physiological colour adaptation of animals. Melanin a polymer of high molecular weight and great stability that is synthesized from phenylalanine and tyrosine (1). Melanophores from fish and frogs have long served as models for a general understanding of intracellular transport and organelle positioning. For this purpose, fish skin biopsies have frequently been used for isolation of pigment cells (2). In fish pigmentation research, skin biopsies have also been used to understand what causes alterations in the skin overall appearance, especially in the context of temporary and permanent modulation of tissue coloration. Here, we review our methodology for the use of skin biopsies in research on fish coloration – from pigment cell physiology to animal behavior. When pigment cell granules are aggregated in the cell centre, most of cell is unpigmented and animal bearing such cells would appear lightly coloured. When pigment granules are uniformly pigmented and animal bearing such cells would appear darkly coloured (3). Melanophores disperse or aggregate their melanosomes when host requires changing its colour in response to the environmental cues such as camouflage or social interaction (4).

## 2. Material and Method

Study area and selected sites for study: Gorakhpur is a city in the eastern part of the state of Uttar Pradesh in India, near the border with Nepal. River Aami is very important river of this region and traverses approximately 102 Kms.

and ultimately confluences in the River Rapti near village Sohgaora of tehsil Bansaon of district Gorakhpur. It is tributary of Rapti river on its right bank receives industrial effluents at several locations in a major stretch starting from Rudhauri to its confluence point at Sohgaora into Rapti river and serves as a lifeline for the people of Siddarth Nagar, Sant Kabir Nagar, Basti and Gorakhpur district in Eastern Uttar Pradesh. The river water, is presently affected severely by industrial pollution ever since the establishment of paper mill at Khalilabad, distillery and sugar unit at Rudhauri at Gorakhpur industrial development area (GIDA), Sahjanva, which is an industrial stretch in Gorakhpur which is important not only for its contribution in Indian economic growth but this rapid growing industrial area has got an important environmental impact which is none other than polluted river Aami, which is backbone of agricultural as well as household needs for people inhabiting near this area. GIDA has many factories which are ranked on state as well as national level and some of them are IGL (India Glycol Limited), Parle, ARP (Azam Rubber Products) footwear as well as powerlooms, plywood and the only jute mill in Uttar Pradesh. The stretch of the river from Rudhauri, Basti to Sohgaora, Gorakhpur has been identified as a polluted stretch.

3 sites or the areas of river were chosen for the study which come under the sources of industrial discharge in the river water from Sahjanva to the final confluence point of Aami to Rapti river at Sohgaora and further work was carried out. The areas chosen for the study are as follow and fishes *Cyprinus carpio* and *mystus vittatus* were collected from the sites chosen. The sites selected for study are below:

Site 1: part of river present near India glycol limited, GIDA, Sahjanva, Gorakhpur

Site 2: river flowing through Adilapur village, where all the different types of industrial effluents are dumped together in the river such as paper wastes, alcoholic chemical, jute and textile mill effluents, metal processing waste materials, hospital and pathological effluents etc.

Site 3: confluence point of 2 rivers i.e. Aami and Rapti near Sohgaora, where Aami meets Rapti river and thus, water of river thus obtained having different physico-chemical properties derived from both the rivers.

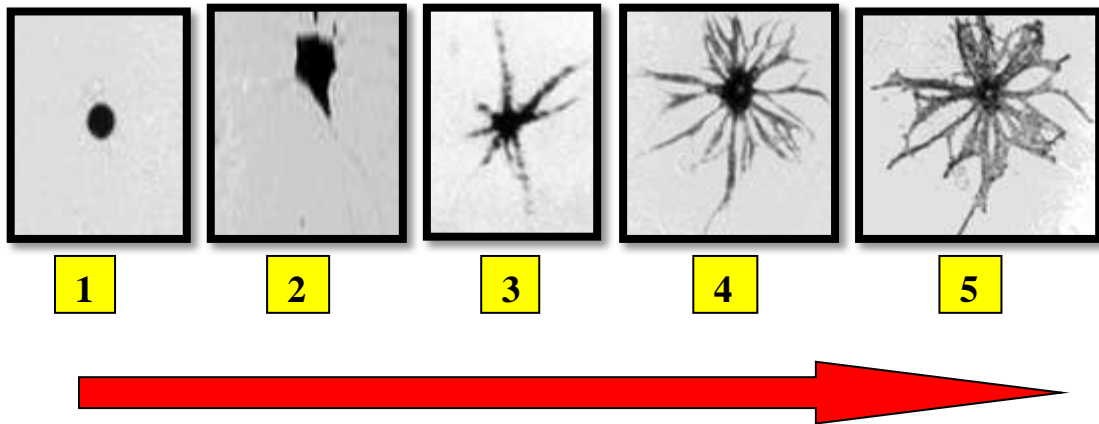
Collection of fishes: The adult fishes of same weight and same length approximately and of same species were captured. It was kept in mind that the species captured were native to that area. Capturing of fish was done with the help of fisherwoman accompanied and afterwards, they were brought in department laboratory as soon as possible.

