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Structure of immune organ in edible catfish, *Mystus gulio*

B. Deivasigamani*
***sigamani_bd@rediffmail.com**

CAS in Marine Biology, Annamalai University, Parangipettai-608 502, India

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Abstract: *The light microscopic study describes the anatomy and histomorphology of head-kidney in bagrid catfish, Mystus gulio. Showing numerous lymphocytes, interrenal cells, reticular cells, postcardinal vein, blood sinuses and melanomacrophage centers.*

Key words: *Head-kidney, Lymphocytes, Interrenal gland, Melanomacrophage, Mystus gulio*
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Introduction

Among vertebrates, fishes are the earliest group to possess a well defined immune system with lymphomyeloid tissues consisting of mixed lymphoid and myeloid elements (Sailendri and Muthukkaruppan, 1975b; Grace and Manning, 1980; Pitchappan, 1980; Rafin and Wignstand, 1981; Pica and Corte, 1987). The main lymphoid organs of fish are thymus, head-kidney, spleen and blood tissue (Corbel, 1975; Ellis, 1977; Zabotkina, 2005; Deivasigamani, 2006). The head-kidney (HK) is a unique, important lymphoid organ in fish (Sailendri and Muthukkaruppan, 1975b; Ellis, 1980). It contains more lymphocytes than spleen and has been shown to be actively involved in antibody production (Sailendri and Muthukkaruppan, 1975a; Zapata, 1979; Ellis *et al.*, 1989; Romano *et al.,* 2002). The role of head-kidney as a major site of production of erythroid, lymphoid and myeloid cells has been established (Smith *et al*., 1970; Zapata, 1979; Romano *et al.,* 2002). The capacity of the head-kidney to trap antigens and produce antibodies has been demonstrated in a few species (Ellis *et al.,* 1989; Zapata and Cooper, 1990).The present study describes the anatomy and histomorphology of head-kidney in bagrid catfish, *Mystus gulio* at the light microscopic level.

Materials and Methods

Live specimens of *Mystus gulio* (Hamilton) of the family bagridae were collected from Ogyampet estuary near Chennai. A total of 60 adult fish of both sexes, weighing 40-45 g (standard length 11-15 cm), were used in the present study. A stock of fish was maintained in the laboratory in a large tank. After acclimatization for at least three days, the fish were transferred to smaller aquarium tanks with continuous aeration. They were fed with bits of earthworms and tubifex.

The fish were immobilized by immersion in ice-cold water for about a minute and then dissected and their immune organ of head-kidney were removed to fixed in Bouin's fluid for about 24 hr, processed, embedded in paraffin wax and cut into 6 μm thick sections. The sections were stained by the hematoxylin and eosin. The imprint was prepared on a clean microslide from cut surface of freshly dissected spleen. They were air-dried and stained by the Leishman stain.

Calibration of microscope was made using stage and ocular micrometers. Measurements of the dimensions of the different types of cells and those of MMCs (melanomacrophage centres) were made under high-power objective magnification (45 X).

Results and Discussion

In *Mystus gulio* the kidney (Fig.1) is a paired but fused retroperitoneal organ that extends as a thick sheet of tissue dorsal in the body cavity in close contact with the osseous vertebral column. The kidney consists of two well-separated segments, an anterior head-kidney (HK) and a posterior trunk-kidney (TK). The trunk-kidney begins as a thin layer near the posterior end of the peritoneal cavity, soon widens and bifurcates into two thick lobes with a marked concavity, made by the pressing air-bladder, at the anterior end. The posterior cardinal veins emerge from the dorsal rim of the concavity close to the spinal column, run parallel, beneath the air bladder and enter into the corresponding half of the HK. The HK's are also fused and appear as a bilobed structure that lies perpendicular to the dorsal body wall and lining the anterior blunt surface of the air bladder. Thus, it is very close to the heart and the pharyngeal chamber. Its association with the trunk-kidney is obscure though the two are well connected by the posterior cardinal veins. The difference between the HK and the TK is that the former is composed of haemolymphoid and endocrine (interrenal, homologue of adrenal) tissues whereas the later is purely a renal tissue. The HK in adult fish weighs about 0.5-0.9 g and measures about 5-10 mm in length and 3-5 mm in width. The lobes of the HK are almost of the same dimensions.

A thin connective tissue capsule covers the HK (Fig. 2-4). The capsule does not seem to extend into the pulp of the HK as trabeculae. However, the HK pulp is supported by a closely knitted network of reticular cells and their fibers (Fig. 5). The entire pulp consists of anastamosing channels of blood by chords of lymphocytes (Fig. 3-7). In cross sections, these channels cells bounded and

