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# Development of melano-macrophage centres (MMCs) in Indian major carps

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#### Abstract

Appearance, development and organization of Melano-Macrophage Centres (MMCs) in Indian major carps. (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) was studied for a period of 360 days. Small patches of melanin-containing cells appeared at about 60 days old in the spleen and kidney of these species. The density of splenic and renal MMCs was observed to increase steadily with age of the fish. The MMCs were less compact and smaller in size in the trunk kidney than in the spleen. The size of the MMCs was highly variable within the fish in the same organ and these centres were distributed in the spleen, pronephros, and mesonephros and opisthonephros but not observed in the liver and thymus in the species. The existence of morphological similarity was observed with respect to size, shape and distribution of MMCs in all the three species of Indian major carps.

Keywords: Melano-macrophage, kidney, lipofuscin, spleen

#### 1. Introduction

Pigment containing cells is a prominent feature of certain soft tissues of fish. The melanin containing cells are commonly encountered in the hematopoietic tissue of fishes. Ultra structurally these cells resemble macrophages and they were often aggregate in large numbers of forming distinct pigment nodules; hence the name melano-macrophage centres <sup>[17]</sup>. In primitive tissues such as the salmonids, pigmented macro-phages are well dispersed in the anterior kidney, liver, spleen and elsewhere <sup>[3, 2]</sup>. While, in higher fishes have a discrete aggregation of pigmented macrophages in the corresponding soft tissues; they are conspicuous and exhibit greater or lesser pigmentation and size varies depending on the species, age and the state of health of fish <sup>[17, 15]</sup>. These centres contain three pigment types: melanin, hemosiderin and lipid pigments (lipofuscin/ceroid), and all three pigment types can be present in the same cell. Lipofuscin appears to be the most common pigment. Melanin is the other major component but hemosiderin can be abundant in teleost spleen under conditions of starvation and disease <sup>[5]</sup>. In fish with morphologically distinct aggregations (Osteichthys), the centres are usually rounded, although they may have some irregularity in outlines and are separated from the surrounding tissue by an argyrophilic capsule (Reticular fibers) and are most often found in close relationship with blood vessels <sup>[19, 13, 14]</sup>, in spleen with ellipsoids, while in liver most commonly in portal vein [17, 13, 16]. The factors that affect the abundance and biology of these have been poorly defined. The current study, try to establish the appearance of these centres during normal development in three species of Indian major carps namely, Catla catla, Labeo rohita and Cirrhinus mrigala during one year growing period.

#### 2. Materials and Methods

Animals: The hatchings of Indian major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were collected after 4 days of hatching at Bhadra Fish Farm, from a particular brood stock of each species. The hatchlings were stocked at cement cisterns of  $25 \text{ m}^2$  area having 1m depth and reared the three species separately. The hatchings were stocked at the rate of  $3000/25 \text{ m}^2$  and were fed daily at 10% of the body weight and later at 5% with rice bran and oil cake at 1:1 ratio. Fishes were segregated at regular intervals of three months and stocked in identical cement cisterns for a period of one year.

A minimum of 10 specimens of each species were collected after hatching daily up to 30 days, at 15 days intervals upto 90 days and later on at monthly intervals till 360 days. The length and weight of the specimens were recorded and fixed in 10% formalin. The standard histological technique <sup>[8]</sup> was followed for the investigation.

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#### 3. Results

To study the appearance, development and organization of melano-macrophage centres (MMC) in the three species of Indian major carps, a histological study was carried out for a period of 360 days. Continuous serial section of samples of the three species collected daily up to day 30post hatch was observed. Small patches of melanin containing cells appeared at about two months of age in the spleen and kidney of these species (plate 1). The density of splenic and renal MMCs was observed to increase steadily with the age fish. In the early stage, the centres were loose, little developed and less organized (Plate 2). The MMCs were less compact and smaller in size in the trunk kidney than in spleen of the three species. Size variations were also observed with respect to their age; the size of these centres in both kidney and spleen increased with age (plate 3 and 4). The splenic MMCs were later more developed and well organized than the renal MMCs. Moreover, the density of splenic MMCs was higher than that of renal MMCs in all three species of Indian major carps.