Diseased as well as injured fishes were removed as soon as possible and experiment was conducted on their blood and tissue samples to assess the alterations due to biochemical parameters included in the study.

10 healthy fishes of nearly approximately same weight and same length of *Cyprinus carpio* and *mystus vittatus* were captured with the help of cast net and brought as soon as possible to natural product laboratory, DDU Gorakhpur University for assessment of the skin pigmentation included in the present study.

Hogben and Slome in 1931 developed a so called melanophore Index (MI) which is in use since the earliest studies of melanophore responses to melanotropins (5). In this microscopic assay, the degree of melanosome dispersion is determined according to 5 point scales, to field a melanophore index that ranges between 1 and 5. This method employs 5 distinct stages representing different degrees of pigment dispersal within the melanophores. The most aggregated state is designated as stage 1 and the most desperado as stage 5, stage 2, 3, and 4 represent intermediate degree of pigment dispersal. As pointed out by several scientists, by using this system an index of the relative dispersal of melanosomes can be obtained from quantitative use (6,7). Based on this system the degree of melanosome

dispersion was determined by visualizing them.the following figure describes the degree of melanosome distribution throughout the melanophore cell .this is called melanophore indexing which is manually accounting the MI number to the melanophore obtained from the skin biopsies of fish chosen for the study.



**Aggregation**

Melanophores

**Dispersion**

**4.Results and Discussion:**

Melanophores can be used successfully as bio marker of water pollutions caused due to various industrial effluents and by products discharged from mills . It was observed that higher concentration of toxic compounds in water might have induced and altered activity of melanophores. Rate of melanosomes dispersion was found to be increased in case of sites which were heavily polluted due to the concentration of toxicant of industrial effluents in water rendering fish skin colour to get dark due to dispersion of melanosomes the most . it was also noticed that site which were less polluted , melanosome dispersion was less rather, aggregated melanophore pigmint was seen in case of melanophores which resulted in slightly or less darker fish skin colour in case.

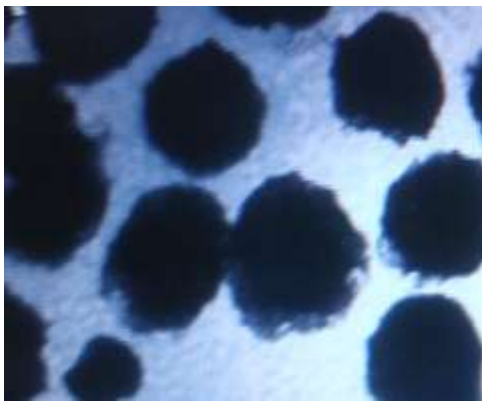
The observation table 1a and 1b below describes the melanophore dispersion and aggregation in the control as well as experimental group of fishes with the the microscopic photograph images picture clearly describe the findings.

Table 1a :Melanophore Index value of the skin done in *Cyprinus carpioto* view level of alterations in pigmentation of skin due to pollutants.

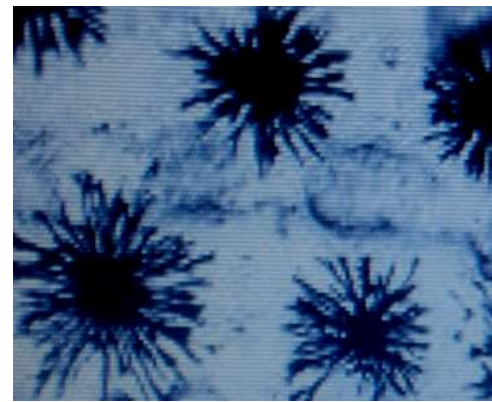
	Control	Experimental group		
		Site 1	Site 2	Site 3
Melanophore dispersal (dispersion/aggregation)	Aggregated melanophore pigmint	highly dispersed pigmint	Highly dispersed	Slightly dispersed
Index number (1 to 5)	1	4	5	2 and 3 both

Table 1b: Melanophore Index value of the skin done in *Mystus vittatus* view level of alterations in pigmentation of skin due to pollutants.