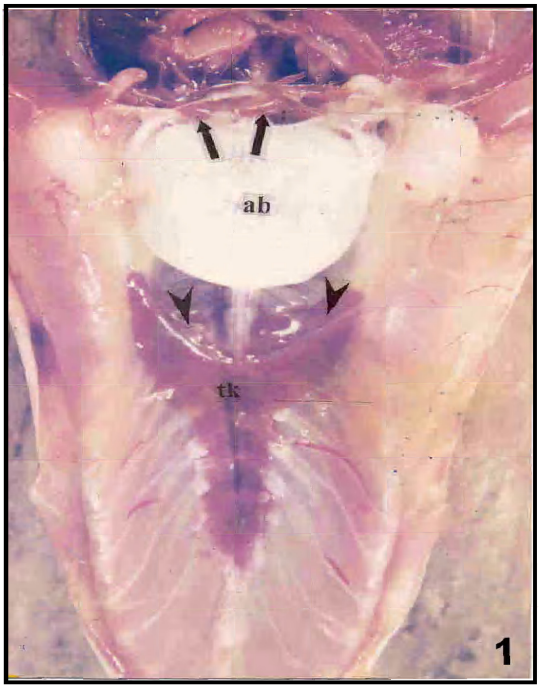

Fig. 1: Dissection of *M. gulio* showing the location of the head-kidney (arrow) in relation to trunk-kidney and air-bladder. The concavity (arrow head) at the anterior surface of the trunk-kidney is made visible by the stretching of the body wall x 1.25

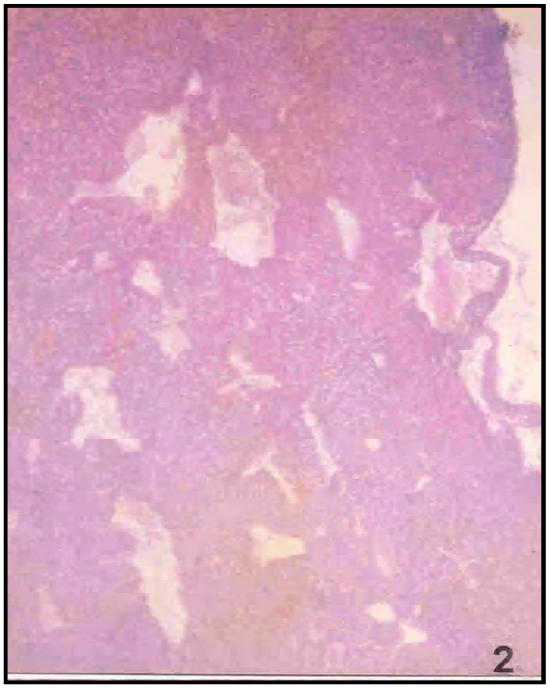

Fig. 2: Section of the HK of *M. gulio* showing the organization of the pulp into channels of blood vessels, chords of lymphocytes and large and small venous sinuses. The entire pulp is covered by a thin capsule. Bouin, HE x 60

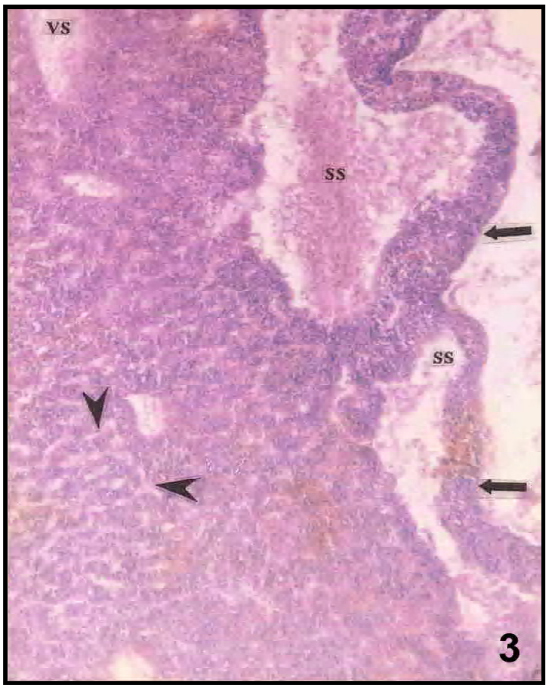

Fig. 3: Higher magnification of part of the section showing the connective tissue capsule (arrow), subcapsular sinus, channels of blood chords of lymphocytes (arrow head), and venous sinuses. Difference in the organization of the pulp between the central and peripheral areas, evident. Bouin, HE x 130

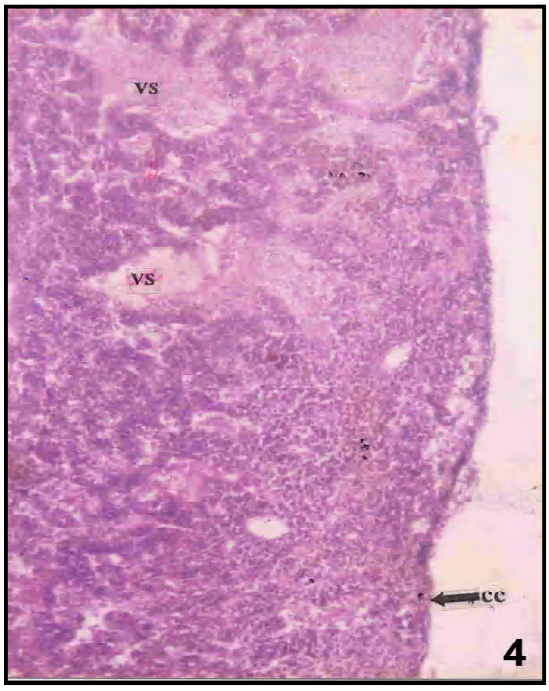

Fig. 4: Section of the HK of *M. gulio* showing the difference in the pulp organization between the peripheral and central areas. Bouin, HE x 130