The MMCs in Indian major carps vary in shape, size, pigment content and organ distribution. The individual cells were often poorly outlined in the clusters. The MMCs were more nodular, usually rounded, but some had irregularity with outline. The number of cells that constituted the centres were not constant and it seemed to vary within species in the same organ of the section during the study. These centres were separated by reticular fibers and were closely associated with the blood vessels. The size the MMCs was highly variable within the fish in the same organ. These centres weredistributedcommonly in the spleen, pronephros, mesonephros and opistho nephros but never observed in the liver and thymus in the species. The existence of morphological similarity was observed with respect to size, shape and distribution of MMCs in all the three species. The pigment content in the centres of melanomacrophage in mrigal was higher than in catla and rohu. Higher density and better organization of MMCs were observed in spleen and kidney in mrigal, followed by rohu and catla.

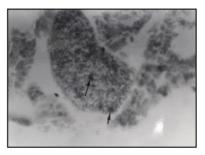


Plate 1: Small patches of melanin containing cells appeared at about two months of age in the spleen and kidney

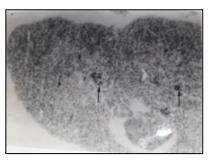


Plate 2: Little developed and less Organized kidney and slpeen in early stages

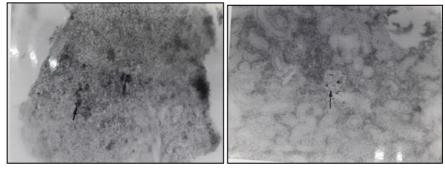


Plate 3, 4: Size of the centres in both kidney and spleen increased with age.

### 4. Discussion

Macrophages are an important component of the immune system of fishes and normally accumulate several kinds of pigment and other materials <sup>[10, 12, 11, 1]</sup>. The predominant pigments are melanin, lipofuscin and hemosiderin. Melanin appears to be derived from phagocytosis of melanin granules or their precursor organelles from melanin containing cells. Lipofuscin appears to be derived from damaged cellular components such as effete mitochondria, through peroxidation of their unsaturated lipids. Hemosiderin is almost certainly derived from the breakdown of hemoglobin from effete erythrocytes <sup>[1, 6]</sup>. In primitive fishes such as salmonids, pigmented macrophages are well dispersed in the anterior kidney, liver, spleen and elsewhere [17, 2], while in advanced bony fishes they are especially abundant in two major haemolymphopoietic organs, the spleen and kidney form distinct aggregates known as pigment nodules <sup>[19]</sup> or

melano-macrophage centres <sup>[17]</sup>. Factors affecting the abundance and biology of the MMCs are scarcely known. The relative abundance in the splenic pulp can be affected by pathological events such as bacteria or protozoan infections, hemolytic anemia of various etiology or iron overloaded <sup>[9, 18]</sup>. In Indian major carps, MMCs appeared on day 60 post-hatch in the spleen and kidney. These centres were loosely organized and less organized upto 3 months of age. The density of the MMCs increased both in kidney and spleen at later stages. The number of MMCs was more in spleen than in the kidney. A similar pattern of appearance and development was described in rainbow trout <sup>[4]</sup>, where 8 weeks-old fry had melano-macrophages in the spleen and the kidney simultaneously. The number increased after 20 weeks and kidney had more MMCs than the spleen. However, in Indian major carps, the number of MMCs was more in the spleen than kidney. The MMCs in the Indian major carps were

heterogeneous in shape and size and were separated by reticular fibres and appeared close to the blood vessels in the spleen of elasmobranchs in sunfish and Lepomis spp<sup>[19, 14]</sup>. The size of the MMCs varies within the fish in the same section of the tissue<sup>[3]</sup>. The number, size and pigment content of MMCs increased steadily with age during the 360 days study period in all the three species of Indian major carps same as <sup>[17, 7, 4]</sup>. On the contrary <sup>[4]</sup> observed the density of splenic and renal melano-macrophages in rainbow trout increased steadily with age. The rate of increase was the highest during the first year of life but decreased later on. However the density of melano-macrophages in spleen was consistently lower than that in kidney. In turbot, MMCs increased during first two years of life, after which they decreased.

#### 5. Conclusion

The present study deals with only the first year of age of the three species. The existence of morphological similarity has been observed in respect of size, shape and distribution of MMCs in all the three species of Indian major carps, but better developed MMCs with high pigment content were observed in the spleen and kidney of mrigal followed by catla and rohu. The possible reasons may be that mrigal is detritivorous in feeding habit and there is a possibility that it might ingests more number of bacteria. This could probably induce intense phagocytosis and enhanced MMC formation.

#### 6. Acknowledgment

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