	Control	Experimental group		
		Site 1	Site 2	Site 3
Melanophore dispersal (dispersion/aggregation)	Aggregation of melanophore pigment	Highly dispersed	Highly dispersed	Slightly dispersed
Index number (1 to 5)	1	3 and 4 both types	4 and 5	2 and 3 both



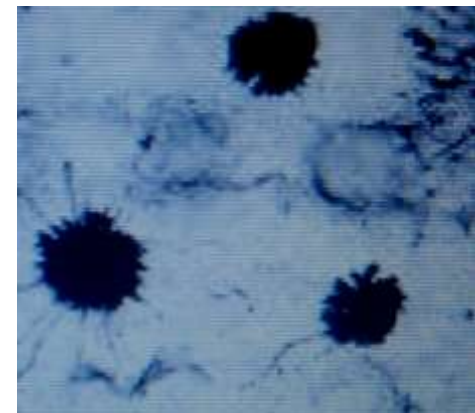
A



B

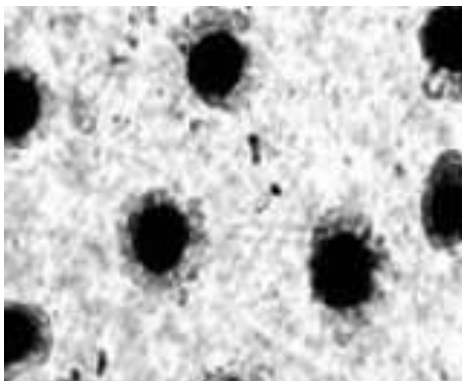


C

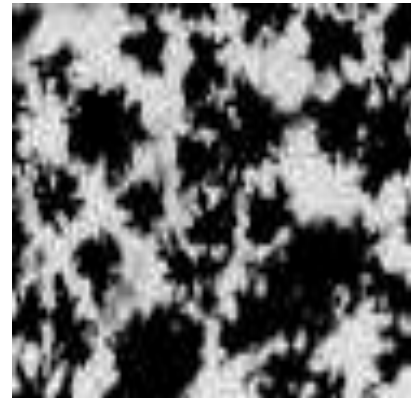


D

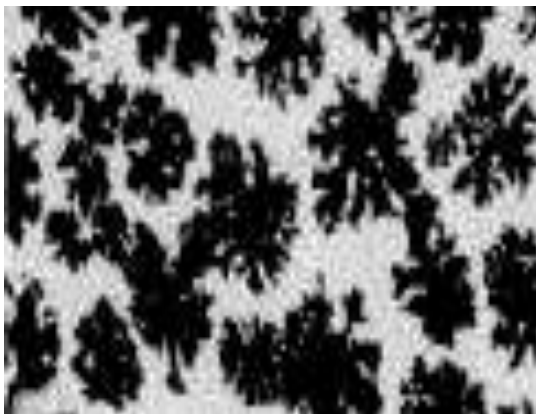
FIGURE 1a : Microscopic images (40x) taken of skin biopsies of *Cyprinus carpio* collected from control group and experimental sites to show aggregation and dispersion of melanin pigment in melanophores of  
 A) control group fishes      B) site 1 fishes      C) site 2 fishes      D) Site 3 fishes



A



B



C



D

FIGURE 1b : Microscopic images (40x) taken of skin biopsies of *Mystus vittatus* collected from control group and experimental sites to show aggregation and dispersion of melanin pigment in melanophores of  
 A) Control group fishes      B) Site 1 fishes      C) Site 2 fishes      D) Site 3 fishes

## 5. Conclusion

skin biopsies can be useful for studies of animal coloration and the mechanisms for colour change. Manipulations and observations of skin biopsies have given us an increased understanding of regulation of rapid colour change in general, and have been successfully applied to the study of colour signals in animals. Similarly, by using abdominal skin biopsies (containing both epidermis and the peritoneum) we have acquired novel insights into the functions of internal pigment cells in transparent fishes. River Aami is the tributary of river Rapti flowing in eastern part of Uttar Pradesh majorly from city Gorakhpur. It is backbone of the rural people dependent on it. It is clear from the above set of experiments and biochemical analysis that condition of the river is not good and it is deteriorating day by day causing people and terrestrial and aquatic animals to face a kind of stress affecting their survival. People who depend on the river for fishing and agricultural activities have to face various problems due to deteriorated quality of the river water. However, seriousness and depth of this problem have realised in previous years and many work as well as



media attention is drawn towards this direction for its conservation from various industrial and domestic pollutants. Hence, the present work is also an attempt made in this direction for conservation of this river by analyzing the river water qualities by physical and chemical parameters and biochemical experiments carried on fish *Cyprinus carpio* and *Mystus vittatus*, a major inhabitant of river in this region shows great adaptability and resilience. I hope, this will provide current health status of this river and its animals for making some conservation programmes in spreading awareness towards our natural resources and sustainable development for future generations.

## 6. Acknowledgement

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## 7. References

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