Abbreviations used: ab = air–bladder, tk = trunk–kidney, vs = venous sinus, ss = sub capsular-sinus, cc = channel-chord

chords appear as follicles, each consisting of a central blood vessel surrounded by a thick layer of closely packed, dark-staining lymphocytes (Fig. 7). The channels of blood cells do not seem to have any connective tissue boundary separating them from the surrounding layers of lymphocytes. The content of these channels are generally erythrocytes (Fig. 6 and 7). However, invasion of these channels by lymphocytes, macrophages and other granulocytes from the surrounding layers is not uncommon (Fig. 8 and 9). All these channels converge towards and open into venous sinuses of varying sizes throughout the HK parenchyma (Fig. 1-3 and 10). The subcapsular sinuses are generally larger than those in the middle. The organization of the pulp into channels and chords is more pronounced in the middle than in the periphery where the lymphocytes are clumped as smaller groups surrounded by thin channels of blood cells (Fig. 3, 4). Generally, the channel-chord organization becomes obliterated closer to the larger sinuses.

In addition to the plexus of channels and chords of cells, there are numerous discrete MMCs of varying sizes (40.6 μm x 20.13 μm to 130.90 μm x 109.10 μm) and irregular shapes distributed throughout the HK, though their frequency is slightly higher in the center (Fig. 10) than in the periphery. Each MMC is an aggregation of at least two types of cells (Fig. 11-14): (i) melanomacrophages in various shades ranging from light yellowish brown to dark blackish brown and (ii) erythrocytes with pycnotic nuclei. In sections stained with Giemsa stain, the distinction between the two cell types is more apparent (Fig. 5), the erythrocytes appearing bluish and the macrophages, dark brownish.

In the early stages of its formation, an MMC includes macrophages in different degrees of pigmentation and erythrocytes (Fig. 11). At this stage, presence of a thin connective tissue strand separating it from the adjacent area is well evident. Further, a few cells-thick layer of lymphocytes surrounds the center. During the subsequent stage, the macrophages become increasingly darker with increased deposition of pigments in their cytoplasm, the connective tissue boundary is not discernible, and the lymphocyte layer grows to several cells-thick (Fig. 12). In advanced stage, most of the macrophages in the MMC appear dark brown to black brown and the surrounding lymphocyte layer has completely disappeared, and, instead, the center is encircled by a number of erythrocytes with pycnotic nuclei (Fig. 13). An MMC in a well-advanced stage has almost all its macrophages fully laden with blackish brown pigments, and is surrounded by thicker layer of erythrocytes with pycnotic nuclei (Fig. 14).

The types of cells observed in the head-kidney *viz*., reticular cells, erythrocytes, lymphocytes of various sizes, monocytes, eosinophils, plasmocytes and macrophages could be identified in the HK histological (Fig. 5) and smear (Fig. 15 and 16) preparations. In addition, a large number of developing cells of RBC's and granulocytes could be seen in the imprints. Melanomacrophages could also be seen in the smear preparation. The dimensions and other characteristics of these cells are almost the same as in the spleen. Structures resembling rosette formation by large lymphocytes around macrophages are very rarely identified (Fig. 17).

In most of the teleosts, the kidney lies along the ventral side of the vertebral column separated from the body cavity by tough connective tissue septum (Ellis *et al.,* 1976; Zapata, 1979). It consists of two segments, the head-kidney representing the pronephros and trunk-kidney, the functional excretory organ in the adults. The two parts are ontogenetically and, in the adult, anatomically distinct. The anatomical distinction is very much pronounced in the case of the catfish, *M. gulio,* as observed in the present study and *M. vittatus* (Gurumani, 1983), the head-kidney being well separated from the trunk-kidney by the air-bladder, and the connection between the two being the posterior cardinal veins. As a result of this separation, the bilobed head-kidney comes to lie on the anterior surface of the air-bladder, very close and dorsal to the heart. The right and the left posterior cardinal veins traverse the respective halves of the head-kidney before joining the common cardinal vein.

Though several workers have studied the histomorphology of the head-kidney of teleosts and indicated its lymphoid nature, most of their works pertain to the endocrine interrenal and chromaffin tissues, homologues of the adrenal cortical and medullary tissues, respectively, of mammals, that lie around the posterior cardinal veins within the head-kidney. Even among those works that deal with immunological or non-immune defense aspects of the HK, only a few have paid attention to the detailed histomorphology of the organ. Thus the literature on this aspect, when compared to that of the spleen, is very meagre.

The HK of *M. gulio* is covered by a rather thin connective tissue capsule that does not extend into the pulp to form a trabecular system as in the spleen. Therefore, ascribing any blood storage and release function to the HK is rather difficult. However, a closely knitted network of reticular cells and their fibers provide the support for the pulp. The pulp itself consists of channels of blood cells, representing red pulp, bounded by chords of lymphoid tissue, mostly lymphocytes, representing white pulp. The blood sinuses are prominent, especially the subcapsular ones. In addition, MMCs of varying shapes, sizes and in different stages of development are seen in the pulp.

The HK of the cichlid teleost *T. mossambica* is encapsulated by thin strands of collagen fibers and includes lymphatic and blood vessels and sinuses (Sailendri and Muthukkaruppan, 1975b). A broad subcapsular or marginal sinus lies directly beneath the capsule that enters at different points into the pulp as trabeculae carrying blood vessels. The HK of this species is mainly a lymphoreticular organ and consists of two different zones, a deeply stained lymphoid zone and a non-lymphoid zone. The lymphoid zone is represented by a number of lymphoid follicles, each of which consists of tightly packed small lymphocytes surrounding large vacuolated cells with

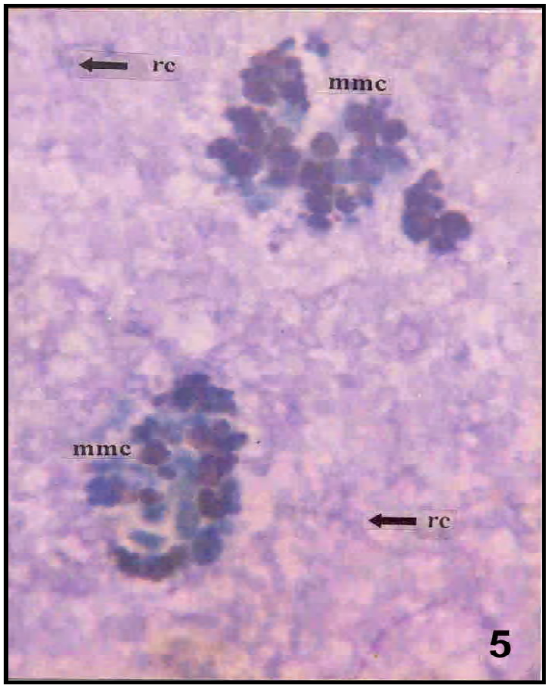

Fig. 5: Section of the HK of *M. gulio* showing network of reticular fibers formed by the reticular cells (arrow). Two MMCs with dark-brown melanomacrophages and bluish erythrocytes are seen. Bouin, Giemsa stain x 600

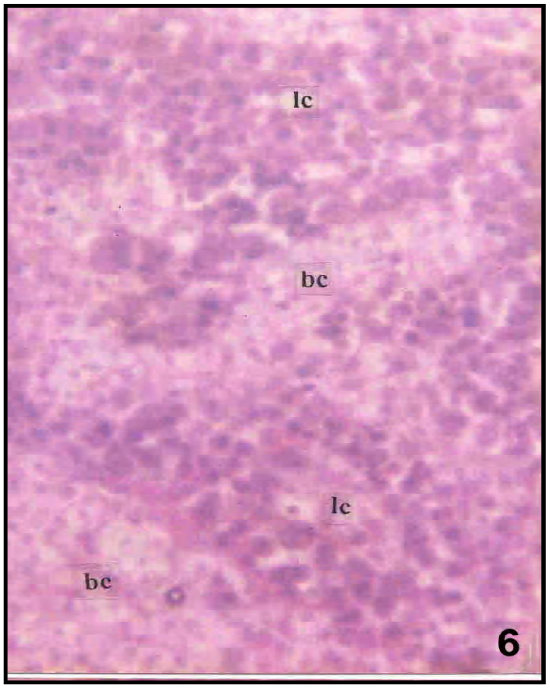

Fig. 6: Section of the HK of *M. gulio* showing at higher magnification the channels of blood containing erythrocytes and chords of lymphocytes. Bouin, HE x 600

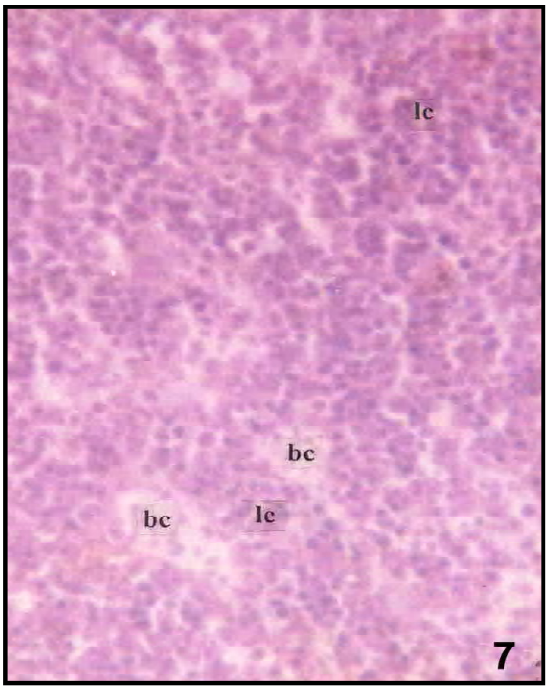

Fig. 7: Section of the HK of *M. gulio* showing a region of the pulp where the blood channel and the lymphocyte chord are cut transversely giving a follicular appearance. Bouin, HE x 600

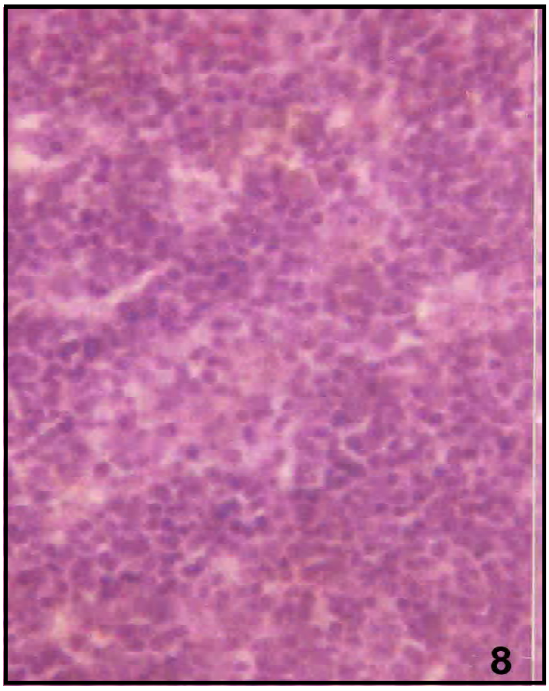

Fig. 8: Section of the HK of *M. gulio* showing a region of the pulp where channel is invaded by lymphocytes. The surrounding lymphocyte layer appears more crowded. Bouin, HE x 600

Abbreviations used: bc = blood channel, lc = lymphocyte chord, mmc = melanomacrophage center, rc = reticular cell

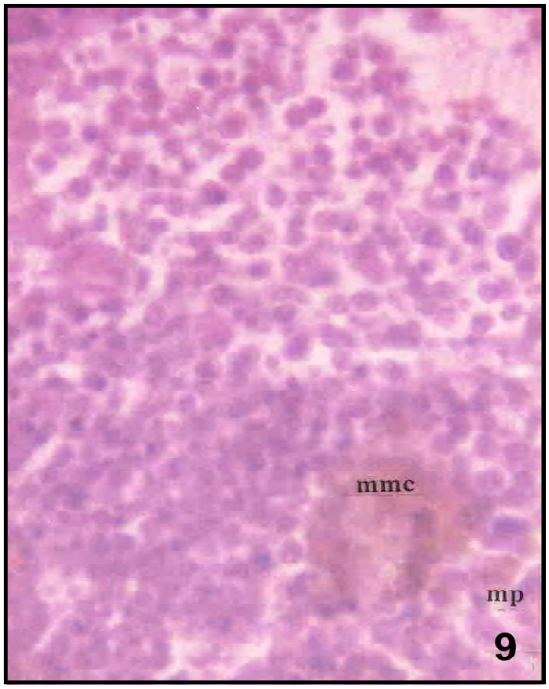

Fig. 9: Section of the HK of *M. gulio* showing at higher magnification a region of the pulp where the blood channel is full of lymphocytes, macrophages and some other granulocytes. Bouin, HE x 600
mmc = melanomacrophage center, mp = macrophage

dark pigments and a few large lymphocytes. The follicle also encloses a number of blood vessels and blood sinuses. The non-lymphoid zone consists of reticular cells, granulocytes and islands of erythrocytes. Lymphatic and blood vessels, and lymph and blood sinuses are also present in this zone. Based on the above lymphoid organization, and on the cellular changes associated with the antigenic stimulation and the presence of antibody-producing cells (Sailendri and Muthukkaruppan, 1975a), it was suggested that the HK of *T. mossambica* bears functional and possibly structural similarity to the mammalian lymph node (Sailendri and Muthukkaruppan, 1975b). However, Zapata (1979), was not in agreement with the above interpretation of equating teleost HK with mammalian lymph node because of the differences in the histological organization of the two structures.

The HK of the plaice, *P. platessa* has a haemopoietic parenchyma supported by a network of reticulo-endothelial cells (Ellis *et al.,* 1976). Thin walled blood vessels communicate with the haemopoietic pulp via fenestrations in their walls. From such blood vessels narrow blood sinuses formed by chords of reticulo-endothelial cells and leucocytes, radiate. The red pulp, comprised of developing and mature erythrocytes and granulocytes, is extensive. Many MMCs, spherical aggregations of melanomacrophages, bounded by a thin fibrous membrane and surrounded by a white pulp devoid of erythrocytes, are found throughout the haemopoietic kidney. Imprints of the plaice kidney showed a large number of developing cells of erythrocytic, granulocytic and lymphocytic cell lines. Thrombocytes were identified only occasionally. Based on the above observations on the HK in the plaice together with their phagocytic properties on colloidal carbon after its intraperitoneal injection, Ellis *et al.* (1976) suggested that this lympho-reticular organ has areas where both macrophages and immunocompetent lymphoid cells may mutually affect each other.

The supporting framework of the HK of the teleosts, *R. rutilus* and *G. gobio* is partly composed of a capsule that surrounds the organ. The capsule is made up of connective tissue consisting largely of collagenous fibers and fibroblasts (Zapata, 1979). Immediately below this sheath, there is a uniform continuous layer of pigment cells. Reticular cells, phagocytic reticular cells and other obligatory elements necessary for proliferation of haemopoietic cells are the cellular elements found the HK stroma. The presence of these elements together with sinusoids and the thin walled arteries in the HK, lead Zapata (1979) to suggest a structural similarity of this organ to the mammalian bone marrow, rather than to the mammalian lymph node.

In the hybrid sturgeon (*Huso huso* X *Acipenser ruthenus*) the lymphoid, or rather haemopietic tissue is found mainly in the anterior part of the kidney (Fänge, 1986). Imprints of the anterior kidney showed numerous blast cells, medium sized lymphocytes, granulocytes, macrophages and erythrocytes. Cells with intensely basophilic cytoplasm and eccentric large nucleus looked like plasmocytes, but were difficult to distinguish from large blast cells. Mitotic stages of blast cells were frequent.

Meseguer *et al*. (1994) and Herraez and Zapata (1986), described the morphology, formation and possible function of melanomacrophages and MMCs in the HK, together with those in spleen and liver, of the teleosts, sea bass *D. labrax* and gilthead seabream *Sparus aurata*. The MMCs had a round, oval or irregular outline and frequently occurred in close association with vasculature of these organs. They consisted of a few cells or large clumps of cells that were sometimes partially encapsulated. These capsules consisted of a single layer of flattened cells containing an elongated central nucleus. Both free and clustered melanomacrophages contained a granular or heterogeneous pigmented material ranging from yellow to dark brown. The granular dark brown pigment was particularly abundant in the HK but virtually absent in the liver of some gilthead seabream specimens. The melanomacrophages are PAS, hemosiderin- and acid phosphatase-positive. Head-kidney melanomacrophages isolated from both the species were able to engulf latex beads and bacteria. These cells were also capable of engulfing melanin granules and cell debris. On the basis of the above observations, Meseguer *et al.* (1994), concluded that at least a sub-population of seabass and gilthead seabream mononuclear phagocytes have a scavenging function and ingest different materials, such as dead or effete cells, mainly

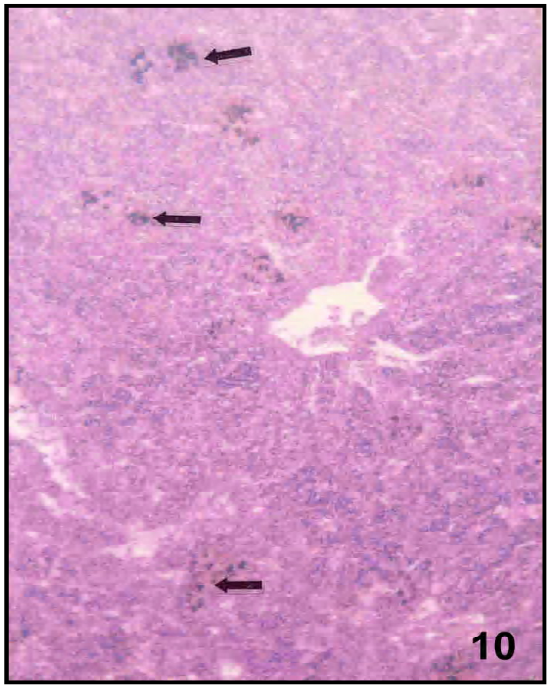

Fig. 10: Section of the HK of *M. gulio* showing the distribution of many MMCs (arrow) in the center. Bouin, HE x 130

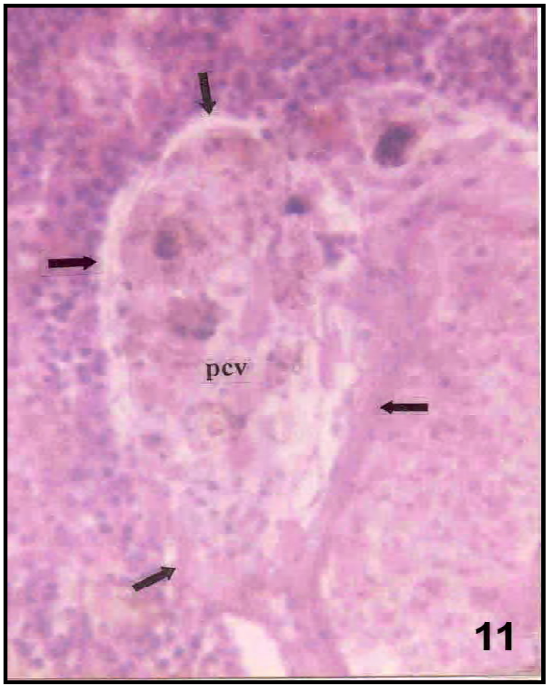

Fig. 11: Section of the HK of *M. gulio* showing an MMC close to a branch of posterior cardinal vein, in its early stage of formation. The macrophages inside the center show varying degrees of pigment deposition. A thin limiting membrane (arrow) and a layer of closely packed lymphocytes around the center visible. Bouin, HE x 600, pcv = posterior cardinal vein

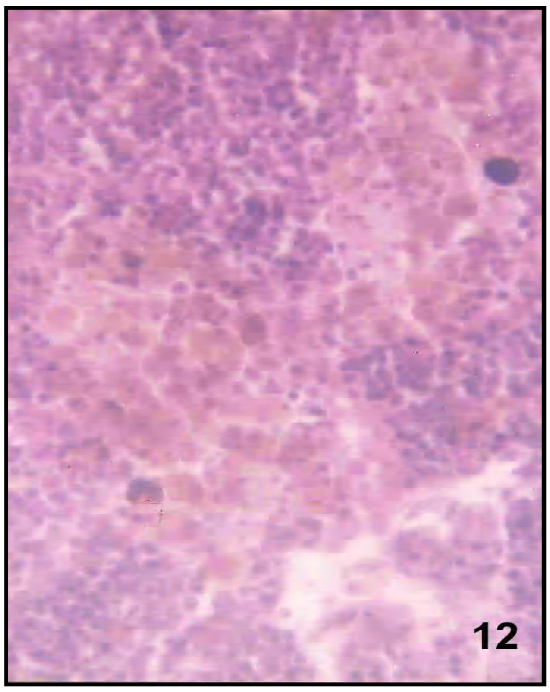

Fig. 12: Section of the HK of *M. gulio* showing two MMCs with many yellow brownish and a few black brownish macrophages. The limiting membrane is not visible. The surrounding lymphocyte layer is thicker. Bouin, HE x 600

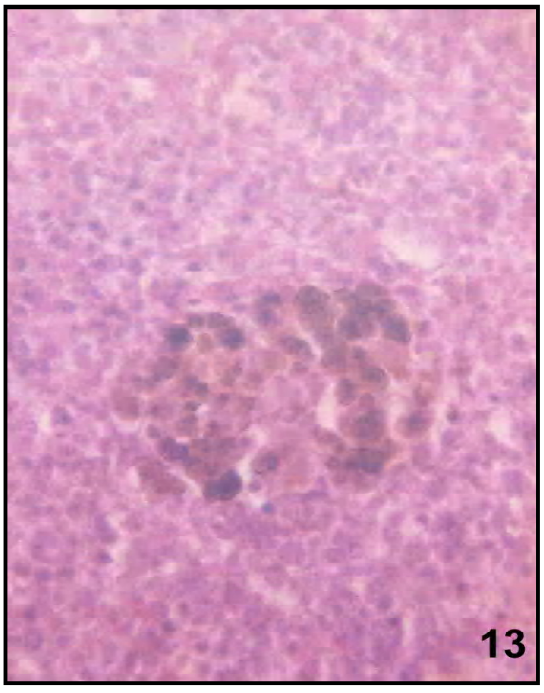

Fig. 13: Section of the HK of *M. gulio* showing an MMC in an advanced stage. Many of the macrophages laden with dark-brown pigments. Limiting membrane not visible. Lymphocyte layer disappeared. Bouin, HE x 600

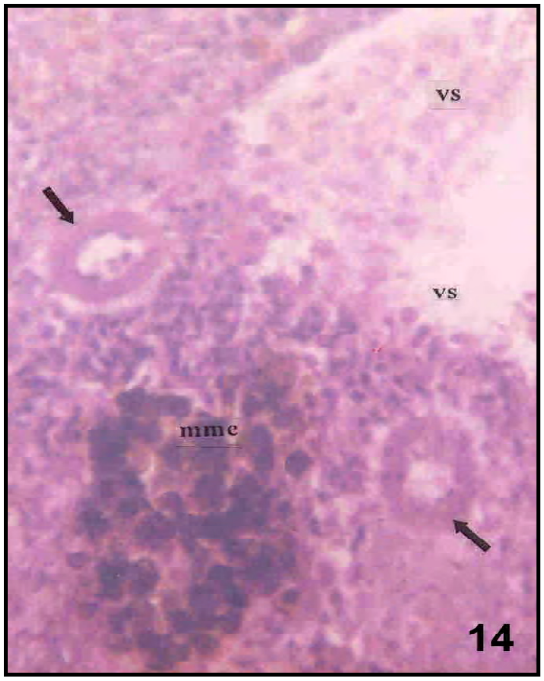

Fig. 14: Section of the HK of *M. gulio* showing an MMC close to two branches of renal artery (arrow) and a venous sinus. Neither limiting membrane nor lymphocyte layer seen. The erythrocytes in the surrounding, with pycnotic nuclei. Bouin, HE x 600

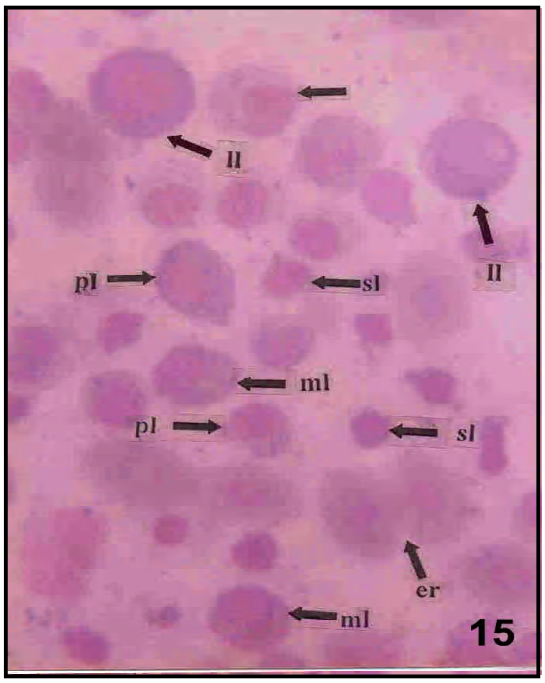

Fig. 15: Imprint of the HK of *M. gulio* showing the various types of cells, erythrocytes, large, medium and small lymphocytes, plasmocytes and monocytes. Leishman stain x 1150

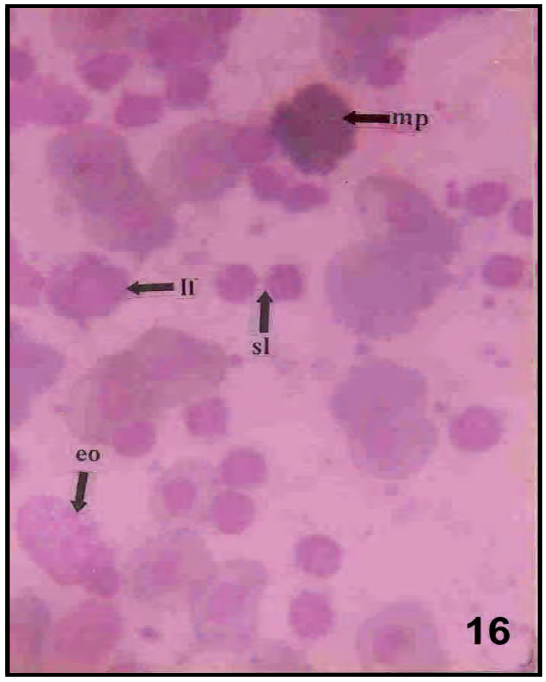

Fig. 16: Imprint of the HK of *M. gulio* showing an eosinophil and a melanomacrophage, besides the other types of cells. Leishman stain x 1150

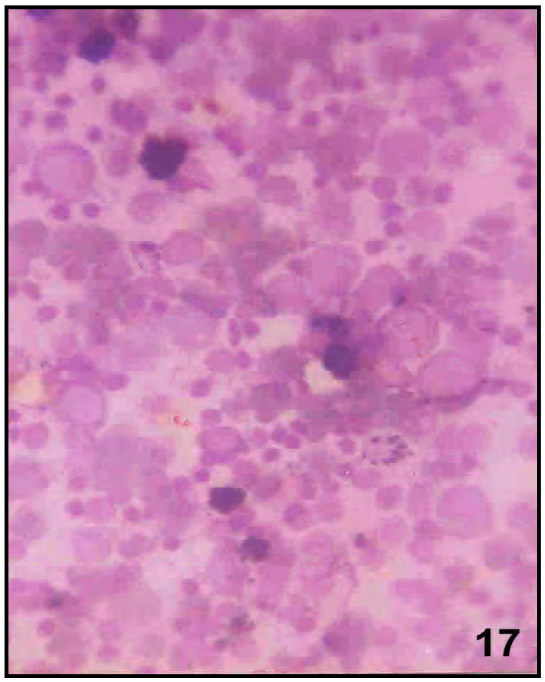

Fig. 17: Imprint of the HK of *M. gulio* showing an rosette formation by large lymphocytes around a macrophage. Leishman stain x 1150

Abbreviations used: eo = eosinophil, er = erythrocyte, ll = large lymphocyte, ml = medium lymphocyte, mmc = melanomacrophage center, mp = macrophage, pl = plasmocyte, sl = small lymphocyte, vs = venous sinus

erythrocytes, cell debris and melanin from melanocytes. The melanomacrophages migrate as free cells, aggregate and accumulate to form the MMCs, the largest being surrounded by fibroblast-like reticular cells. During this process, a part of the engulfed material is partially digested by the lysosomic hydrolase and processed for reutilization. Thus, the melanomacrophages in the HK together with those in the spleen of these teleosts are mainly involved in iron and haemoglobin metabolism and tissue catabolism.

The HK of *M. gulio* showed well organized sinuses and channels of red pulp, chords of white pulp and numerous MMCs in different stages of formation. In addition, immature cells of erythrocytes and other granulocytes, and rosette forming cells could be identified in HK imprint preparations. Based on these observations and in the light of the information available in the literature, it may be suggested that the HK of *M. gulio* has haemopoietic, immune and phagocytic functions.

Acknowledgments

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